


**CONCISE REVIEW**

# The potential of induced pluripotent stem cells for discriminating neurodevelopmental disorders

Ricarda Stock<sup>1</sup> | Pauline Jeckel<sup>1</sup> | Udo Kraushaar<sup>1</sup> | Richard Wüst<sup>2</sup> |  
Andreas Fallgatter<sup>2</sup> | Hansjürgen Volkmer<sup>1</sup> 

<sup>1</sup>Department of Molecular Biology, NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany

<sup>2</sup>Department of Psychiatry, University of Tübingen, Tübingen, Germany

**Correspondence**

Hansjürgen Volkmer, Department of Molecular Biology, NMI at the University of Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany.

Email: volkmer@nmi.de

**Abstract**

Studying human disease-specific processes and mechanisms *in vitro* is limited by a lack of valid human test systems. Induced pluripotent stem cells (iPSCs) evolve as an important and promising tool to better understand the molecular pathology of neurodevelopmental disorders. Patient-derived iPSCs enable analysis of unique disease mechanisms and may also serve for preclinical drug development. Here, we review the current knowledge on iPSC models for schizophrenia and autism spectrum disorders with emphasis on the discrimination between them. It appears that transcriptomic analyses and functional read-outs are the most promising approaches to uncover specific disease mechanisms *in vitro*.

**KEYWORDS**

autism, calcium imaging, electrophysiology, iPSC, schizophrenia, transcriptome

## 1 | INTRODUCTION AND CLINICAL OBSERVATIONS

To date, neurodevelopmental disorders (NDDs) are considered as a heterogeneous group of diseases, all implicating an impairment in the development and growth of central nervous system. Among other NDDs, autism spectrum disorder (ASD) and schizophrenia (SZ) stand out due to their partly clinical similarities and their frequency diagnosed in population (SZ ~1%; ASD ~2%). In the case of SZ, more than 50% of patients receiving diagnosis have long-term psychiatric problems, an unemployment rate of ~90% and decreased life expectancy by ~20 years.<sup>1,2</sup> With a disease onset during childhood as a striking criterion, ASD patients show impairments such as delayed development in childhood or disturbed social interactions throughout lifetime.<sup>3</sup>

Besides both being heritable psychiatric diseases with a multifactorial etiology and so far no available curable treatment, a clinical overlap in parts is evident.<sup>4</sup> Both diseases can show similar

symptoms, including neuropsychological and cognitive impairment, social withdrawal and anxiety, isolated play preference, poor performance in school, disturbed social-communication function, or decreased intelligence in subgroups.<sup>5-7</sup>

For ASD diagnosis, major criteria are (a) restricted, repetitive behavior, behavioral rigidity, odd or intense interests and (b) persistent deficits in social communication, interactions, and communicative behavior must be fulfilled. Additionally, the onset of at least some symptoms must be evident with an age of 18 to 24 months. Further ASD shows a gender preference toward male sex with a ratio of 4-5:1.<sup>8,9</sup> SZ in contrast is mostly diagnosed during young adolescence (male ~18-25 years and female ~25-35 years) while showing no specific gender differences in prevalence. For diagnosis of SZ, at least two symptoms such as hallucinations, delusions, disorganized speech, grossly or catatonic behavior, and negative symptoms must be fulfilled over a certain point of time.<sup>10</sup>

So far an effective treatment appears challenging, and only relief of impairing symptoms can be addressed. In both cases, a

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combination of pharmacotherapy, biological therapies, and interventions such as cognitive behavioral therapy, family-based therapy, speech therapy, or support of daily structure is a major part of a comprehensive treatment.<sup>11,12</sup> In both diseases, pharmacological treatment management remains uncertain, finally relying on *try and error*. Trying to grasp and explain these two complex disease groups based on the genetic background and nosological descriptions, or findings from pharmacological treatments seem to be insufficient. This indicates a need for more profound tools helping to understand and treat the diseases.

