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Genetics of Alcohol Use Disorder: A Role for Induced Pluripotent Stem Cells?

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Abstract

Alcohol use disorder (AUD) affects millions of people and costs nearly 250 billion dollars annually. Few effective FDA-approved treatments exist, and more are needed. AUDs have a strong heritability, but only a few genes have been identified with a large effect size on disease phenotype. Genome wide association studies (GWASs) have identified common variants with low effect sizes, most of which are in non-coding regions of the genome. Animal models frequently fail to recapitulate key molecular features of neuropsychiatric disease due to the polygenic nature of the disease, partial conservation of coding regions, and significant disparity in non-coding regions. By contrast, human induced pluripotent stem cells (hiPSC) derived from patients provide a powerful platform for evaluating genes identified by GWAS and modeling complex interactions in the human genome. hiPSCs can be differentiated into a wide variety of human cells, including neurons, glia and hepatic cells, which are compatible with numerous functional assays and genome editing techniques. In this review, we focus on current applications and future directions of patient hiPSC-derived CNS cells for modeling AUDs in addition to highlighting successful applications of hiPSCs in polygenic neuropsychiatric diseases.

Keywords

AUD; hiPSCs; GWAS; addiction; neuropsychiatric disease

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INTRODUCTION

In 2013, alcohol use disorder (AUD) affected >16.3 million people over the age of 18 (Murray and Lopez, 2013) and cost society nearly \$250 billion in 2010 (Sacks et al., 2015). For the past 20+ years, evidence has been accumulating for a strong genetic component to AUD. Family and twin-based studies estimated a 45%-65% heritability (Heath et al., 1997; Kendler et al., 1994; Pickens et al., 1991), and a recent meta-analysis also estimated the heritability to be ~49% (Verhulst et al., 2015). In animal studies, selectively bred (Bell et al., 2012) and genetically engineered rodent models (Crabbe et al., 2006) of alcohol consumption also support a genetic component to AUD. In addition to genetics, environmental exposure also contributes to disease development and severity. For example, early life stress has been shown to play a significant role in AUD (Clarke et al., 2011). Heritability of neuropsychiatric disorders like AUD is notoriously complex and has been the subject of intense investigation (for reviews, see Geschwind and Flint, 2015; McConnell et al., 2017). Studying AUD from a genetic perspective is particularly challenging due to variation in severity of disease phenotype. For example, an individual could meet either two to three of eleven diagnostic criteria in the DSM-V for a “mild” diagnosis, 4-5 for a “moderate” diagnosis, or six or more for a “severe” diagnosis (American Psychiatric Association, 2013). Such clinical heterogeneity is likely reflective of the genetic complexity underlying AUD. Identification of genes involved in AUD is crucial for understanding disease mechanisms and designing targeted treatments.

Genome-wide association studies (GWASs) seek to identify common variants, usually single nucleotide polymorphisms (SNPs), that are associated with common phenotypes (for review of GWAS, see: Bush and Moore, 2012). Most GWAS data have been obtained through chip-based microarray technologies such as Illumina® or Affymetrix®, while targeted sequencing approaches have been used to follow up regions of interest identified in chip microarrays (DiStefano and Taverna, 2011). GWAS studies are correlational and any effects of most SNPs on disease phenotype are small, so large sample sizes are necessary to produce enough power to meet a stringent P value (e.g., 5×10^{-8}) and to estimate SNP effect size. Currently, meta-analysis, combining multiple datasets, is used to generate the largest possible sample.

SNPs may occur within protein-coding sequences, introns, regulatory regions, or between genes. Missense variants or other alterations of coding sequences can have profound effects on protein function. For example, linkage analysis of cystic fibrosis inheritance in families has identified multiple mutations in *CFTR* (cystic fibrosis transmembrane conductance regulator), with the most common variant resulting in the deletion of a phenylalanine (Kerem et al., 1989). Some SNPs reside in the coding region but do not change the amino acid sequence (i.e., synonymous) whereas others are found in non-coding regions, as is the case for many common variants associated with AUD (Clarke et al., 2011, 2017; Zuo et al., 2014). Here, the functional consequence of a disease-associated SNP is more difficult to assess and could result in a change in the expression level of a disease-relevant protein or a change in splicing/regulation of a transcript. Such SNPs may constitute expression quantitative trait loci (eQTLs), which have profound effects on transcript abundance. However, association of a SNP with a phenotype does not necessitate that the SNP plays a

causal role; this may be an indication that the SNP is in linkage disequilibrium (LD) with an unidentified causal variant. Because many variants are not found in conserved protein-coding sequences or may be in LD with unknown sequences, functional studies in human cells are needed to examine the significance of GWAS findings and unravel functionality (see Figure 1 for GWAS to hiPSC-based study pipeline).

