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Modeling schizophrenia pathogenesis using patient-derived induced pluripotent stem cells (iPSCs)

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Abstract

Schizophrenia is a chronic disabling mental disorder that affects about 1% population world-wide, for which there is a desperate need to develop more effective treatments. In this *minireview*, we summarize the findings from recent studies using induced pluripotent stem cells to model the developmental pathogenesis of schizophrenia and discuss what we have learned from these studies. We also discuss what are the important next steps and key issues to be addressed to move the field forward.

Keywords

Schizophrenia; iPSC; neural progenitor cell; differentiation

1. Introduction

Schizophrenia (SCZ) is a highly heritable [1] neurodevelopmental disorder [2] that is characterized by positive symptoms (e.g., hallucinations and delusions), negative symptoms (e.g., apathy, anhedonia) and cognitive symptoms (e.g., impairments of memory, executive functions and attention) [3]. Worldwide, 1% of the adult population suffer from this disorder, and individuals with SCZ are at high risk for substance abuse [4], suicide [5] and lifelong disability [6]. Even with antipsychotic treatment, cognitive and negative symptoms persist throughout life [7], preventing normal functioning. Furthermore, many patients discontinue antipsychotic medications due to their adverse sideeffects [8]. Given the fact that SCZ remains the seventh most costly medical disorder to our Society, it is imperative to develop novel efficacious treatments and even preventive interventions for SCZ. As SCZ is a uniquely human disorder characterized by hallucinations and thought disorder, animal

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models cannot recapitulate all SCZ pathophysiology, though certain aspects of SCZ pathophysiology can be replicated [9,10]. Furthermore, many therapeutics developed in animal models have failed in human clinical trials [11,12], thus it is critical to develop novel, human-specific, disease-relevant models of SCZ to develop and test novel therapeutics.

2. Developmental model for Schizophrenia

In vivo imaging and *postmortem* studies in SCZ have shown consistent brain abnormalities, such as reduced cortical volume, atrophic dendrites and altered gene expression, especially in cortical pyramidal neurons [13–15] and cortical interneurons [16–21], as well as abnormal structure of dopaminergic (DA) neurons [22,23] and dis-regulation in the ventral tegmental area (VTA) DA system [24]. Determining the cause of these abnormalities can be confounded by psychotropic drug treatment, malnutrition and substance abuse (Fig. 1). Since the disease process begins long before the onset of overt symptoms of psychosis [2,25], understanding disease progression during brain development could shed light on SCZ's etiology and serve as the basis for preventive therapeutics. Many SCZ putative risk genes are expressed prenatally or early in pluripotent stem cell (PSC) differentiation [26–28], highlighting the importance of studying developmental process to clarify the etiology of SCZ. Many genetic as well as environmental models of SCZ show more pronounced SCZ-like behavioral and morphological changes with perturbations during early development, indicating the significance of developmental disturbance in generating SCZ-like abnormalities [29–32]. However, the ability to study these critical pathologic events in the developing human nervous system once seemed unimaginable since after symptom onset in young adulthood, it is not possible to go back in time to study what happened during fetal brain development (Fig. 1). Recent advances in research on iPSC technologies [33] offer the possibility of generating disease-relevant developing tissues (Fig. 1), making it possible to study developmental abnormalities in brain tissue with the exactly same genetic background as the patients diagnosed with SCZ. Thus, iPSC-derived human developmental brain cells will play a critical role to accurately recapitulate cellular pathology of SCZ and permit the screening of novel chemical agents to correct functional abnormalities or rectify the cellular pathology characteristic of SCZ.

3. Strengths and limitations of iPSC modeling

Development and refinement of iPSC technology so far allows reliable and robust reprogramming into hES-like cells with most of the variance among iPSCs derived from individual differences, not clonal line differences [76,77]. Integration-mediated reprogramming methods could be a concern because of the possibility of generating copy number variants (CNVs) in iPSCs. The use of footprint-free reprogramming methods such as modified RNA methods [78] would be more suitable to study the effect of fine genetic variation of SCZ. Also, the same kind of donor cell sources should be used to minimize tissue-specific epigenetic memory effect.