## 2 | INDUCED PLURIPOTENT STEM CELL AS A NOVEL TOOL TO STUDY NDDs

The establishment of valid models for the understanding of NDDs and drug development remains a challenge. The majority of neurobiological and neuropsychiatric test systems relies on time consuming and cost intensive animal models that lack the ability to precisely reflect the human brain. Human alternatives are limited and restricted to analyses of postmortem tissue or in vivo neuroimaging.<sup>13</sup>

The potential of induced pluripotent stem cells (iPSCs) revolutionized the field of human in vitro test systems upon discovery in 2007.<sup>14</sup> iPSCs comprise the whole complex genome of the original donor with every specific mutation leading to a distinct phenotype. Especially for studying highly heterogeneous diseases with several associated risk genes, iPSCs can be used as a tool to study pathological processes.

iPSCs for neuropsychiatric disorders are applied by different approaches. Somatic cells can be used for reprogramming providing the full genetic background of individual patients.<sup>15-20</sup> The immanent variability of different patients can be alleviated by control iPSCs from unaffected first degree relatives. Alternatively, targeted mutation, for example, by Crispr/Cas9 provides genetically defined models which are advantageous since they enable isogenic approaches for highest control of the genetic background. On the other hand, the strong effects observed coincide with a certain ambiguity. For instance, SHANK3 mutations may account for ASD, schizophrenia, and other psychiatric disorders which precludes the identification of disease-specific mechanisms (for review, see Reference 21). Likewise, mutation of tuberous sclerosis complex 1 or 2 serves as an ASD model. However, the mutation is also associated with tumors in different organs, epilepsy, attention deficit hyperactivity disorder, and cognitive disabilities.<sup>22</sup>

Here, data are collected to illustrate how experimental data sets obtained from iPSC-derived neurons reflect the disease pathology of ASD and SZ forms with no known genetic cause here referred to as idiopathic. Furthermore, the point is addressed whether a specific discrimination between ASD and SZ has been achieved in vitro.

## 3 | TRANSCRIPTOME ANALYSIS

For SZ and ASD, transcriptomic analysis developed as a valuable tool for the discrimination of disease phenotypes and a deeper

### Significance statement

Published data are encouraging and established induced pluripotent stem cell-derived neurons as a robust and valuable tool for a deeper understanding of the precise mechanisms of neuropsychiatric diseases, and may ultimately pave the way for biomarker research and drug development.

understanding of disease-related genes and molecular pathways.<sup>23,24</sup> However, transcriptome profiling of patients and iPSC-derived neuronal models is biased through the genetic heterogeneity of different individuals. For this reason, the comparison of independent data sets obtained from different patients is essential to identify characteristic genes for ASD or SZ.

So far, two reports account for the comparison of deregulated transcripts in iPSC-derived neurons from patients with idiopathic SZ which included a sample size of three healthy controls vs three patients<sup>25</sup> and four healthy controls vs four patients.<sup>26</sup> Both transcriptome analyses, for example, identified lymphoid enhancer binding factor 1 (LEF1) and erb-b2 receptor tyrosine kinase 3 (ERBB3) that became upregulated in patients with SZ (Table 1). LEF1 recruits  $\beta$ -catenin as a downstream transcription activator of the WNT signaling pathway, a crucial disease pathway in schizophrenia.<sup>33,34</sup> Genome-wide association studies confirmed *NRG1-ErbB* signaling as an important pathway for schizophrenia.<sup>35</sup> ERBB family members are receptor tyrosine kinases recognized by the ligand neuregulin1 (NRG1<sup>36</sup>); Nrg1 signaling is implicated in cortical GABAergic and glutamatergic circuit development.<sup>37,38</sup> Nrg1 is a ligand of *ErbB* receptor tyrosine kinases *ErbB3* and *ErbB4*.<sup>39</sup> While ERBB4 appears as an important factor in SZ,<sup>40,41</sup> the role of *ERBB3* is controversial.<sup>42,43</sup> Dysregulation of *ERBB3* transcriptome analysis may be explained by compensatory effects of deficient ERBB4 signaling. iPSC lines with DISC1 mutations were also used as a model for schizophrenia.<sup>27</sup> However, only five genes in neurons differentiated from DISC1 iPSC were deregulated in idiopathic models (Table 1) suggesting that the monogenic model is at least partially different from idiopathic models.