Elucidating the functional consequences of genetic variants and how they contribute to disease risk is critical for understanding neuropsychiatric diseases such as AUD. Animal models, particularly in rodents, have been widely implemented to investigate genetic findings from human studies, but they have several key limitations. While rodent models of psychiatric disease provide important behavioral insight, they typically focus on manipulating a single gene, and often fail to recapitulate the full scope of molecular and cellular phenotypes. This is perhaps best illustrated by the plethora of Alzheimer's disease rodent models, none of which demonstrates combination of plaques, tangles, and neurodegeneration found in human post-mortem tissue (Elder et al., 2010), unless mutant genes are expressed in combination, a condition not found in humans (Oddo et al., 2003). The lack of adequate animal models hampers clinical studies, with many potential therapies failing in early phase clinical trials (Becker and Greig, 2010). In the context of AUD, animal models typically focus on consumption phenotypes (Lynch et al., 2010). However, recent genetic data suggest that the shared heritability for consumption (Bierut et al., 2012; Clarke et al., 2017) and alcohol dependence (Clarke et al., 2011; Mbarek et al., 2015; Zuo et al., 2014) is low. Furthermore, animal models typically focus on modeling variants of protein-coding genes, but a large number of AUD-linked SNPs are not in coding sequences (Zuo et al., 2014) and non-coding sequences tend to be species specific (Batzoglou et al., 2000).

Collecting sufficient quantity or quality of human brain cells for biochemical and genetic analyses has been difficult. The development of human induced pluripotent stem cells (hiPSCs) provides a strategy to overcome this and is a compelling complement to animal models. hiPSCs are derived from donor somatic cells by reprogramming into a pluripotent state (Takahashi et al., 2007). These stem cells can then be differentiated into a desired cell type, e.g. an excitatory neuron. Once differentiated, cells derived from hiPSCs have the inherent advantage of containing all genes expressed endogenously under the correct tissue-specific promoters as well as non-coding regulatory sequences. Although variability within the whole genome, among multiple donors, poses a challenge to understanding single gene effects, hiPSCs are amenable to a variety of gene editing methods for probing individual or combinatorial gene contributions to a disease. Isogenic lines, which are genetically manipulated cells that have a single genetic background, can be generated to isolate the effects of one or more variants among simple variants or single SNPs. Gene knockouts in isogenic lines can be created to investigate the effects of reduced gene expression.

It is generally believed that specific cell types and neuronal circuits within the brain mediate the acute response to alcohol as well as the long-term changes that occur in AUD. Advances in the stem cell field has enabled creation of different types of central nervous system (CNS) cells, including excitatory forebrain neurons (Bardy et al., 2015; Chanda et al., 2013; Ho et al., 2016), dopaminergic neurons (Caiazzo et al., 2011; Swistowski et al., 2010), serotonergic neurons (Vadodaria et al., 2017), mixed populations of inhibitory and excitatory

neurons (Nadadthur et al., 2017), medial ganglionic eminence cells (Ahn et al., 2016), astrocytes (TCW et al. 2017), oligodendrocytes (Ehrlich et al., 2017), microglia (Abud et al., 2017), brain microvascular endothelial cells and pericytes (Yamamizu et al., 2017), as well as three dimensional (3D) cell culture (including neurospheres and brain organoids) (Choi et al., 2014; Pa ca et al., 2015). These cells have already been successfully applied to model complex polygenic diseases such as Alzheimer's disease (Liao et al., 2016; Takamatsu et al., 2014; Wray et al., 2013), schizophrenia (Brennand et al., 2011; K. Brennand et al., 2015; Toyoshima et al., 2016), and bipolar disorder (Mertens et al., 2015b), demonstrating distinct cellular and molecular phenotypes. Furthermore, co-culture systems have great potential for elucidating the contribution of a cell type to a disease phenotype as well as cell-autonomous and non-autonomous effects. Thus, hiPSC studies show great promise for helping to elucidate the genetic underpinnings of human diseases such as AUD.

In this critical review we discuss GWAS studies of AUDs and the potential of hiPSC derived cells for investigating key genetic findings. We also provide some examples of successful applications of hiPSCs for modeling other genetically complex neuropsychiatric diseases. We conclude with an overview of the advantages, caveats, and future directions of using hiPSCs for studying AUD.