One of the most critical steps in successfully utilizing iPSC-derived neural tissues with consistent and reproducible results is to overcome heterogeneity and stochasticity of differentiation, which can result in unreliable assay results. This will be overcome by the

development of efficient differentiation protocols and/or isolation strategies for specific cell populations (e.g., GABAergic, dopaminergic, etc.). Single cell transcriptome analysis could help to reduce the problem of heterogeneity in differentiation, but it has a limitation in that relatively low sequencing depth could be prohibitive for novel gene discovery, especially for low abundance genes. In addition, rigorous quality control of both iPSC and derived cells will be required to generate reproducible results, avoiding any cell culture artifacts caused by abnormal karyotypes or contamination with pathogens.

While iPSCs can provide unlimited quantities of developing human neurons for disease modeling, the fact that they follow their *in vivo* developmental timeline during *in vitro* differentiation [34] could prevent the study of adult neurons (years in culture). In the case of SCZ, such a developing neuronal population could identify targets for preventive treatments *in utero* rather than disease-modulating targets of mature neurons after symptom onset. However, the discovery of protocols to generate mature neurons would allow us to overcome these limitations. For example, a Ngn2-based glutamatergic neuronal differentiation protocol developed by the Sudhof laboratory bypasses the embryonic precursor stage and directly generates mature neuron [35]. Thus, the field awaits protocols to efficiently generate each mature neuronal subtype to recreate the disease phenotype in mature neurons. Alternatively, directly converting from fibroblast to neurons has been shown to maintain age markers, unlike iPSCs that undergo resetting the clock back to embryonic stage [36], This could be alternative method to obtain mature neurons to recreate disease phenotype rather than disease predisposition.

While these limitations are being overcome, iPSC technology definitely provides an unprecedented opportunity to study disease cellular phenotypes, by making it possible to generate unlimited quantities of disease-relevant cells from patients for the development of novel therapeutics. For example, IPSC-based disease modeling has led to drug re-purposing in ALS [37], The investigators found hyperexcitibility of iPSC-derived ALS motor neurons that could be reversed by Retigabine, resulting in better survival of the ALS motor neurons. Retigabine was previously approved by the FDA for the treatment of epilepsy, and is now in clinical trial in ALS, encouraging the effort to use iPSC-derived progenies for development of novel therapeutics including drug screening and drug repurposing.

4. Recent iPSC-based cellular models of SCZ

4.1. Subject selection criteria and generation of tissue types for disease modeling

In recent years, an increasing number of studies have used iPSC-derived neural cells to model early developmental pathology of the SCZ brain. Subject selection criteria vary in these studies, spanning from specific genetic abnormalities like CNVs such as 22q11.2 deletion [38–40], 15q11.2 deletion [41] or CNTNAP2 deletion [42] and mutations such as DISC1 mutation [43,44] to SCZ patients with no identified genetic risk factor drawn from clinical populations [45–48]. Childhood onset SCZ (COS) [49], has also been studied since COS exhibits more severe psychopathology as compared to adult onset SCZ and thus would likely have a more robust cellular phenotype.

In light of the heterogeneity of SCZ etiology, cell lines derived from patients with highly penetrant CNVs will allow the identification of specific disease relevant abnormalities more clearly. However, knowledge gained from these subgroups of patients may not be broadly applicable to general patient population where no highly penetrant risk genes have been identified but rather where more than a hundred allelic risk variants have been identified, each of which confers a modest (<5%) risk for SCZ [50]. Stratification of subjects by sex, race, symptomatic features such as prominent negative symptoms or polygenic risk score [51] would help to minimize heterogeneity of subject populations.

In these studies, the neural populations generated, range from neural progenitor cells (NPC) [41–43,45,46,48,49,52] to mixed neural populations [38–40,47,53] and to more specific neural subtypes such as highly enriched glutamatergic neurons [44]. These iPSC-derived neural populations recapitulate the normal developmental time frame and more closely resemble fetal neural progenitors and neuronal populations, suitable for analysis of developmental abnormalities. As discussed above, in light of the heterogeneity and stochastic behavior of iPSC differentiation in general, generating well-defined and homogeneous differentiated progenies is critical for reliable and reproducible outcomes for cellular modeling of SCZ. Recent advances in iPSC differentiation protocols to generate highly homogeneous cell populations [54–56] will help achieve this goal.

