Understanding neurodevelopmental disorders using human pluripotent stem cell-derived neurons

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Abstract

Research into psychiatric disorders has long been hindered by the lack of appropriate models. Induced pluripotent stem cells (iPSCs) offer an unlimited source of patient-specific cells, which in principle can be differentiated into all disease-relevant somatic cell types to create in vitro models of the disorder of interest. Here, neuronal differentiation protocols available for this purpose and the current progress on iPSCs-based models of schizophrenia, autism spectrum disorders and bipolar disorder were reviewed. We also discuss the impact of the recently developed CRISPR/Cas9 genome editing tool in the disease modeling field. Genetically engineered mutation of disease risk alleles in well characterized reference "control" hPSCs or correction of disease risk variants in patient iPSCs has been used as a powerful means to establish causality of the identified cellular pathology. Together, iPSC reprogramming and CRISPR/CAS9 genome editing technology have already significantly contributed to our understanding of the developmental origin of some major psychiatric disorders. The challenge ahead is the identification of shared mechanisms in their etiology, which will ultimately be relevant to the development of new treatments.

INTRODUCTION

Neurodevelopmental disorders are a group of conditions typically manifest early in life and are characterized by developmental deficits that produce impairments of personal, social, academic or occupational functioning. Neurodevelopmental (or neuropsychiatric) conditions include autism spectrum disorders, intellectual disabilities, schizophrenia and bipolar disorder, and have a substantial genetic component (7, 19, 66, 104).

For a long time, psychiatric research mainly relied on post-mortem studies and animal models. The first approach has the major limitation of representing the end-stage of the disease, without being informative about its origin and pathological progression. More-over, changes observed in post mortem tissues may be secondary effects of a patient's prolonged use of medications. Animal models, predominantly rodents, are available for several psychiatric disorders, especially autism spectrum disorders and schizophrenia, but they have so far failed to show significant predictive validity for drug discovery (56, 71). This may be because of the inability of model organisms to represent unique higher human functions and consequently, to recapitulate all the symptoms characterizing a particular disorder (63). For example, despite the numerous rodent models available

for schizophrenia, these are mostly representative of the psychotic aspects of the disease, but do not reliably reproduce the cognitive and negative symptoms, such as impaired working memory, anhedonia and social withdrawal (37). In the case of bipo-lar disorder, there are no animal models available to represent both the manic and depressive extremes characterizing the disorder (11).

 In just a decade from the derivation of the first line (84, 85), induced pluripotent stem cells (iPSCs) have become a fundamental tool for modeling human development and diseases, as well as for drug discovery. Like their mouse counterparts, human iPSCs are believed to have the same self-renewal and pluripotency properties of human embryonic stem cells (hESCs), but are derived (reprogrammed) from somatic cells, such as skin fibroblasts (84), keratinocytes (1), dental pulp (86, 98) or blood (48). The reprogramming is achieved by forcing the expression of key pluripotency genes such as OCT4, SOX2, c-MYC andKLF4insomatic cells, where the reprogramming factors start a self-regulatory loop that initiates and maintains pluripotency (58, 67). Expression of these reprogramming factors can be induced via viral transduction (6, 69), transfection of polycistronic plasmids (61), mRNAs (92) or direct delivery of recombinant proteins (103). More-over, treatment with specific combinations of small molecules has been shown to greatly increase the reprogramming efficiency (32, 78).

The use of adult somatic cells as starting material means that iPSCs are free from the ethical concerns that surround the use of hESCs, and that they can be derived from individuals carrying genetic variants that predispose to an increased risk of human dis-eases. Working on iPSCs, and PSCs in general, has become increasingly important in psychiatric research thanks to the ability to study the consequences of a large number of disease-associated mutations in the phenotypically relevant cell types. This is playing an important role in advancing our understanding of the cellular mechanisms underlying psychiatric disorders and reinforcing the hypothesis of their developmental origin.

GENERATION OF DISEASE RELEVANT NEURONAL CELL TYPES FROM HUMAN PSCS

The brain contains thousands of neuronal types that differ in terms of neurotransmitter identity, electrophysiological properties and afferent/efferent connectivity (62). Different neurological diseases often exhibit pathologies specific to certain brain regions and/or cell types. This means that the possibility of observing and, there-fore, being able to correct a specific phenotype, is strictly dependent on the presence of the appropriate neuronal type in the system used. Therefore, an important consideration in modeling human neurological diseases is the generation of neural cell types targeted by the disease of interest.

A number of neuronal cell types have been implicated in psychiatric disorders: cortical projection neurons (23), inhibitory inter-neurons (23, 44), hippocampal neurons (25), dopaminergic neurons (23) and striatal medium spiny neurons (26, 80). Protocols to generate these neuronal types have been developed and are reviewed in this paragraph. Many lines of evidence demonstrate that in vitro PSC differentiation, to a large extent, mimics vertebrate development. In the context of neuronal conversion, PSCs firstly exit the pluripotent state and acquire a neuroectoderm fate. Therefore, the majority of neuronal differentiation protocols start with the induction of a specific regional neuroepithelial phenotype (region-specific progenitors) from which the target cells arise. This is mostly achieved by artificially recapitulating the signaling environment that the region-specific progenitors normally experience in vivo, by adding an appropriate combination of "inductive" molecules. The aim is to induce a cascade of transcription resembling normal

development, leading to the expression of a combinatorial set of transcription factors characteristic to the desired neural progenitor phenotype.

Currently, a popular method for generating neural cells from hPSCs is via monolayer differentiation protocol by dual SMAD inhibition (14). Normal central nervous system development follows an anterior first–posterior later temporal fashion. As such, the first neuroepithelial cells generated from PSCs exhibit features of forebrain regional identity. These anterior progenitors readily mature into neurons with predominantly cortical glutamatergic identity (9, 24, 79). Cell types of all cortical layers appear in a sequence reminiscent of in vivo corticogenesis, from deep layers to superficial ones. The efficiency of this approach was shown to be very high, with glutamatergic neurons accounting for 80% to nearly 100% of the cells in culture.