In contrast to limited data sets for SZ transcriptomes, six publications have compared transcriptome data of iPSC-derived neurons from idiopathic ASD to healthy controls (Table 1).<sup>25,28-32</sup> For example, ATP8A1 is a catalytic component of a P4-ATPase flippase complex and involved in cell migration. Compared to controls, elevated ATP8A1 protein was found in the hippocampus and temporal lobe of juvenile autistic subjects.<sup>44</sup> Another interesting candidate gene which showed up in four publications is the transcription factor aristaless-related homeobox (ARX). ARX is essential for normal brain development, in particular in the migration and maintenance of interneurons.<sup>45</sup> ARX mutations are connected to a wide spectrum of NDDs including autism.<sup>46-48</sup> In conclusion, transcriptomic analyses came up with some ASD or SZ risk genes found in the respective patient populations. The smaller number of deregulated mRNAs as compared to the large number of risk genes identified in genomic studies may be explained by the much smaller patient

**TABLE 1** Consistently deregulated genes in iPSC-derived neurons in (A) SZ or (B) ASD neurons

A. Schizophrenia						
Gene	Grunwald et al <sup>25</sup>		Roussos et al <sup>26</sup>		Wen et al <sup>27</sup>	
DCT		x		x		x
LEF1		x		x		-
ACTN2		x		x		x
COL14A1		x		x		x
EN1		x		x		-
ERBB3		x		x		-
GBP3		x		x		-
KCNA4		x		x		x
OLFML1		x		x		-
PMP2		x		x		-
RASSF9		x		x		x
RGPD2		x		x		-
SAMD9L		x		x		-
ZDBF2		x		x		-
B. Autism						
Gene	Liu et al <sup>28</sup>	Grunwald et al <sup>25</sup>	DeRosa et al <sup>29</sup>	Schafer et al <sup>30</sup>	Mariani et al <sup>31</sup>	Marchetto et al <sup>32</sup>
ATP8A1	x	-	x	x	x	-
AGTR1	-	x	-	x	x	x
ARX	-	x	x	x	x	-
FOXA1	-	x	x	x	x	-
SHH	-	x	x	x	x	-
ASPN	-	-	x	x	x	x

cohorts under inspection. No commonly deregulated transcript has been found suggesting transcriptomic analysis as an important tool to discriminate between ASD and SZ.

#### 4 | DEVELOPMENTAL PHENOTYPES: NEURITE LENGTH AND SYNAPTIC CONNECTIVITY

NDDs are signified by early developmental deficits such as impaired neuronal connectivity or altered synaptogenesis which can be reproduced in iPSC-derived neurons in vitro.<sup>49</sup> For schizophrenia, post-mortem tissue analysis and in vivo neuroimaging in human individuals reported reduced brain volumes,<sup>50</sup> decreased numbers of dendritic spines,<sup>13,51</sup> and diminished expression of postsynaptic marker PSD-95.<sup>52,53</sup> Accordingly, iPSC-derived cortical neurons showed a reduction in neuronal connectivity, neurite outgrowth, and synaptic markers (PSD-95, glutamate receptors) in idiopathic SZ.<sup>25,54-56</sup> Reduced synaptogenesis was also shown for dopaminergic neurons and cortical interneurons.<sup>57</sup> Likewise, deficits in hippocampal neurogenesis were reported for dentate gyrus granule neurons accompanied by reduced neurotransmitter release and neuronal activity.<sup>58</sup> In 22q11.2 deletion models for SZ, no reduction of synapse densities was observed which is

opposite to the DISC1 model showing decreased synaptic punctae in accordance with the idiopathic models.<sup>27,59</sup>