4.2. Disease-relevant cellular abnormalities

Disease-relevant cellular phenotypes have been observed in these iPSC-derived neural cells. For NPCs derived from idiopathic SCZ cohort, increased expression of the Wnt signaling pathway [52], abnormal nuclear FGFR1 pathway [48], increased protein translation [45], neuronal migration deficits and oxidative stress [46] have been reported. Topol et al. described a molecular substrate of disrupted neuronal migration, decreased mir9 expression, and found that transfection of mir9 reversed the migration deficit [49]. It remains to be determined how the NPC migration deficit contributes to the pathogenesis of SCZ and whether correction of the migration deficit could be a novel preventive therapeutic target for SCZ. Disruption of gene pathways that are critical for neurodevelopment [48,52] comports well with the developmental hypothesis of SCZ pathogenesis.

In mixed neuron cultures from iPSCs derived from patients with 22q11.2 deletion syndrome, altered miRNA expression, consistent with postmortem findings, was observed [40]. In addition, genes involved in cell cycle, apoptosis and MAPK pathway were affected [38]. Consistent with this finding, another group also observed disruption of the MAPK pathway in 22q11.2 deletion syndrome in mixed neuronal populations [39], though there was differences in miRNA findings between these two studies [39,40]. This discrepancy may have resulted from small sample size, heterogeneous differentiation or the influence of gene variants not within the CNV. Controls that better match the genetic background (sibling or isogenic control) and more homogeneous differentiation could reduce such discrepancies. The disrupted neurogenesis presumably resulting from dysregulation of the MAPK pathway was partially reversed by treating with the p38 inhibitor, SB203580 [39].

Two studies with iPSCs harboring the DISC1 mutation utilized isogenic control lines with identical genetic background. Srikanth et al [32] reported that DISC1 NPCs show an

abnormal forebrain specification and high levels of Wnt signaling accompanied by increased neural proliferation, which could be reversed by early inhibition of Wnt. Wen et al. [41] examined the mature neuronal phenotype and observed a synaptic release deficit using 90% homogeneous glutamatergic neuronal population, which was reversed by isogenic correction of DISC1 mutation. It will be important to compare the similarities and differences between this robust isogenic experimental system and the results from idiopathic SCZ patients to determine the degree to which the result from single gene mutation could be used as a platform to develop novel treatments for idiopathic SCZ.

Overall, it is encouraging that now we can make neurons (or other relevant tissues that are specifically affected by the disease) from patients with the exact same genetic makeup as their living brain using this technology. This is especially so, considering the fact that the complex genetics of SCZ, with over a hundred risk alleles, each having modest effect, are difficult to replicate in an experimental animal. Patient-derived neurons generated from iPSCs faithfully reproduce their complex genetics, thereby permitting the study of pharmacologic interventions that could affect disease progression as well as symptoms. Encouragingly, some of the disease relevant phenotypes in patient iPSC-derived neurons have been shown to be reversed by targeted pharmacologic treatments. However, the preliminary findings, albeit promising, are based on quite small numbers of patients (2 to 14). Given the genetic heterogeneity of SCZ, the observed phenotypes need to be validated in much larger samples of patients and controls to determine how extensively these final common pathways of neural pathology map onto the SCZ phenotype. Nevertheless, patient subgroup-specific phenotypes could be utilized to develop personalized treatments, once the underlying mechanism of the abnormal phenotype is elucidated and effective interventions could be identified.

5. Where do we go from here?

5.1. Disease-relevant tissue types to unravel SCZ pathogenesis

One of the most consistently affected neuronal types in SCZ is the medial ganglionic eminence (MGE)-derived parvalbumin (PV^+) or somatostatin (SST^+) expressing GABAergic interneurons, as shown in numerous post-mortem studies [57]. In accordance with these findings, experimental evidence suggests a role for altered GABA neurotransmission in SCZ disrupting cortical gamma oscillations and thus causing cognitive deficits in patients [58]. Consistent with these findings, $GABA_A$ agonists were shown to restore gamma band activity in SCZ patients, accompanied by improved cognitive functions [59], whereas blocking of $GABA_A$ in prefrontal cortex resulted in SCZ-like cognitive, behavioral, and dopaminergic abnormalities [60]. Furthermore, interneuron-specific developmental disturbances result in a SCZ-like phenotype, including deficits in dopaminergic systems in adult mice [29], suggesting a role for impaired cortical interneuron development in SCZ pathogenesis. By default, most of the mixed neuronal populations generated from iPSCs have excitatory glutamatergic neurons as a major component, and thus suitable for studying glutamatergic pathology but are not optimal for the study of other SCZ-relevant cell types. Recent advances in protocols for generating homogeneous population of interneurons from iPSCs

[34,56] will help to study interneuron-specific pathogenic mechanisms. This is currently the focus of the research in the authors' laboratory.