The specification of the other neuronal fates requires the use of additional morphogens to mimic the in vivo environment of the corresponding brain region. For example, the derivation of GABAergic interneurons requires the activation of sonic hedgehog (SHH) signaling (46, 47), in some cases combined with WNT inhibition (57, 64), to induce the medial ganglionic eminence (MGE) fate demonstrated by the expression of transcription factor NKX2.1. However, despite the induction of a high percentage of NKX2.11 MGE-like progenitors, efficient generation of mature interneurons, including the two major subtypes, somatostatin (SST) and parvalbumin (PV), have so far proved challenging. On the other hand, caudal ganglionic eminence (CGE)-derived interneurons expressing calretinin have been derived with a higher efficiency (>70%), by exposing late neural progenitors to activin A, a member of the TGF-b superfamily (13).

When applied to forebrain neural progenitors at an earlier time window, activin A induces the specification of a lateral ganglionic eminence (LGE) fate, leading to the production of 40%–50% of medium spiny neurons (4). Other strategies for deriving this type of neurons rely on the use of SHH, alone or in combination with WNT inhibition (45, 49, 65), in a similar way to MGE protocols. Dopaminergic neurons, which are born in the ventral midbrain, can be differentiated from hPSCs by exposing the neural precursors to different combinations of WNT agonists with SHH (20, 39, 41) or FGF8 (96). In addition, FGF signaling blockade using an ERK/MEK inhibitor on exit of pluripotency, followed by addition of SHH and FGF8 has also been reported to induce authentic mid-brain dopamine neurons (34). The yield of tyrosine hydroxylase positive cells is elevated in all these cases, reaching above 80%. Finally, only one protocol has been reported so far for the production of hippocampal neurons (101). For these cells, the application of WNT3a and BDNF to dorsal forebrain progenitors seems to be essential to induce the expression of PROX1, a marker for dentate gyrus hippocampal neurons.

Monolayer differentiation has proved itself a highly efficient and reliable paradigm for generating a number of neuronal types. Indeed, monolayer based forebrain glutamatergic neuron differentiation has been the platform of choice for the majority of disease modeling articles published so far (10, 11, 15, 30, 38, 53, 74, 77, 81, 94, 100). However, the monolayer culture system has limitations in visualizing potential alterations in the cytoarchitecture of the derived "brain tissue." This shortcoming can be overcome, to a certain extent, by differentiating PSCs in three-dimensional (3D) structures called organoids (43, 55, 72), although at the expense of higher variability, both within and between organoids, and in different preparations. To date, only Mariani et al have used telencephalic organoids for analyzing the developmental abnormalities with iPSCs derived from patients with idiopathic autism (54).

The generation of "induced neurons" (iNs) by direct reprogramming represents an alternative to conventional PSC neural differentiation. iNs are produced by forcing the expression of a defined set

of transcription factors crucial for the acquisition of neuronal fate in somatic cells or PSCs (2, 31, 42, 68, 99). iN protocols for the generation of specific neuronal types have also been published (12, 17, 70, 83, 89, 97). However, the iN approach may not be suitable for modeling diseases where pathogenesis occurs at neural progenitor stage, because it bypasses the process of neural progenitor specification, proliferation and differentiation choice toward distinct neuronal and glial fates. Moreover, in contrast to hPSC neural differentiation, the number of iNs that can be generated from the donor somatic cells is limited because of the restricted proliferative capacity of somatic cells prior to senescence. These shortcomings combined may explain why there are no studies published to date using iN technology to model psychiatric disorders.

CURRENT STATUS OF IPSCS-BASEDRESEARCH FOR PSYCHIATRICDISORDERS

Brain imaging studies have demonstrated changes in the anatomy and neuronal activity in patients suffering from psychiatric disorders, while post-mortem studies have revealed aberrant cell-lar morphology (8, 22, 33, 95). The iPSC technology has provided an invaluable tool to investigate the cellular basis of such alterations and elucidate the molecular pathways that may be targeted for drug discovery.

The first proof-of-principle study using iPSCs was published by Brennand et al in 2011 (11). The authors reported that neurons derived from schizophrenic patients' iPSCs differ from those of the controls in neuronal connectivity, morphology and gene expression. This work was followed by several other schizophrenic iPSC studies reporting the emergence of earlier developmental abnormalities.

Robicsek et al differentiated schizophrenia and control iPSCs into glutamatergic and dopaminergic neurons and found defects in the maturation of both cell types, with the dopamine lineage more severely affected (74). The authors also reported differences in mitochondrial distribution and function. This aspect of the phenotype was more pronounced in dopaminergic progenitors than the glutamatergic cells. This could suggest the presence of a higher vulnerability for dopamine cells to oxidative stress, but it could also reflect, at least in part, their extremely impaired maturation.

Together with alterations of mitochondrial membrane potential and neuronal morphology, other defects often reported in neuronal cells from schizophrenic patients iPSCs are relative to WNT signaling and migration (10, 88) (see also Table 1).

All the studies listed above were based on heterogeneous cohorts of schizophrenic patients, selected only on the basis of their diagnosis, without knowledge of their genetic risk variants. This probably played a significant part in the high experimental variability evident in some of the results.

It is known that schizophrenia has a strong genetic component, with rare Copy Number Variations (CNVs) significantly increasing the risk of developing this disorder [reviewed by Kirov (40)]. Strat-ifying patients based on the presence of specific genetic mutations could help to reduce the degree of variability associated with iPSC work and discover new mechanisms that otherwise may be masked by the heterogeneity of the patients' samples.