GWAS OF ALCOHOL USE DISORDERS

Alcohol (i.e. ethanol) produces differential effects in multiple brain areas, including reward pathways involving the ventral tegmental area, nucleus accumbens, and prefrontal cortex (for review see Harrison et al., 2017). GWAS has the potential to reveal previously unknown genetic variants affected by ethanol or genes modulating those proteins. Multiple GWAS studies have been used to probe alcoholism risk, defining affected cohorts by a variety of criteria, including DSM criteria for alcohol dependence (Bierut et al. 2010; Dick et al. 2008; Edenberg et al. 2010; Kendler et al. 2011), online survey AUD identification test (AUDIT) (Sanchez-Roige et al., 2017), and reward-related theta oscillations (a highly heritable neuroelectric endophenotype) (Clarke et al., 2011, 2017; Kang et al., 2012). However, some SNPs fail to meet significance cutoffs after multiple test correction, because the heterogeneity and complexity of factors contributing to alcohol use disorder risk requires large sample sizes to parse out significant associations (e.g. Treutlein et al., 2009). Several consortia across the world are gathering expanded datasets from alcohol use disorder families, including Australian Twin Family Study of AUDs (OZALC) and Collaboration of Genetics of Alcoholism (COGA) in the United States. For more information about resource access visit <https://niaaagenetics.org/>. For a comprehensive summary of alcohol dependence genetics see (Edenberg and Foroud, 2014; Tawa et al., 2016).

Among nonsynonymous SNPs consistently meeting significance thresholds for association with alcohol dependence are those in the genes encoding alcohol dehydrogenase (ADH) and alcohol aldehyde dehydrogenase (ALDH) (Bierut et al., 2012; Edenberg, 2007; Sanchez-Roige et al., 2017), which have been previously identified through linkage analysis (Edenberg et al., 2006) and biochemistry (Smith et al., 1971; von Wartburg et al., 1965). These proteins play a key role in alcohol metabolism, and their variants have a profound effect on alcohol consumption in rodents and humans (Edenberg, 2007). Genetically dictated

differences in metabolism, either due to ADH or ALDH function, indicate that liver function may contribute AUD manifestation (Smith et al., 1971; von Wartburg et al., 1965). Linkage analysis by COGA has also enabled fine mapping of targeted chromosomal regions (Wang et al., 2004). Eight SNPs associated with alcohol dependence ($p < 0.01$) were identified, with four located near the *ACN9* gene, which codes for a mitochondrial intermembrane protein of gluconeogenesis (Dick et al., 2008). Investigation of mitochondrial abnormalities in patient derived neurons containing these SNPs is a potential avenue of research. Additionally, patient-derived liver cells can be generated from hiPSCs (Pashos et al., 2017; Warren et al., 2017) and these may be valuable for studying AUD-related variants linked with alcohol metabolism.

Comparison with a UK BioBank GWAS study of alcohol consumption suggests that genes influencing consumption and dependence may differ (Clarke et al., 2017, 2011). The study used self-reported consumption measures and identified eight independent significantly associated loci, including replicating *AHD1B*, *ADH1C*, and *ADH5* loci and two loci in *KLB* (Clarke et al., 2017). *KLB* is involved in consumption regulation and has been identified in a recent meta-analysis GWAS of European population alcohol consumption (Schumann et al., 2016). Clarke et al. additionally identified four novel loci linked to consumption but not found in dependence studies, including *GCKR*, *CADM2*, *TNFRSF11A*, and *PXDN*. The divergence of consumption and dependence suggests a fundamental involvement of the brain in driving alcohol related behavior.

Focusing on neurocognitive aspects of AUDs, other groups have investigated the neuroelectric endophenotype of frontal theta reward event related oscillations (EROs) in addition to clinical diagnoses or self-reported measures (Klimesch et al., 2001), (Kamarajan et al., 2006), (Kang et al., 2012). Theta EROs are implicated in inhibitory control, conscious awareness, episodic and recognition memory, and evaluating loss and gain in gambling paradigms (Klimesch et al., 2001). Decrease in reward response theta EROs is a highly heritable endophenotype observed in AUD patients and their adolescent offspring at risk for the disorder (Kamarajan et al., 2006). A family-based study of theta EROs in AUD patients, their offspring, and healthy controls identified several polymorphisms in the *KCNJ6* gene, with the most significant SNP (rs702859, imputed) at $p = 4.7 \times 10^{-10}$ constituting a synonymous mutation in exon 4 and other genotyped and imputed SNPs located in intronic regions (Kang et al., 2012). *KCNJ6* encodes the G protein-gated inwardly rectifying potassium channel GIRK2, which plays an important role in regulating neuronal excitability by stabilizing the membrane potential (Lüscher and Slesinger, 2010). *KCNJ6* is expressed in cholinergic, GABAergic, glutamatergic, and, most prominently, dopaminergic neurons (del Burgo et al., 2008). Additionally, GIRK2 has been shown to be directly activated by alcohol (Aryal et al., 2009; Bodhinathan and Slesinger, 2014; Glaaser and Slesinger, 2017). Eleven significant SNPs in *KCNJ6* were also identified in an association study between adult drinking, adolescent drinking, and early life stress interactions (Clarke et al., 2011). The variant SNPs identified in *KCNJ6* do not result in amino acid changes, suggesting potential differences in gene expression or distal regulatory effects on other genes. The theta ERO differences in AUD patients suggest potential differences in neuronal activity, which can be investigated *in vitro*, providing a phenotype to overlay the complex genetic interactions. Identification of common cellular phenotypes either at baseline or upon treatment with