Similarly, ASD is linked to abnormal dendritic structures with altered morphological spines which might coincide with deregulated functional connectivity.<sup>60,61</sup> Most of the identified genes have been shown to contribute to neurite outgrowth and length, dendritic spine number and structure and eventually to impaired neuronal connectivity.<sup>62</sup> In iPSC-derived neurons from a small cohort of idiopathic ASD patients, neurite outgrowth assays revealed reduced numbers of neurons with neurites and decreased length of neurites.<sup>25</sup> Likewise, synaptic dysregulation and increased neurogenesis may contribute to FOXG1-mediated overproduction of GABAergic inhibitory neurons.<sup>32</sup> As a consequence, excitatory/inhibitory imbalance may be linked to the development of ASD.<sup>31</sup> ASD models based on genetically defined mutations come up with conflicting results in neurite outgrowth assays. CGG repeat expansion of FMR1 decreases neurite outgrowth in accordance with the idiopathic models while TSC1 mutation was found to increase neurite outgrowth.<sup>63,64</sup> Likewise, mutation of MeCP2, serving as an iPSC-derived model for Rett syndrome, as well as 22q13 deletion in Phelan-McDermid syndrome recapitulate reduced synaptic punctae densities found in idiopathic ASD iPSC-derived models.<sup>65,66</sup>

When analyzing neuronal iPSC-based cell cultures to compare SZ- and ASD-related developmental phenotypes, most analyses come

**TABLE 2** Phenotypes of schizophrenia SZ and ASD neurons observed in different reports

Parameter	Phenotypes of schizophrenia	Literature	Phenotypes of autism spectrum disorders	Literature
Mitochondrial defects	Altered oxygen metabolism with increased oxidative stress	55-57	Not reported	Not reported
Proliferation	Not reported	Not reported	Accelerated cell cycle of 3D brain organoids	31
	Not reported	Not reported	Increased neuronal progenitor cell proliferation	32
Neuronal migration, arborization and connectivity	Diminished migration	54	Not reported	Not reported
	Arborization deficits	56	Not reported	Not reported
Neurite number and length	Reduced number and length	21,54,55	Reduced number and length	21
Deficient neurogenesis	Reduced hippocampal neurogenesis	58	Abnormal neurogenesis	32
Synaptic dysregulation	Reduced PSD-95 levels	21,54	Reduced PSD-95 levels	21
	Reduced glutamate expression	54	Not reported	Not reported
Neurotransmitter release	Reduced release levels	58	Not reported	Not reported
Neuronal activity	Reduced neuronal activity	58,65	Reduced neuronal activity	29,32
	Reduced Ca <sup>++</sup> peak frequency and increased peak area	21	Reduced Ca <sup>++</sup> peak amplitude	21

up with similar results, such as decreased neurite outgrowth, synaptic connectivity, or abnormal neurogenesis in idiopathic models (Table 2). While being instructive for developmental deficits, these assays do not contribute to a precise discrimination between SZ and ASD.

## 5 | FUNCTIONAL IMPACT

Deficient network activity in iPSC-derived neurons from SZ and ASD patients suggests a functional preservation in iPSC-derived neuronal cultures *in vitro*. The idiopathic ASD phenotype is associated with the inability of iPSC-derived neurons to generate higher frequencies of action potentials (APs).<sup>28</sup> This alteration in turn has an impact on synaptic output and, as a consequence, on the computation within neuronal networks. Indeed, genes associated with synaptic function appeared to be deregulated in iPSC-derived neurons from patients with ASD.<sup>31</sup> Accordingly, network activity is strongly reduced when investigated using microelectrode arrays (MEAs).<sup>29</sup> Like data directly obtained from ASD patients,<sup>67,68</sup> the balance between excitatory and inhibitory input is disrupted illustrating once again the meaningfulness of this *in vitro* approach.<sup>31</sup>