In addition to abnormalities in glutamatergic neurons and GABAergic interneurons, DA neurons have long been implicated in the pathophysiology of SCZ [61]. Neuronal populations enriched with DA neurons were used to model SCZ [53,62,63]. These studies employed different DA induction protocols that generated a maximum 30% enrichment of DA neurons (15–25% of total cells for [53], 10–30% of total neurons for [62] and undefined minor % in [63]), resulting in inconsistent result among studies. Thus, development of more robust phenotype specification protocol for midbrain DA neurons, especially VTA DA neurons over substantia nigra (SN) DA neurons (reviewed in [64]) or development of protocols to isolate desired cell populations after iPSC differentiation [65] will help to generate more robust data that can be better compared across different laboratories. In addition to the neuronal subtypes, glial cells are also implicated in SCZ pathology [66]. With the protocol available to generate astrocytes [67] or oligodendrocytes [68] from human iPSCs, it will be possible to study cell-intrinsic and autonomous schizophrenia pathogenic mechanism on these disease-relevant tissues.

Finally, generation of homogeneous, well-controlled specific neural subtypes, that are critical for robust analysis of cell intrinsic mechanism, can be also essential to reconstitute specific circuit-dependent pathology. Strategically co-culturing relevant neural components together in a 3D system, would circumvent the limitations of the organoid approach, which tends to be cellularly heterogeneous and stochastic [69]. The creation of well-defined neural circuits constructed from homogeneous components would ensure reproducibility of the modeling of circuit-based abnormalities.

5.2. Connection with genetic studies

SCZ is a disorder of complex genetics, where many genes with small effects interact together with environmental risk factors to bring about the pathogenesis. Thus, it would be essential to identify common gene networks underlying heterogeneous interactions of diverse risk factors to gain further insights into SCZ pathogenesis and identify novel and common therapeutic targets. A recent genome wide association study of the risk for SCZ identified 108 genomic loci with genome-wide significance ($P < 5\times10^{-8}$), opening up the potential to identify such SCZ-selective risk gene networks [50]. However, the functional effects of many of these loci have not yet been elucidated. More than 99% of genetic variants lie outside coding regions, presumably in gene-regulatory sites [70]. Thus, it is essential to identify their functional effects during SCZ pathogenesis in disease-relevant brain tissues, which is generally not feasible from postmortem tissues, but now is attainable through iPSC technology. One caveat for this strategy to find the genetic link in iPSC-derived neural population is that any given risk gene presumably has small effects and thus, it will require large number of subjects to achieve needed statistical power [71]. This is quite different from gene knock-out methods with which can readily yield significant differences with small number of samples.

Minimizing heterogeneity of the differentiated progeny will be important to reduce unnecessary variance. To further reduce variance, identification of groups of subjects with

several shared or overlapping risk genes by genotyping would likely yield more robust results. In addition, taking advantage of publicly available large data sets such as commonmind portal (https://www.commonmind.org) or GTEx portal (http://www.gtexportal.org) for eQTL analysis can compensate for small sample size inherent for iPSCs studies. They aid in the identification of eQTLs of risk genes and the association of these eQTLs with schizophrenia risk in larger data sets. However, results from these postmortem adult brain studies may not always comport with the developmental tissues from iPSCs. Recently, with the aim of studying phenotype effect of genotypes with iPSC-derived specific cell types, the NextGen consortium was formed [72]. Their initial proof of principle studies showed that indeed iPSC-derived specific cell types can be used for this purpose, accurately recreating *in vivo* phenotype [73]. This type of collaborative large-scale study could help reach greater statistical power needed for eQTL studies.

5.3. Cell-environment interaction

Although SCZ is a highly heritable disease, environmental risk factors (e.g., maternal immune activation, folate deficiency and cannabis use) are also important for SCZ pathogenesis, as shown by both epidemiological and animal model studies [74,75]. Thus, more a complete understanding of SCZ pathogenesis will require further elucidation of the interaction of risk genes with environmental risk factors. However, testing the effect of each environmental factor on specific schizophrenia-relevant cell types cannot be performed in living human brains. iPSC technologies do provide such well-defined disease-relevant developmental cell populations to analyze the cell intrinsic effect of each environmental factor. By facilitating the molecular identification of disturbances in gene expression pathways by environmental factors in well-defined system, the PSCs provide excellent tools for biochemical and mechanistic studies. Such an environmental analysis could be further performed in healthy control as compared to schizophrenia neuronal subtypes, to study possible gene-environmental interactions.