ethanol could identify targets for pharmacotherapy to normalize divergences in electrophysiological activity.

hiPSC MODELS OF ALCOHOL USE AND ALCOHOL-ASSOCIATED GENE VARIANTS

Lieberman and colleagues used hiPSCs to investigate the biological effects of alcohol on human brain cells (Lieberman et al., 2012). hiPSCs derived from four alcohol dependent individuals and three social drinkers were differentiated into a mixed population of neurons and astrocytes, and then exposed to acute or chronic ethanol. Electrophysiological recordings revealed that acute alcohol exposure attenuated NMDA (excitatory) responses in samples from both healthy and alcohol dependent individuals. Chronic exposure, however, led to an upregulation of NMDA subunit transcripts in dependent but not healthy subjects, indicating a compensatory mechanism for NMDA-R attenuation in the transition from acute to chronic alcohol exposure (Lieberman et al., 2012). This pilot study demonstrated the feasibility of studying the effects of alcohol in hiPSC-derived neurons from healthy and AUD-affected individuals.

Focusing on a genetically defined cohort, a subsequent hiPSC study explored a *GABRA2* variant (rs279858*C) (Lieberman et al., 2015) which has been repeatedly associated with AUD (Covault et al., 2004, 2008; Edenberg et al., 2004; Ittiwut et al., 2012; Li et al., 2014). *GABRA2* encodes the alpha subunit of the pentameric GABA_A receptor, and is located in chromosome 4p12, tandem to three other GABA_A subunits. rs279858*C is a synonymous mutation in exon 5, with no effect on protein structure. Other GABA_A subunits are located on chromosomes 5q34, 15q11, and Xq28 (Steiger and Russek, 2004). mRNA encoding GABA_A subunits on 4p12 as well as subunits located on other chromosomes were measured in 36 neural lines from alcoholic and non-alcoholic donors (Lieberman et al., 2015). Carriers of rs279858*C had significantly lower levels of all GABA_A subunits located on 4p12, but not other chromosomal regions (Lieberman et al., 2015). However, protein levels in postmortem cortex did not vary by rs279858 genotype, suggesting the polymorphism's effects may be most profound during neural development (Lieberman et al., 2015). In a more recent study, 24-hour and 7-day exposure of healthy hiPSCs and NPCs to ethanol activated an inflammasome pathway (NLRP3), priming an innate immune-like response and impairing distribution of lysosomes and mitochondria (De Filippis et al., 2016). Additionally, ethanol exposure did not have any effect on proliferative capacity of those cells, but did negatively impact the number of mature neurons that could be generated from exposed NPCs (De Filippis et al., 2016). These studies underscore the capacity of hiPSC-derived cells to delineate developmental components of AUDs.

A recent investigation of GABA_A function in a genetically heterogenous cohort of alcohol dependent (AD) and alcohol-exposed control (CTL) subjects revealed no alteration in GABA-evoked current in directly differentiated, excitatory-enriched neurons following chronic alcohol treatment (Lieberman et al., 2018). The lack of GABA adaptation could be due to the absence of GABAergic signaling in the excitatory-enriched neural cultures, since three-week exposure to chronic ethanol significantly increased transcription levels of

GABRA1 and *GABRG2* in both AD and CTL groups. *GABRD* only showed a modest increase (24%) for the AD but not the CTL group (Lieberman et al., 2018), which is consistent with *GABRD* suggested as an AUD-candidate gene (Rodd et al., 2007). The comparison of differential effects of ethanol on human cells derived from healthy and alcohol dependent subjects illustrates how to use hiPSC-derived cells to study AUDs.

EXISTING hiPSC MODELS OF NEUROPSYCHIATRIC DISEASE

Applications of hiPSCs for investigating AUDs are still in their nascent stage, but promising results have been obtained from several hiPSC models of neuropsychiatric disease, including schizophrenia (Toyoshima et al. 2016; Brennand et al. 2015; Lin et al. 2016), bipolar disorder (Mertens et al., 2015b; Stern et al., 2017), and Alzheimer's disease (Liao et al., 2016; Mahairaki et al., 2014; Yang et al., 2016). Although hiPSC models have been applied to a large number of different types of neurological disorders, we focus here on a polygenic subset.