With regard to impaired synaptic transmission in genetically defined models, miniature and spontaneous excitatory postsynaptic potentials (mEPSC and sEPSC, respectively) as well as their inhibitory pendants mIPSC and sIPSCs are detected at reduced frequency and occasionally smaller amplitude compared to control, in a variety of different models with mutations in MeCP2, ATRX, AFF2, KCNQ2, SCN2A, ASTN2, and PTCH-D1.<sup>65,69-71</sup> The finding that SHANK3 mutation decreases synaptic transmission while SHANK2 mutation elicited increased neuronal transmission requires further examination.<sup>72,73</sup>

For idiopathic SZ models, the impact on neuronal network functionality seems to be less prominent. Overall single-cell

electrophysiological properties including membrane resistance and AP generation remain conserved compared to control, but synaptic transmission was negatively affected in idiopathic forms of SZ.<sup>54</sup> Analysis of neuronal networks with MEAs and patch clamp recordings of hippocampal cultures revealed significantly reduced number of spikes and network bursts as well as deficits in spontaneous and evoked activity compared to healthy controls.<sup>74</sup> In a genetically defined DISC1 model, a loss of glutamatergic synapses and a reduction of sEPSC frequency was confirmed, accordingly.<sup>27</sup>

Imaging of intracellular calcium signals in iPSC-derived SZ neurons revealed decreased peak frequency with significantly increased peak areas,<sup>25</sup> while no significant differences in calcium signaling were reported earlier.<sup>54</sup> Most interestingly, SZ and ASD can be discriminated by distinct neuronal calcium activity patterns.<sup>25</sup> iPSC-derived ASD neurons showed, in contrast to previously published results,<sup>32</sup> no reduction of peak frequency compared to healthy controls, whereas the peak amplitude ( $\Delta F/F_0$ ) was significantly decreased in comparison to healthy controls and SZ-derived cells. The peak area was unaffected in ASD-neurons, while SZ-neurons showed an increased peak area accompanied by a decrease in peak frequency.<sup>25</sup>

Although the number of studies dealing with idiopathic forms of NDDs are scarce, functional assays may provide a possibility to discriminate SZ from ASD phenotypes in iPSC-derived neurons.

## 6 | iPSC-DERIVED NEURONS FOR DRUG TESTING

The impact of different biochemical agents and antipsychotic drugs was analyzed in only few iPSC-based experiments. In the case of SZ-models, loxapine or the mood stabilizer valproic acid rescued gene expression or release of excessive reactive oxygen species, respectively.<sup>54,57</sup>

**TABLE 3** MHC II-related genes upregulated after clozapine treatment

Gene	Gene ID	Description
CD74	ENSG0000019582	Major histocompatibility complex, class II, antigen gamma chain
CIITA	ENSG00000179583	Major histocompatibility complex, class II, transactivator
HLA-DOA	ENSG00000204252	Major histocompatibility complex, class II, DO alpha chain
HLA-DPA1	ENSG00000231389	Major histocompatibility complex, class II, DP alpha chain 1
HLA-DQA1	ENSG00000196735	Major histocompatibility complex, class II, DQ alpha chain 1
HLA-DRA	ENSG00000204287	Major histocompatibility complex, class II, DR alpha chain
HLA-DRB1	ENSG00000196126	Major histocompatibility complex, class II, DRB1 beta chain
HLA-DRB5	ENSG00000198502	Major histocompatibility complex, class II, DRB5 beta chain