Wen and colleagues took the genetics orientated approach by analyzing the effect of Disrupted In SChizophrenia 1 (DISC1) mutations, which are known to co-segregate with major psychiatric illnesses (60, 94). iPSC lines were derived from two patients carrying the same frameshift mutation in the DISC1 gene and three unaffected individuals. The authors reported altered morphology and electrophysiological properties in DISC1 neurons, as well as the expression of genes related to

synaptic transmission, neural development and major mental disorders. They also established the causality between DISC1 mutations and the changes observed by repeating some of the analysis in several isogenic cell lines. These were derived by correcting the DISC1 mutation in one of the mutant lines and by introducing the same frameshift deletion present in patients in two control lines. Both synaptic and vesicular release properties were restored to normal levels in the cell line in which the DISC1 gene sequence had been corrected, while the control lines carrying DISC1 deletion recapitulated the original mutant phenotype. However, the majority of CNVs associated to psychiatric disorders contain multiple genes. This genetic feature makes iPSC modeling particularly advantageous over the generation of animal models. Microdeletion of the 15q11.2 locus has been reported by several studies as an important risk factor for schizophrenia (82, 87). Yoon and colleagues demonstrated that iPSCs derived from 15q11.2 deletion carriers had significant defects in neural rosette formation (100). The 15q11.2 region contains four genes, CYFIP1 was proposed to be the likely responsible gene for the observed phenotypes. Indeed, increasing CYFIP1 expression by lentiviral transduction in differentiating 15q11.2del iPSCs rescued the abnormal expression of apical polarity markers, while reduction of CYFIP1 expression by shRNA in a control line mimicked the phenotype observed in the deleted progenitors. The authors extended their quest into CYFIP1 function by shRNA knockdown of Cyfip1 in mouse embryos via in utero electroporation, which resulted in incorrect localization of radial glia cells and nascent neurons. However, the consequences of CYFIP1 disruption in post-mitotic neurons were not explored.
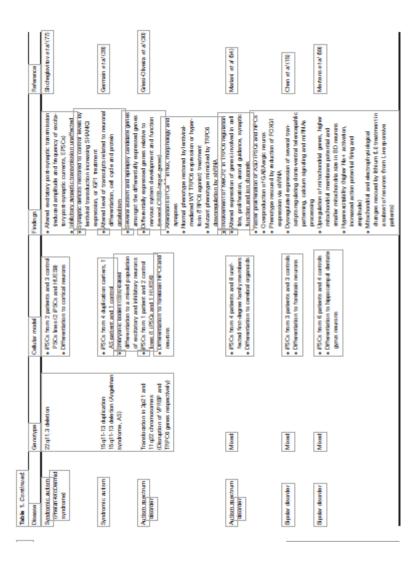
As for autism spectrum disorders, in vitro modeling was first applied to syndromic autism, such as Rett syndrome (RTT) and Phelan-McDermid syndrome (PMDS). RTT syndrome is one of the most common causes of mental retardation mainly affecting girls and is caused by mutations of the methyl CpG binding protein 2 (MECP2) (3). iPSCs based investigations into RTT reported defects in neuronal maturation in patients' cells (38, 53) (see also Table 1). In particular, Marchetto et al demonstrated the presence of morphological alterations and reduced number of glutamatergic synapses in RTT neurons (53). This defect could be rescued by IGF1, a neurotrophic factor capable of promoting synaptogenesis. The same group subsequently reported the involvement of MeCP2 in regulating the expression of TRPC6, one of the genes disrupted by a translocation recently found in an autistic patient (30).

Similar cellular phenotypes were reported in in vitro models of PMDS. Shcheglovitov et al investigated the cellular phenotypes of iPSCs-derived neurons from two patients carrying heterozygous deletion of chromosome 22q13.3, the mutation responsible for PMDS (77). Cortical neurons derived from these iPSCs displayed impaired excitatory synaptic transmission, while the properties of their inhibitory synapses were not affected. These deficits could be either rescued by increasing the expression of SHANK3, a gene included in the deleted locus, or by IGF1 treatment, which did not affect SHANK3 levels.

Taking a different approach, Mariani et al derived iPSCs from four probands with idiopathic autism, carrying no known genetic mutation previously associated with autism spectrum disorders, and unaffected family members (54). Transcriptomic analysis of telencephalic organoids derived from patients and control cells revealed many differentially expressed genes, mainly relative to cell fate, proliferation, axonal guidance, synaptic function and ion channels. Consistently with these results, probands' cells showed dysregu-ated cell cycle and overproduction of inhibitory neurons, a phenotype that could be rescued by attenuating FOXG1 levels.

In the context of bipolar disorder, after performing a microarray analysis of iPSC-derived neurons from three patients and three controls, Chen and colleagues found a similar alteration in the expression of transcription factors regulating dorsoventral telencepahlic patterning (15). These

results, however, do not significantly overlap with those from Mertens et al, the only other publication employing iPSCs for bipolar disorder modeling to date (59). Their work, based on hippocampal neurons derived from bipolar patients, showed that the hyperexcitability and abnormal mitochondria size of these cells could be rescued by lithium treatment, while alteration of mitochondrial membrane potential could not be improved.