Nicotine Dependence

Several GWAS studies of human smokers have identified SNPs associated with risk of nicotine dependence (Bierut et al., 2008; Saccone et al., 2009) or cigarette consumption (Caporaso et al., 2009; Consortium et al., 2010). Bierut et al (2008) found a significant SNP (rs16969968, $p=0.007$) in the cholinergic nicotinic receptor $\alpha 5$ subunit, changing Aspartate (Asp) (major allele) to Asparagine (Asn) (minor allele) at position 398 (Bierut et al., 2008). Genotyping in African American (AA) and European-American (EA) participants revealed differential associations between populations, but the SNP was most significantly associated with nicotine dependence in the combined samples (Bierut et al., 2008). Recently, Oni and colleagues examined hiPSC-derived neurons from patients with and without the *CHRNA5* Asn-398 allele (Oni et al., 2016). Patient somatic cells from the Collaborative Genetic Study of Nicotine Dependence (COGEND) were reprogrammed into hiPSC and differentiated into primarily dopaminergic neurons, with about 20% glutamatergic neurons. Neurons containing the Asn-398 allele exhibited an increase in the amplitude and frequency of spontaneous excitatory post synaptic currents, compared to Asp-398 neurons (Oni et al., 2016). Glutamatergic neurons, but not DA neurons, displayed similar baseline electrophysiological characteristics across genotypes, but had an increased excitatory response to nicotine stimulation and faster receptor desensitization in the Asn-398 variant (Oni et al., 2016). Analysis of the dopaminergic neuron transcriptome by RNAseq revealed significant functional enrichment for pathways specific for calcium signaling, axon guidance, and ligand-receptor interaction (Oni et al., 2016). Due to the young age of these hiPSC-derived neurons, these results suggest the *CHRNA5* risk variant may underlie predisposition to nicotine dependence (Oni et al., 2016).

Another investigation of this polymorphism in iPSC-derived dopaminergic neurons demonstrated that Asn-398 variant has a decreased sensitivity to the receptor agonists (Deflorio et al., 2017). However, increases in current in response to agonists were observed (Deflorio et al., 2017), consistent with findings in glutamatergic but not dopaminergic neurons by Oni et al., 2016. The difference in findings of the variants' effects on receptor

function by neuron type between these studies could be attributed to differences in iPSC differentiation protocols. While these studies elucidate functional consequences of a GWAS variant, they also highlight the need for replication and standardization of differentiation protocols.

Schizophrenia

Schizophrenia (SCZ) is a debilitating neuropsychiatric disorder with a strong developmental component, numerous perturbations in synaptic and network functions, and a polygenic risk profile (Adriano et al., 2012; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). One of the earliest studies of SCZ used hiPSCs derived neurons from a genetically heterogeneous cohort of four SCZ patients and performed gene expression profiling, extensive characterization of neurite outgrowth and synaptic connectivity, as well as pharmacological treatment with antipsychotics (Brennand et al., 2011). SCZ patient cells differentially expressed 596 unique genes and showed deficits in synaptic connectivity, compared to controls, which were partially ameliorated by the antipsychotic drug Loxapine (Brennand et al., 2011). Furthermore, several groups investigated 22q11.2 hemizygous microdeletion, one of the few single loci associated with a large increase in risk for SCZ (see Bassett and Chow, 2008), in hiPSC derived cells. Neurospheres derived from the 22q11.2 deletion patients were smaller in size, had reduced expression levels of markers for cellular proliferation and survival, and an increased astrocyte to neuron ratio, consistent with observations in postmortem brains (Toyoshima et al., 2016). Integrative network analysis of hiPSC-derived neurons from eight cases with 22q11.2 microdeletion and ten healthy controls identified two sub-networks which are disrupted by 22q11.2 deletions: a cell cycle network mediated by CD45, disrupted in embryonic development, and a PRODH-mediated network, potentially contributing to disrupted brain function in adolescence when SCZ symptoms most often begin to emerge (M. Lin et al., 2016). Thus, human SCZ variants can reveal cellular developmental mechanisms linked with the disorder.