5.4. Conclusion (Future Direction)

iPSC-derived neural tissues provide unprecedented opportunity to study SCZ pathogenesis using neural cultures comprised of cells with the same genetic background as the human patients from whom they have been derived. An immediate next step would be to develop highly enriched, well-defined neuronal cell lines to characterize the cellular pathology of SCZ. This will require either using more robust differentiation protocols and/or isolation of specific progenies by using cell surface markers or reporter expression. Obvious candidates include excitatory neurons as well as other SCZ-relevant cell types such as interneurons, DA neurons and oligodendrocytes. These pure populations of well-defined cells will provide disease-relevant phenotypes at transcriptomic level and at the cellular level, providing a platform for determining whether re-purposed or new chemical entities can reverse the phenotype. The next step will be systematic reconstitution of well-defined cellular components to recreate circuit-based phenotypes, which are now beginning to be explored [79]. Reconstitution of neural circuit from defined components will overcome the variability and the poorly controlled cellular heterogeneity of organoids.

By utilizing patient derived iPSCs, we are beginning to identify pathogenic mechanisms of SCZ during brain development. But to have meaningful impact on treatment development, we will need to considerably expand the number of subjects studied in order to identify how broadly represented are genetically and environmentally determined final common pathways for the pathogenesis for SCZ. Considering the immense cost of iPSC studies, collaborative efforts bringing together multiple laboratories and/or the creation of a public iPSC repository will greatly facilitate reaching this goal.

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Abbreviation

CON	healthy control
SCZ	schizophrenia
COS	child onset schizophrenia
NPC	neural progenitor cell
DISC1	disrupted in Schizophrenia 1
CYFIP1	cytoplasmic FMR1 interacting protein 1
CNTNAP2	contactin-associated protein-like 2
DA	dopaminergic

SAD	schizoaffective disorder
MAPK	mitogen-activated protein kinase

Highlights

- iPSCs provide SCZ-relevant tissues genetically identical to patient brain tissues.
- SCZ iPSC-derived neural cells show disease-relevant phenotypes.
- SCZ iPSCs provide a model system to dissect the pathogenetic mechanism of SCZ with.
- Further development in technology will ensure reliable disease modeling using iPSCs.