Table 1. Continued

Disease	Genotype	Cellular model	Findings	Reference
Syndromic autism (Phelan-McDermid syndrome)	22q13.3 deletion	• iPSCs from 2 patients and 3 control PSC lines (2 iPSCs and HUES8) • Differentiation to cortical neurons	• Altered excitatory post-synaptic transmission (reduced amplitude and frequency of excitatory post-synaptic currents, EPSCs); inhibitory synaptic transmission unaffected • Synaptic deficits restored to control levels by lentiviral transduction increasing SHANK3 expression, or IGF1 treatment	Shcheglovitov et al (77)
Syndromic autism	15q11-13 duplication; 15q11-13 deletion (Angelman syndrome, AS)	• iPSCs from 4 duplication carriers, 1 AS patient and 1 control • Differentiation to a mixed population of excitatory and inhibitory neurons	• Altered level of transcripts related to neuronal differentiation, cell cycle and protein catabolism	Germain et al (78)
Autism spectrum disorder	Translocation in 3p21 and 11q22 chromosomes (Disruption of MBD5 and TRPC6 genes respectively)	• iPSCs from 1 patient and 2 control lines (1 iPSCs and 1 HUES6) • Differentiation to nonneuronal iPSCs and neurons	• Several autism and epilepsy candidate genes amongst the differentially expressed genes • Differentially expressed genes relative to nervous system development and function • Alterations of CREB-target genes • Alterations of Ca²⁺ influx, morphology and synapses • Normal phenotype restored by lentiviral-mediated WNT TRPC6 expression or hyper-tonin if TRPC6 agonist treatment • Mutant phenotype mimicked by TRPC6 downregulation by shRNA • Involvement of FOXG1 in TRPC6 regulation	Griesi-Oliveira et al (10)
Autism spectrum disorder	Mixed	• iPSCs from 4 patients and 8 unaffected first-degree family members • Differentiation to cerebral organoids	• Abnormal expression of genes involved in cell fate, proliferation, axonal guidance, synaptic function and ion channels • Faster proliferation of AGD iPSCs and NPCs • Overproduction of GABAergic neurons • Phenotype rescued by reduction of FOXG1 expression via shRNA	Mariani et al (84)
Bipolar disorder	Mixed	• iPSCs from 3 patients and 3 controls • Differentiation to forebrain neurons	• Dysregulated expression of several transcripts regulating dorso-ventral telencephalic patterning, calcium signaling and miRNA processing • Upregulation of mitochondrial genes, higher mitochondrial membrane potential and smaller mitochondria size in BD neurons	Chen et al (15)
Bipolar disorder	Mixed	• iPSCs from 6 patients and 4 controls • Differentiation to hippocampal dentate gyrus neurons	• Hyperexcitability (higher Na+ activation, increased action potential firing and amplitude) • Mitochondrial and electrophysiological changes rescued by lithium in treatment in a subset of neurons from Li-responsive patients	Mertens et al (59)

In the interest of space, Table 1 summarizes the above works together with additional studies not individually discussed in this mini review. It is evident that differences exist between studies within the context of the same disorder. These discrepancies may attribute to different differentiation protocols, culture systems and analysis methods. Nevertheless, some common themes emerge from these studies. In general, genes involved in nervous system development are reported to be affected by many studies and, in line with this, several phenotypes are already present at the neural progenitor stage. In particular, altered WNT signaling and mitochondria dysfunction seem to be frequently reported in patient-derived cells (10, 11, 59, 74, 81, 88, 90). They may represent shared mechanisms in the etiology of neurodevelopmental psychiatric disorders.

CRISPR-BASED GENOME EDITING AS A POWERFUL ALTERNATIVE TO MODELNEURODEVELOPMENTAL DISORDERS

iPSCs represent a virtually unlimited and bankable source of patient-specific cells that can be differentiated into many disease-relevant neuronal types. Therefore, they allow the investigation of cellular phenotypes in cohorts of patients sharing a specific genetic mutation or the same clinical manifestation with unknown genetic background. This is particularly advantageous in the context of those disorders for which a genetic cause has not been identified, such as idiopathic autism cases (5). However, iPSC-based studies can suffer from high variability because of the differences in the genetic background of different patients, reprogramming methods and culture conditions (75). Working with genetically modified hESCs or a well characterized reference line of hiPSCs and their isogenic controls can represent an alternative to the use of patient iPSCs. This strategy will avoid the variability linked to the different genetic backgrounds of distinct patient iPSC lines and reduce the necessary work load of studying multiple patient iPSC lines required for identifying true phenotype and establish causality.

The CRISPR (Clusters of Regularly Interspaced Short Palindromic Repeats)/Cas9 technology allows genome editing more easily and efficiently than traditional gene targeting via Homologous Recombination (18, 21, 52, 73). CRISPR/Cas9 is a type II CRISPR system [reviewed by Makarova et al (51)], which is naturally present in many bacteria as an immunity mechanism to protect them against foreign DNA (27, 35). It consists of the Cas9 nuclease and a guide RNA (gRNA), a chimeric RNA molecule combining a CRISPR RNA (crRNA) and a transactivating RNA (tracrRNA), which together direct the Cas9 to cleave the target DNA sequence. This mechanism also requires the presence of a protospacer adjacent motif (PAM) upstream of the binding region (35). As a genome editing tool, CRISPR/Cas9 can be used to target virtually any genomic sequence next to a PAM site, by simply designing appropriate gRNAs. The generation of a double strand break (DSB) by the Cas9 induces cellular DNA repair mechanisms, like non-homologous ends joining (NHEJ), which is likely to introduce indels disrupting the targeted DNA sequence, or homology directed repair (HDR), if a donor construct is present. This system has been optimized for its application to human cells, including hPSCs, by transfecting a single or multiple vectors to co-express the Cas9 nuclease and the gRNAs (16, 18, 36, 52). As an alternative to the generation of a DSB by the wild type Cas9 enzyme, a mutated nickase version has also been developed, to facilitate HDR and reduce off-target mutations (18). Delivery of the CRISPR/Cas9 components via lentiviruses (76, 91), or adenoviruses (50), has also shown a high efficiency in targeting the human genome. To further improve the flexibility and rapidity of genome editing in hPSCs, Danwei Huangfu's group developed the iCRISPR platform, consisting of hESCs lines with doxycycline-inducible Cas9 expression (indicated as iCas9) (29). Transfection of iCas9 cells with gRNAs, derived via in vitro transcription, lead to over 40% efficiency for single gene targeting or around 10% for triple gene targeting.

Genome editing technologies are evolving fast and, as previously discussed, they represent a valid alternative to the use of iPSCs, because of the reduced variability associated with the use of isogenic cell lines. Alternatively, CRISPR/Cas9 could also be used to correct a specific genetic mutation in patient-derived cells. In this case, the rescue of the phenotype would provide the definitive proof of the connection between disease and genotype. When investigating the effects of CNVs including several genes, the creation of isogenic models would be excessively time consuming, but it should at least be considered to confirm the causality link between the gene(s) of interest within a CNV and the phenotype observed in differentiated iPSCs. In conclusion, the careful planning of in vitro modeling experiments and parallel use of engineered hESCs and iPSCs, allows the generation of very

elegant systems for the investigation of the cellular pathology underlying complex neurodevelopmental disorders.