Bipolar Disorder

Bipolar disorder (BD) is characterized by recurring episodes of alternating mania and depression, has a complex pathophysiology and a polygenic risk, in addition to reduction in neural volumes and alterations in neurotransmission (Consortium 2009; Boies et al., 2017). Chen et al. (2014) examined changes in gene expression throughout the course of directed differentiation from hiPSCs to mixed-population neurons derived from three patients and three age-matched controls. Overall no significant expression differences were observed in the pluripotent stage, but BD neurons expressed significantly more membrane receptors and ion channels, especially those involved in calcium signaling (Chen et al., 2014). Total RNAseq analyses of three-week-old hippocampal dentate gyrus granule cell-like neurons revealed enhancement in multiple mitochondrial genes in BD neurons. Flow cytometry and mitochondrial membrane potential measurements suggested an enhanced mitochondrial function. Patch-clamp recordings and calcium imaging at baseline and following exposure to lithium, a commonly used pharmacotherapy for BD, revealed lower threshold for action potentials and higher firing frequencies for BD neurons (Mertens et al., 2015b). Interestingly, BD neurons from lithium responders displayed a reduction in hyperexcitability following lithium treatment, in contrast to non-responder neurons (Mertens et al., 2015b).

RNAseq analyses showed a partial normalization of mitochondrial gene levels in the lithium responder group (Mertens et al., 2015b). A subsequent hiPSC study of dentate gyrus-like neurons from BD patients confirmed hyperexcitability of BD neurons and differential reductions in hyperexcitability following lithium treatment (Stern et al., 2017). Furthermore, differences in spiking shape were analyzed revealing that lithium responders had a larger sodium current than controls, whereas non responders had smaller sodium currents (Stern et al., 2017). These studies illustrate how selected hiPSC-derived neurons can respond to appropriate medications and predict patient response, which is notoriously variable in concordance with disorder heterogeneity (Drozda et al., 2014).

Alzheimer's Disease

Humans with Alzheimer's disease (AD) exhibit progressive cognitive impairment, neurodegeneration, formation of amyloid beta ($A\beta$) plaques, and neurofibrillary tangles - features that have not been fully recapitulated in animal models (Elder et al., 2010). Linkage studies have uncovered mutations in certain genes causing autosomal dominant forms of AD (ADAD), including presenilin (*PSEN1*, *PSEN2*), amyloid precursor protein (*APP*), and a moderately penetrant polymorphism, *APOE-e4* (for AD genetics overview, see (Bagyinszky et al., 2014)). *APOE* isoforms have differential effects on disease progression: whereas *APOE2* is protective and *APOE3* is neutral, *APOE4* appears to constitute a major risk factor for AD (Strittmatter et al., 1993). Investigation of *APOE* isoforms in iPSC-derived human neurons has provided novel insights into molecular pathways of neural *APOE*, such as the differential efficacy of *APOE* isoforms in activating an unusual *MAPK* cascade regulating *APP* gene regulation (Huang et al., 2017). Another study ascribes a neurotoxic function for *APOE4* that can be ameliorated with a small-molecular structure corrector, predicting a novel and potentially protective AD therapy (Wang et al., 2018). An innovative single-cell analytical platform for detecting secreted $A\beta$ and soluble amyloid precursor protein-alpha (sAPP α) revealed heterogeneity in secretion profiles from cells harboring APP mutations (Liao et al., 2016). These include subpopulations of cells that secrete high levels of $A\beta$, but not sAPP α , cells that secrete both $A\beta$ and sAPP α (enriched for GABAergic markers), and cells that secrete $A\beta$ levels (enriched for astrocyte markers) (Liao et al., 2016). Finally, 3D organoid models of AD have been able to recapitulate both amyloid deposition, and hyperphosphorylated tau accumulation in somato-dendritic compartments, both from patients with familial mutations (Raja et al., 2016) and from patients with late onset disease (Lee et al., 2016).

Together, these studies highlight the utility of hiPSC-derived CNS cells for exploring genetically complex neuropsychiatric disorders. Table 1 lists recent publications utilizing hiPSCs for investigating polygenic neuropsychiatric diseases (for reviews, see (Ardhanareeswaran et al., 2017; Prytkova and Brennand, 2017; Shi et al., 2017; Soliman et al., 2017)). Though some caveats and limitations exist (discussed below), the rapidly advancing field of hiPSC differentiation and associated analytical platforms is rife with potential.