Application of  $\alpha$ -Lipoic Acid/Acetyl-L-Carnitine reversed deficits in oxidative phosphorylation and subsequent deficits in arborization of cortical, iPSC-derived interneurons from SZ patients.<sup>56</sup> In contrast, application of commonly used antipsychotics, such as clozapine, haloperidol, and olanzapine, did not rescue reduced neurite lengths in SZ-neuronal progenitor outgrowth assays.<sup>25</sup> These findings question the suitability of neurite outgrowth assays for high-throughput drug screening. It is of note that clozapine treatment of neurons derived from a healthy individual revealed increased expression of several MHC class II-associated genes (Table 3)<sup>25</sup> suggesting a possible link of clozapine treatment to inflammatory mechanisms. In vitro, the same set of genes was found to be upregulated in iPSC-derived neurons without drug treatment that were obtained from a patient who was previously treated with clozapine. How clozapine treatment affects MHC class II-related gene expression remains to be elucidated.

In the case of iPSC-derived ASD-models, the promising drug IGF-1 that is currently in clinical trials for ASD, addresses reduced synaptic function and impaired neuronal connectivity by targeting the  $\beta$ -catenin/BRN2 transcriptional cascade.<sup>32</sup>

## 7 | SUMMARY AND PERSPECTIVES

In conclusion, data from transcriptomic analyses suggest a valid representation of disease-specific gene expression in iPSC-derived neurons. For target validation in drug development for improved therapies, this enables the identification of disease pathways and their mechanistic validation in a relevant genetic background. Additionally, high-throughput protocols have been developed which allow for phenotypic testing of drug libraries in human neurons improving previous approaches with neural tumor cell lines.<sup>75</sup> Whether iPSC-derived neurons provide a tool for the prediction of drug action in patients requires further analysis. Limitations are the immature phenotypes achieved which preclude analysis of neurons of a maturation state corresponding to the disease on-set. Additionally, variabilities are observed at different levels. Phenotypic assays, for example, calcium imaging resulted in conflicting results among different studies. These findings may be explained by discrepancies due to variable properties of iPSC lines or different genetic backgrounds, exacerbated by typically low patient numbers included in iPSC-based analyses. Varying differentiation protocols may

account for further variability. Since NDDs are highly heterogeneous, precise stratification of patients included in an iPSC study is required for providing consistent and reproducible results. For example, transcriptomes of childhood-onset schizophrenia patients did not reproduce any of the significantly deregulated genes identified for idiopathic schizophrenia in Table 1.<sup>76</sup> Finally, regarding discrimination among different NDDs in vitro, combination of different approaches for more complex information might be helpful.<sup>77</sup>

Comparison of genetically defined models with idiopathic models revealed both similar and divergent findings. Therefore, the validity of these models to unravel disease-specific mechanisms needs to be further evaluated. Nevertheless, the results published so far encourage to step further and develop iPSC-derived neurons as a robust and valuable tool for the deeper understanding of precise mechanisms of neuropsychiatric diseases, that may ultimately pave the way for biomarker research and drug development. Especially biomarker or drug candidates may be mechanistically validated by testing patient-derived neurons.

The development of 3D organoids begins to leave disadvantages of artificial 2D neuronal network behind, reducing functional variability and resembling more in vivo like neuronal activity patterns.<sup>78</sup> Although such brain organoids are superior to common 2D cultures, they still display an immature phenotype being far from, for example, an adolescent phenotype to model the onset of schizophrenia. Therefore, additional work for the incorporation of myelinating and vascular cells into organoids is essential.<sup>79</sup> A further hurdle is the establishment of functional assays for organoids. It is desirable to develop simple tools for life cell imaging, for example, optogenetic approaches, as well as robust tools for electrophysiological recordings of cells within the organoid.

### CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

### AUTHOR CONTRIBUTIONS

R.S., U.K., R.W.: manuscript writing; P.J.: assembly of data, manuscript writing; A.F.: final approval of manuscript; H.V.: conception and design, final approval of manuscript.

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## ORCID

Hansjürgen Volkmer  <https://orcid.org/0000-0002-3867-0603>

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