CONCLUDING REMARKS

Patient iPSC-based disease modeling has in a short time already shed light on the cellular pathology and molecular basis of neuropsychiatric disorders. This experimental model holds great promise despite the limitations discussed. We anticipate that CRISPR/CAS9 mediated genome editing of disease risk alleles in hPSCs will become a popular alternative in the disease modeling field, especially for diseases with monogenic mutations. For CNVs involving multiple genes, genome editing provides a means to demonstrate causality underpinning phenotype. Fully harnessing the potential of PSC-based disease modeling will benefit from the development of network models that contain different neuronal cell types (eg, cortical projection neurons and interneurons) and nonneuronal cells (eg, glial), and the improvement of high throughput culture systems for efficient drug screening.

REFERENCES

1. Aasen TR, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R et al (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 26:1276–1284.

2. Ambasudhan R, Talantova M, Coleman R, Yuan X, Zhu S, Lipton SA et al (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. Cell Stem Cell [Internet] 9:113–118. Available at: https://doi.org/10.1016/j.stem.2011.07.002

3. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet [Internet] 23:185–188. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10508514

4. Arber C, Precious SV, Cambray S, Risner-Janiczek JR, Kelly C, Noakes Z et al (2015) Activin A directs striatal projection neuron differentiation of human pluripotent stem cells. Development

[Internet] 142:1375–1386. Available at: http://dev.biologists.org/content/142/7/1375.long

5. Ardhanareeswaran K, Coppola G, Vaccarino F (2015) The use of stem cells to study autism spectrum disorder. Yale J Biol Med [Internet] 88: 5–16. Available at: http://www.pubmedcentral.nih.gov/articlerender. fcgi?artid54345539&tool5pmcentrez&rendertype5abstract

6. Ban H, Nishishita N, Fusaki N, Tabata T, Saeki K, Shikamura M et al (2011) Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc Natl Acad Sci U S A [Internet] 108:14234–14239. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21821793

7. Beneyto M, Lewis DA (2011) Insights into the neurodevelopmental origin of schizophrenia from postmortem studies of prefrontal cortical circuitry. Int J Dev Neurosci [Internet] 29:295–304. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53319737 &tool5pmcentrez&rendertype5abstract

8. Black JE, Kodish IM, Grossman AW, Klintsova AY, Orlovskaya D, Vostrikov V et al (2004) Pathology of layer V pyramidal neurons in the prefrontal cortex of patients with schizophrenia. Am J Psychiatry 161:742–744.

9. Boissart C, Poulet A, Georges P, Darville H, Julita E, Delorme R et al (2013) Differentiation from human pluripotent stem cells of cortical neurons of the superficial layers amenable to psychiatric disease modeling and high-throughput drug screening. Transl Psychiatry [Internet] 3:e294. Available at:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53756296&tool5pmcentrez&rendertype 5abstract

10. Brennand K, Savas JN, Kim Y, Tran N, Simone A, Hashimoto-Torii K et al (2015) Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. Mol Psychiatry [Internet] 20:361–368. Available at: https://doi.org/10.1038/mp.2014.22%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid54182344&tool 5pmcentrez&rendertype5abstract%5Cnhttp://www.nature.com/doifinder/10.1038/mp.2014.22

11. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S et al (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature [Internet] 473:221–225. Available at:http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53392969&tool5pmcentrez&rendertype5abstract

12. Caiazzo M, Dell'Anno MT, Dvoretskova E, Lazarevic D, Taverna S, Leo D et al (2011) Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature [Internet] 476: 224–227. Available at: http://www.ncbi.nlm.nih.gov/pubmed/ 21725324

13. Cambray S, Arber C, Little G, Dougalis AG, de Paola V, Ungless MA et al (2012) Activin induces cortical interneuron identity and differentiation in embryonic stem cell-derived telencephalic neural precursors. Nat Commun [Internet] 3:841. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22588303

14. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol [Internet] 27:275–280. Available at: http://www.pubmedcentral.nih. gov/articlerender.

fcgi?artid52756723&tool5pmcentrez&rendertype5abstract

15. Chen HM, DeLong CJ, Bame M, Rajapakse I, Herron TJ, McInnis MG et al (2014) Transcripts involved in calcium signaling and telencephalic neuronal fate are altered in induced pluripotent stem cells from bipolar disorder patients. Transl Psychiatry [Internet] 4: e375. Available at: http://www.nature.com/tp/journal/v4/n3/abs/tp201412a.html

16. Cho SW, Kim S, Kim JM, Kim J-S (2013) Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat Biotechnol [Internet] 31:230–232. Available at: http://www.ncbi. nlm.nih.gov/pubmed/23360966

17. Colasante G, Lignani G, Rubio A, Medrihan L, Yekhlef L, Sessa A et al (2015) Rapid conversion of fibroblasts into functional forebrain GABAergic interneurons by direct genetic reprogramming. Cell Stem Cell [Internet] 17:719–734. Available at: https://doi.org/10.1016/j. stem.2015.09.002

18. Cong L, Ran F, Cox D, Lin S, Barretto R (2013) Multiplex genome engineering using CRISPR/Cas systems. Science (80-) [Internet]. Available at: http://www.sciencemag.org/content/339/6121/819.short

19. Cristino AS, Williams SM, Hawi Z, An J-Y, Bellgrove MA, Schwartz CE et al (2014) Neurodevelopmental and neuropsychiatric disorders represent an interconnected molecular system. Mol Psychiatry [Internet] 19:294–301. Available at: http://www.ncbi.nlm. nih.gov/pubmed/23439483

20. Denham M, Bye C, Leung J, Conley BJ, Thompson LH, Dottori M (2012) Glycogen synthase kinase 3b and activin/nodal inhibition in human embryonic stem cells induces a pre-neuroepithelial state that is required for specification to a floor plate cell lineage. Stem Cells 30: 2400–2411.