ADVANTAGES OF hiPSC MODELS

Human imaging, postmortem analyses, and animal studies of substance use disorders have demonstrated region-specific changes in neurons, astrocytes, oligodendrocytes, and microglia (for reviews, see Niciu et al., 2014; Zahr and Pfefferbaum, 2017). A significant advantage of hiPSC models is the expanding availability of various CNS cell types that can be derived from patients. This offers a valuable toolbox for exploring human cellular phenotypes within a specific genotype. A variety of reprogramming methods exist for generating specific CNS cells from hiPSCs, including direct induction of hiPSCs (Ahn et al., 2016; Close et al., 2017; Livesey et al., 2016; Lu et al., 2016; Muffat et al., 2016; Swistowski et al., 2010; Tcw et al. 2017b; Yu et al., 2014), neural progenitor cells (NPCs) (Ehrlich et al., 2017; Ho et al., 2016) or fibroblasts (Ladewig et al., 2012; Pang et al., 2011; Vadodaria et al., 2017; Yang et al., 2011). Alternatively, directed differentiation of hiPSCs involves long-term cultures with differentiation-specific growth factors and is generally thought to more closely resemble *in vivo* conditions but also to produce heterogeneous cell types (Maroof et al., 2013). This approach is suitable for exploring developmental components of AUDs in a mixed excitatory and inhibitory neuronal population. By contrast, induction of cell type specific genes significantly reduces the timeline and increases homogeneity of cell populations (Ho et al., 2016). We summarize some of these protocols in Table 2. Thus one advantage of hiPSCs is the availability of different reprogramming methods which allows the examination of the same genotype in different contexts.

Though most hiPSC-derived neurons are grown as monolayers, researchers have developed 3D cultures and brain organoids to produce neuronal ensembles that more closely resemble human brain, e.g., layers of cortex (for review, see Quadrato et al., 2016). These cortico-spheroids typically exhibit a mix of neurons and astrocytes (Pacca et al., 2015), and particularly exciting is the recent description of chimeric organoids, which contain integrated excitatory and inhibitory cortical neurons mimicking interneuron migration observed *in vivo* (Birey et al., 2017; Mariani et al., 2015; Xiang et al., 2017). Finally, a promising development for drug screening applications lies in the recent creation of a human blood brain barrier (BBB) model from hiPSCs that recapitulates drug BBB permeability in humans (Yamamizu et al., 2017). The inherently different function of rodent and human BBB (Aday et al., 2016) accounts for some of the failed CNS therapeutic trials (Alavijeh et al., 2005) as well as being implicated in neurodegenerative and psychiatric diseases (Desai et al., 2007; Saito and Ihara, 2014). These more complex hiPSC-derived cultures provide an important approach for modeling disease specific circuitry and drug delivery.

Lastly, the development of human isogenic lines provides a method for testing specific variants and their effects on a phenotypic trait under conditions of reduced variability. Comparing multiple hiPSCs derived neurons from genetically unrelated humans, or even siblings, can result in large variability in measurements that are unrelated to the disease phenotype (Faulconbridge et al., 2017; Lin et al., 2015). With the discovery of efficient genome-editing techniques (Ran et al., 2013), it is now possible to genetically manipulate hiPSCs with precision and efficiency (see Figure 2) (Bassett, 2017; Bertero et al., 2016). Indeed, several studies have successfully utilized clustered regularly interspaces short palindromic repeats (CRISPR)/Cas9 nuclease genome editing (Wang et al., 2017), CRISPR

activation/repression (Ho et al., 2017), and shRNA for manipulating gene expression levels (Bertero et al., 2016; Sancho-Martinez et al., 2016). With these techniques, investigators can manipulate expression of single or several genes, as well as generate control cells with identical genetic (isogenic) backgrounds (for detailed review of genome editing techniques see: Bassett, 2017).

ANALYSIS OF hiPSC-DERIVED CELLS

hiPSC-derived neurons are amenable to a variety of functional analyses. Given that perturbations in neuronal excitability are thought to underlie many psychiatric disorders (Anticevic and Murray, 2017), the ability to investigate excitability and network activity of human cells is a crucial step for understanding the disease. Whole-cell patch-clamp electrophysiology provides single-cell measurements of neuronal activity, with details on excitatory and inhibitory synaptic inputs. This is crucial for determining dysfunctions in particular channels implicated in alcoholism (for example, GABA_A (Lobo and Harris, 2008). Several techniques are available for examining neuronal network activity, such as with microelectrode array (MEA) and Ca²⁺ imaging. MEAs can be used to study population activity at different time points throughout development. hiPSC-derived neurons are cultured directly on top of microelectrodes lining the bottom of the plate, and can be subjected to field stimulation or photostimulation techniques (Hales et al., 2010; Obien et al., 2015). Calcium imaging, using cell permeable dye (e.g. Fluo4-AM) or a genetically encoded indicator (e.g. GCaMP6) (Grienberger and Konnerth, 2012), offers single cell resolution as well as information on network activity. Compared to electrophysiology and MEA, Ca²⁺ imaging has a slower temporal resolution, in which action potential spikes are integrated over time. However, this temporal resolution has been sufficient to monitor activity in neuronal networks (Grienberger and Konnerth, 2012). The development of optical sensors for changes in voltage may overcome this limitation in the future (Marshall and Schnitzer, 2013).