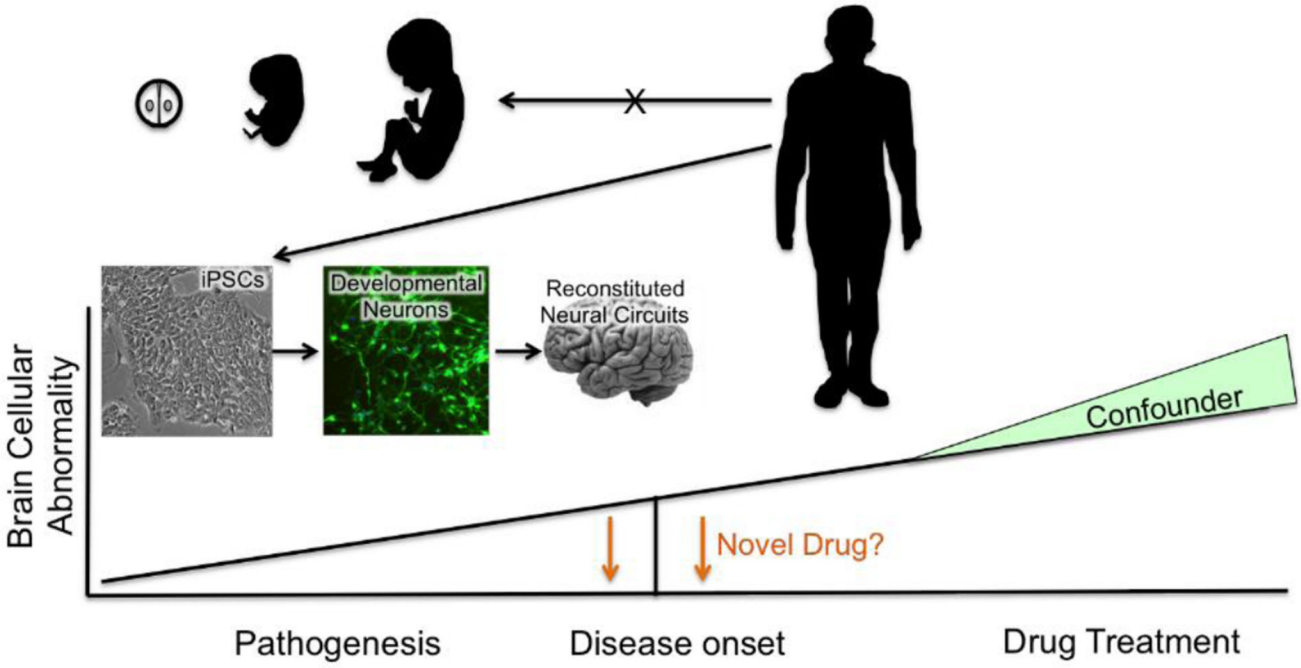

Fig. 1. Modeling pathogenesis of SCZ using induced pluripotent stem cell technology

SCZ pathogenesis can be studied by deriving patient-specific and disease-specific neural cell types using iPSC technology: this allows for the study of cell-innate abnormalities as a monoculture system (developmental neurons) and the study of circuit-based abnormalities with multi-cell type co-culture system (reconstituted neural circuit). Postmortem tissues from schizophrenia patients have been extensively used to gain understanding of schizophrenia-specific brain abnormalities. However, treatment history, substance abuse and poor nutrition can confound interpretation of postmortem findings. iPSC-derived disease-relevant tissues do not present such a problem. Observed cellular abnormalities in these model systems will provide an opportunity to develop novel therapeutics to reverse the observed phenotypes and possibly preventive interventions.

Table 1

Summary of recent iPSC-based SCZ disease modeling studies

Reference	Subjects	Generated cell types	Phenotypes	Recovery
Lin *et al.*, 2016. BMC Syst. Biol. [28]	7 CON 8 SCZ and SAD (all 22q11.2 del)	mixed Neurons	Disrupted mRNA expression in cell cycle, survival and MAPK pathways	
Toyoshima *et al.*, 2016. Transl Psychiatry [29]	3 CON, 2 SCZ (22q11.2 del)	NPCs and Neurons	Neural differentiation and migration ↓ miR-17/92 and miR-106a/b ↓ p38α ↑	p38α inhibitor
Zhao *et al.*, 2015. PLoS One [30]	6 CON, 1 SCZ, 3 SAD, 2 COS (all 22q11.2 del)	mixed Neurons	miRNA disruption consistent with postmortem study	
Yoon *et al.*, 2014. Cell Stem Cell [31]	3 CON, 3 COS (15q11.2 del)	NPCs	CYFIP1 and WAVE2 ↓ Disrupted Adherent junctions and apical polarity	CYFIP1 expression
Lee *et al.*, 2015. NPJ Schizophr [32]	6 CON, Trio (mother, carrier Father, SCZ daughter with CNTNAP2 del)	mixed NPCs	Exon 14–15 CNTNAP2 mRNA ↓ Neurosphere migration ↓	
Srikanth *et al.*, 2015. Cell Rep [33]	1 CON (WT and isogenic DISC1 mutant)	mixed NPCs	Canonical WNT signaling and proliferation ↑ Disturbed NPC fate decision	WNT antagonist
Wen *et al.*, 2014. Nature [34]	1 CON, Family (2 CON, 2 DISC1 mutation)	Glut neurons	Synaptic vesicle release ↓	Isogenic gene correction
Topol *et al.*, 2015. Transl Psychiatry [35]	6 CON, 4 SCZ	mixed NPCs	Protein synthesis ↑ in NPCs but not in neurons	Rapamycin
Brennard *et al.*, 2015. Mol Psychiatry [36]	6 CON, 4 SCZ	mixed NPCs	Migration ↓ Oxidative stress ↑	
Roussos *et al.*, 2016. JAMA Psychiatry [37]	4 CON, 4 SCZ	Mixed Neurons	Activity-dependent gene expression ↓	
Topol *et al.*, 2016. Cell Rep [38]	6 CON, 4 SCZ Replication (10 CON, 10 COS)	mixed NPCs	MiR-9 ↓ in NPCs but not in neurons Migration ↓	miR-9 expression
Topol *et al.*, 2015. Biol. Psychiatry [41]	6 CON, 4 SCZ	mixed NPCs	Canonical WNT signaling ↑	
Hartley *et al.*, 2015. Mol Psychiatry [42]	3 CON, 4 SCZ	mixed Neurons	DA neuron differentiation ↔	