21. Ding Q, Regan SN, Xia Y, Oostrom LA, Cowan CA, Musunuru K (2013) Enhanced efficiency of human pluripotent stem cell genome editing through replacing TALENs with CRISPRs. Cell Stem Cell 12: 393–394.

22. Dinstein I, Pierce K, Eyler L, Solso S, Malach R, Behrmann M et al (2011) Disrupted neural synchronization in toddlers with autism. Neuron [Internet] 70:1218–1225. Available at: https://doi.org/10. 1016/j.neuron.2011.04.018

23. Donegan JJ, Lodge DJ (2016) Cell-based therapies for the treatment of schizophrenia. Brain Res [Internet]. Available at: https://doi.org/ 10.1016/j.brainres.2016.08.010

24. Espuny-Camacho I, Michelsen KA, Gall D, Linaro D, Hasche A, Bonnefont J et al (2013) Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. Neuron [Internet] 77:440–456. Available at: http://www.ncbi. nlm.nih.gov/pubmed/23395372

25. Frey BN, Andreazza AC, Nery FG, Martins MR, Quevedo J, Soares JC et al (2007) The role of hippocampus in the pathophysiology of bipolar disorder. Behav Pharmacol 18:419–430.

26. Fuccillo MV (2016) Striatal circuits as a common node for autism pathophysiology. Front Neurosci 10:27.

27. Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) PNAS Plus: Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci 109: E2579–E2586.

28. Germain ND, Chen P-F, Plocik AM, Glatt-Deeley H, Brown J, Fink JJ 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 [Internet] 5:44. Available at: http://www.molecularautism.com/content/5/1/44

29. Gonzᴀlez F, Zhu Z, Shi ZD, Lelli K, Verma N, Li QV et al (2014) An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells. Cell Stem Cell 15:215–226.

30. Griesi-Oliveira K, Acab A, Gupta AR, Sunaga DY, Chailangkarn T, Nicol X et al (2014) Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons. Mol Psychiatry [Internet] 20:1–16. Available at: http://www.nature.com/doifinder/10.1038/mp. 2014.141%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25385366

31. Hu W, Qiu B, Guan W, Wang Q, Wang M, Li W et al (2015) Direct conversion of normal and Alzheimer's disease human fibroblasts into neuronal cells by small molecules. Cell Stem Cell [Internet] 17:204–212. Available at: https://doi.org/10.1016/j.stem.2015.07.006

32. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE et al (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol

[Internet] 26:795–797. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18568017

33. Hutsler JJ, Zhang H (2010) Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res [Internet] 1309:83–94. Available at: https://doi.org/10.1016/j.brainres.2009.09.120

34. Jaeger I, Arber C, Risner-Janiczek JR, Kuechler J, Pritzsche D, Chen I-C et al (2011) Temporally controlled modulation of FGF/ERK signaling directs midbrain dopaminergic neural progenitor fate in mouse and human pluripotent stem cells. Development [Internet] 138:4363–4374. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53177308&tool5pmcentrez&rendertype5abstract

35. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA – guided. Science 337:816–822.

36. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J (2013) RNA-programmed genome editing in human cells. Elife 2013:1–9.

37. Jones C, Watson D, Fone K (2011) Animal models of schizophrenia. Br J Pharmacol 164:1162–1194.

38. Kim KY, Hysolli E, Park IH (2011) Neuronal maturation defect in induced pluripotent stem cells from patients with Rett syndrome. Proc Natl Acad Sci U S A [Internet] 108:14169–14174. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21807996

39. Kirkeby A, Grealish S, Wolf DA, Nelander J, Wood J, Lundblad M et al (2012) Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep [Internet] 1:703–714. Available at: https://doi.org/10.1016/j.celrep.2012.04.009

40. Kirov G (2015) CNVs in neuropsychiatric disorders. Hum Mol Genet 24:R45–R49.

41. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z et al (2011) Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature [Internet] 480:547–551. Available at: https://doi.org/10.1038/nature10648

42. Ladewig J, Mertens J, Kesavan J, Doerr J, Poppe D, Glaue F et al (2012) Small molecules enable highly efficient neuronal conversion of human fibroblasts. Nat Methods 9:575–578.

43. Lancaster MA, Knoblich JA (2014) Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc [Internet] 9:2329–2340. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid54160653&tool5pmcentrez&rendertype5abstract

44. Lewis DA, Curley AA, Glausier JR, Volk DW (2012) Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci [Internet] 35:57–67. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53253230&tool5pmcentrez&rendertype5abstract

45. Li X-J, Zhang X, Johnson MA, Wang Z-B, Lavaute T, Zhang S-C (2009) Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells. Development 136:4055–4063.

46. Liu Y, Liu H, Sauvey C, Yao L, Zarnowska ED, Zhang S-C (2013) Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. Nat Protoc [Internet] 8:1670–1679. Available at: http://www.pubmedcentral.nih.gov/articlerender.

fcgi?artid54121169&tool5pmcentrez&rendertype5abstract

47. Liu Y, Weick JP, Liu H, Krencik R, Zhang X, Ma L et al (2013) Medial ganglionic eminence-like cells derived from human embryonic stem cells correct learning and memory deficits. Nat Biotechnol [Internet] 31:440–447. Available at: https://doi.org/10.1038/nbt.2565

48. Loh Y, Agarwal S, Park I, Urbach A, Huo H, Heffner GC et al (2009) Generation of induced pluripotent stem cells from human blood. Hematop Stem Cells 113:1–3.

49. Ma L, Hu B, Liu Y, Vermilyea SC, Liu H, Gao L et al (2012) Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. Cell Stem Cell [Internet] 10: 455–464. Available at: https://doi.org/10.1016/j.stem.2012.01.021

50. Maggio I, Holkers M, Liu J, Janssen JM, Chen X, Gonc¸alves MAFV (2014) Adenoviral vector delivery of RNA-guided CRISPR/Cas9 nuclease complexes induces targeted mutagenesis in a diverse array of human cells. Sci Rep [Internet] 4:5105. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid54037712&tool5pmcentrez&rendertype5abstract

51. Makarova KS, Haft DH, Barrangou R, Brouns SJJ, Charpentier E, Horvath P et al (2011) Evolution and classification of the CRISPR–Cas Systems. Nat Rev Microbiol [Internet] 9:467–477. Available at: http://www.nature.com/doifinder/10.1038/nrmicro2577%0Ahttp://www.nature.com.myaccess.library.utoronto.ca/nrmicro/journal/v9/n6/abs/nrmicro2577.html%5Cnfiles/319/Makarovaetal2011-Evolutio-nandclassificationoftheCRISPR?Cassys.pdf

52. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE et al (2013) RNA-guided human genome engineering via Cas9_Sup. Science (80-) [Internet] 339:823–826. Available at: http://www.sciencemag.org/content/early/2013/01/03/science.1232033.abstract

53. Marchetto MCN, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y et al (2010) A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell [Internet] 143:527–539. Available at: https://doi.org/10.1016/j.cell.2010.10.016

54. Mariani J, Coppola G, Zhang P, Abyzov A, Provini L, Tomasini L et al (2015) FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. Cell [Internet] 162:375–390. Available at: https://doi.org/10.1016/j.cell.2015.06.034

55. Mariani J, Vittoria M, Palejev D, Tomasini L, Coppola G, Szekely AM et al (2012) Modeling human cortical development in vitro using induced pluripotent stem cells. Proc Natl Acad Sci 109:12770–12775.

56. Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T(2009) Removing obstacles in neuroscience drug discovery: the future path for animal models. Neuropsychopharmacology 34173:74–89.

57. Maroof AM, Keros S, Tyson JA, Ying S-W, Ganat YM, Merkle FT et al (2013) Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell [Internet] 12:559–572. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53681523&tool5pmcentrez& rendertype5abstract

58. Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K et al (2007) Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol 9:625–U26.

59. Mertens J, Wang Q-W, Kim Y, Yu DX, Pham S, Yang B et al (2015) Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. Nature [Internet] 527:95–99. Available at: http://www.nature.com/nature/journal/v527/n7576/full/nature15526.html%5Cnhttp://www.nature.com/nature/journal/v527/n7576/pdf/nature15526.pdf

60. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA et al (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9: 1415–1423.

61. Montserrat N, Garreta E, González F, Gutiⓔerrez J, Eguizⓐabal C, Ramos V et al (2011) Simple generation of human induced pluripotent stem cells using poly-??-amino esters as the non-viral gene delivery system. JBiolChem286:12417–12428.

62. Nelson SB, Sugino K, Hempel CM (2006) The problem of neuronal cell types: a physiological genomics approach. Trends Neurosci 29: 339–345.

63. Nestler E, Hyman S (2010) Animal models of neuropsychiatric disorders. Nat Neurosci [Internet] 13:1161–1169. Available at: http://www.nature.com/neuro/journal/v13/n10/abs/nn.2647.html

64. Nicholas CR, Chen J, Tang Y, Southwell DG, Chalmers N, Vogt D et al (2013) Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. Cell Stem Cell [Internet] 12:573–586. Available at: https://doi.org/10.1016/j.stem.2013.04.005

65. Nicoleau C, Varela C, Bonnefond C, Maury Y, Bugi A, Aubry L et al (2013) Embryonic stem cells neural differentiation qualifies the role of Wnt/??-Catenin signals in human telencephalic specification and regionalization. Stem Cells 31:1763–1774.

66. O'Shea KS, McInnis MG (2016) Neurodevelopmental origins of bipolar disorder: IPSC models. Mol Cell Neurosci [Internet] 73: 63–83. Available at: https://doi.org/10.1016/j.mcn.2015.11.006

67. Pan G, Li J, Zhou Y, Zheng H, Pei D (2006) A negative feedback loop of transcription factors that controls stem cell pluripotency and self-renewal. FASEB J 20:1730–1732.

68. Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ et al (2011) Induction of human neuronal cells by defined transcription factors. Nature 476:220–223.

69. Park I-H (2008) Disease-specific induced pluripotent stem cells. Cell [Internet] 134:877–886. Available at: http://resolver.scholarsportal. info/resolve/00928674/v134i0005/877_dipsc.xml

70. Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A et al (2011) Direct conversion of human fibroblasts to dopaminergic neurons. PNAS [Internet] 108:5819. Available at: https://doi.org/10. 1073/pnas.1105135108%5Cnhttp://www.pnas.org/content/108/25/ 10343.short

71. Pratt J, Winchester C, Dawson N, Morris B (2012) Advancing schizophrenia drug discovery: optimizing rodent models to bridge the translational gap. Nat Rev Drug Discov [Internet] 11:560–579.

72. Qian X, Nguyen HN, Song MM, Hadiono C, Ogden SC, Hammack C et al (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell [Internet] 165:1238–1254. Available at: https://doi.org/10.1016/j.cell.2016.04.032

73. Ran F, Hsu P, Wright J, Agarwala V (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc [Internet] 8:2281–2308. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/24157548%5Cn
http://www.nature.com/nprot/journal/v8/n11/abs/nprot.2013.143.html

74. Robicsek O, Karry R, Petit I, Salman-Kesner N, M€uller F-J, Klein E et al (2013) Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol Psychiatry [Internet] 18:1067–1076. Available at:
http://www.ncbi.nlm.nih. gov/pubmed/23732879

75. Sandoe J, Eggan K (2013) Opportunities and challenges of pluripotent stem cell neurodegenerative disease models. Nat Neurosci [Internet] 16:780–789. Available at:
http://www.ncbi.nlm.nih.gov/pubmed/23799470

76. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Heckl D et al (2014) Genome - scale CRISPR - Cas9 knockout screening in human cells. Science (80-) 343:84–87.

77. Shcheglovitov A, Shcheglovitova O, Yazawa M, Portmann T, Shu R, Sebastiano V et al (2013) SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. Nature

[Internet] 503:267–271. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24132240

78. Shi Y, Desponts C, Do JT, Hahm HS, Sch€oler HR, Ding S (2008) Induction of pluripotent stem cells from mouse embryonic fibroblasts by oct4 and klf4 with small-molecule compounds. Cell Stem Cell 3: 568–574.

79. Shi Y, Kirwan P, Livesey FJ (2012) Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. Nat Protoc 7:1836–1846.

80. Simpson EH, Kellendonk C, Kandel E (2010) A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia. Neuron [Internet] 65:585–596. Available at:
https://doi.org/10.1016/j.neuron.2010.02.014

81. Srikanth P, Han K, Callahan DG, Makovkina E, Muratore CR, Lalli MA et al (2015) Genomic DISC1 Disruption in hiPSCs Alters Wnt Signaling and Neural Cell Fate. Cell Rep [Internet] 12:1414–1429. Available at: https://doi.org/10.1016/j.celrep.2015.07.061

82. Stefansson H, Rujescu D, Cichon S, Pietil€ainen OPH, Ingason A, Steinberg S et al (2008) Large recurrent microdeletions associated with schizophrenia. Nature [Internet] 455:232–236. Available at: http://www.pubmedcentral.nih.gov/articlerender.
fcgi?artid52687075&tool5pmcentrez&rendertype5abstract

83. Sun AX, Yuan Q, Tan S, Xiao Y, Wang D, Khoo ATT et al (2016) Direct induction and functional maturation of forebrain GABAergic neurons from human pluripotent stem cells. Cell Rep [Internet] 16: 1929–1941. Available at: https://doi.org/10.1016/j.celrep.2016.07.035

84. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872.

85. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676.

86. Tamaoki N, Takahashi K, Tanaka T, Ichisaka T, Aoki H, Takeda-Kawaguchi T et al (2010) Dental pulp cells for induced pluripotent stem cell banking. J Dent Res [Internet] 89:773–778. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20554890

87. The International Consortium Schizopernia (2008) Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature [Internet] 455:237–241. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid53912847&tool5pmcentrez&rendertype 5abstract

88. Topol A, Zhu S, Tran N, Simone A, Fang G, Brennand KJ, Altered WNT (2015) Signaling in human induced pluripotent stem cell neural progenitor cells derived from four schizophrenia patients. Biol Psychiatry 29–34.

89. Victor MB, Richner M, Hermanstyne TO, Ransdell JL, Sobieski C, Deng PY et al (2014) Generation of human striatal neurons by microRNA-dependent direct conversion of fibroblasts. Neuron [Internet] 84:311–323. Available at: https://doi.org/10.1016/j.neuron. 2014.10.016

90. Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W et al (2015) CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. Mol Autism [Internet] 6:55. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid54612430&tool5pmcentrez&rendertype 5abstract

91. Wang X, Wang Y, Wu X, Wang J, Wang Y, Qiu Z et al (2015) Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase- defective lentiviral vectors. Nat Biotechnol 33:175–178.

92. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F et al (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7:618–630.

93. Wen Z, Nguyen HN, Guo Z, Lalli M. a, Wang X, Su Y et al (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature [Internet] Available at: http://www.nature.com/doifinder/10.1038/nature13716

94. Wen Z, Nguyen HN, Guo Z, Lalli MA, Wang X, Su Y et al (2014) Synaptic dysregulation in a human iPS cell model of mental disorders. Nature [Internet] 515:414–418. Available at: http://www. nature.com/nature/journal/v515/n7527/full/nature13716.html%5Cnhttp://www.nature.com/natur e/journal/v515/n7527/pdf/nature13716.pdf

95. Whitfield-Gabrieli S, Thermenos HW, Milanovic S, Tsuang MT, Faraone SV, McCarley RW et al (2009) Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proc Natl Acad Sci U S A [Internet] 106:1279–1284. Available at: http://www. pubmedcentral.nih.gov/articlerender.fcgi?artid52633557&tool5pmcentrez&rendertype5abstract

96.Xi J, Liu Y, Liu H, Chen H, Emborg ME, Zhang SC (2012) Specification of midbrain dopamine neurons from primate pluripotent stem cells. Stem Cells 30:1655–1663.

97.Xu Z, Jinag H, Zhong P, Yan Z, Chen S, Feng J (2016) Direct conversion of human fibroblasts to dopaminergic neurons. Mol Psychiatry [Internet] 21:62–70. Available at: https://doi.org/10.1073/pnas.1105135108%5Cnhttp://www.pnas.org/content/108/25/10343.short

98. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT-J (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev [Internet] 19:469–480. Available at: http://www.liebertonline.com/doi/abs/10.1089/scd.2009.0314

99. Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y et al (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476:228–231.

100. Yoon K-J, Nguyen HN, Ursini G, Zhang F, Kim N-S, Wen Z 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 [Internet] 15:79–91. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24996170

101. Yu DX, Di Giorgio FP, Yao J, Marchetto MC, Brennand K, Wright R et al (2014) Modeling hippocampal neurogenesis using human pluripotent stem cells. Stem Cell Reports [Internet] 2:295–310. Available at: https://doi.org/10.1016/j. stemcr.2014.01.009

102. Zhao D, Lin M, Chen J, Pedrosa E, Hrabovsky A, Fourcade HM et al (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 10:1–24.

103. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T et al (2009) Generation of Induced Pluripotent Stem cells using recombinant proteins. Cell Stem Cell 4:381.

104. Ziats MN, Rennert OM (2016) The evolving diagnostic and genetic landscapes of autism spectrum disorder. Front Genet 7:1–6. (APR):