In addition to functional activity studies, hiPSC-derived neurons can be processed for RNAseq to study up and down regulation of transcripts, immunohistochemistry to observe the localization of proteins, and Western analyses to quantitate protein expression levels. Since many alcohol-related variants are believed to be eQTLs (Mamdani et al., 2015; Zhou et al., 2017), genome-wide transcript analysis is likely to reveal regulatory targets and these processes are expected to be cell type specific. With this wide selection of techniques, researchers can elucidate differences in activity in healthy, disease affected, genetically manipulated, and pharmacologically treated human cells. Thus, hiPSC-derived neurons can provide unique insight into developmental events of AUD on a transcriptomic and functional level. Furthermore patient-derived cells are an effective experimental platform for manipulating disease phenotypes and testing pharmacotherapy response.

CAVEATS AND LIMITATIONS OF hiPSC MODELS

While hiPSC models offer a promising approach to studying AUD, there are several limitations worth noting. First, one would ideally study neurons at a mature stage comparable to neurons in adults. However, hiPSC-derived neurons are immature and lack

some of the complex connectivity of adult neurons. Recently, transcriptional profiling of neurons and astrocytes derived from hiPSCs indicated these cells most closely resemble fetal cells (Brennand et al. 2015; TCW et al. 2017). Optimization of components in the differentiation culture medium has helped promote the presence of more synaptically mature neurons (Bardy et al., 2015). Second, while it is advantageous to focus on one cell type, the interactions with other cell types play an important role in development and functionality.

This limitation has been recently addressed by the development of co-culture systems and the production of organoids (Quadrato et al., 2016). Co-culture of neurons on rat astrocytes resulted in synchronized network activity within three months of plating, as measured with MEAs (Odawara et al., 2014). Furthermore, co-cultures of human primary astrocytes and a mixed excitatory-inhibitory population of hiPSC-derived cortical neurons can lead to network synchronization and connectivity in three to four weeks (Kuijlaars et al., 2016). Considering distinct electrical activity in EEG and fMRI are associated with alcoholism (Huang et al., 2018; Klimesch et al., 2001; Pandey et al., 2012), this is an important development for modeling predisposition.

Another limitation of hiPSC studies is that connections between neurons are inherently random, unlike neuronal networks that form *in vivo*. Micropatterning and microfluidic devices offer a potential solution for organizing circuits and exercising granular control over the cells' microenvironment (for review, see Brunello et al., 2013). Microfluidic local perfusion chambers have been successfully implemented with dissociated primary neurons from rodents. For example, a microfluidic local perfusion chamber has been developed to manipulate distinct synaptic regions, directing synapse formation in distinct parallel rows through microgrooves connecting two distinct neuronal populations (Taylor et al., 2010). hiPSC-derived neurons are compatible with micropatterning for long term growth and circuit organization. Neurons adhere to regularly spaced adherent surfaces with neurites crossing cell-repellant surfaces to form connections (Burbulla et al., 2016). This system facilitates long-term studies of mitochondrial dynamics, axonal transport, and dynamic network formation (Burbulla et al., 2016). Compartmentalized microfluidic devices have been utilized to create interconnections between excitatory, inhibitory, and dopaminergic hiPSC-derived neurons, mimicking reward circuits (Fantuzzo et al., 2017). This is a key development for studying AUDs, considering accumulating evidence pointing towards differential reward processing in alcohol dependent individuals (Kamarajan et al., 2006, 2015; Müller-Oehring et al., 2013).

Epigenetic contributions, known to be important in AUD, pose another major challenge to utilizing hiPSC models. Incomplete tissue-specific erasure and aberrant *de novo* methylation during reprogramming somatic cells have effects on conversion efficiency and gene expression (Kim et al., 2010, 2011; Lister et al., 2011; Nazor et al., 2012; Oni and Hart, 2016). Epigenetic reset during conversion of somatic cells to a pluripotent state, even with residual methylation signatures, erases age-related marks (Lapasset et al., 2011) and may have an impact on other epigenetic marks contributing to AUD phenotype. One work-around for this could be to directly induce neurons from fibroblasts, which retain age-related epigenetic marks (Mertens et al., 2015a). In AUD, alcohol metabolism has a profound effect on epigenetic markers (Boschen et al., 2018; Krishnan et al., 2014; Zakhari, 2013), which

may vary between CNS and peripheral cells, and careful consideration of reprogramming methods and epigenetic marks is essential for adequate disease modeling.

Verification of patient genotype and understanding of copy number variation is another essential step with hiPSC based studies. For example, characterization of hiPSCs from patients with 9p24.1 duplications/triplications, a copy number variant associated with psychotic disorders (Malhotra et al., 2011), revealed chromosomal instability during reprogramming in the form of extrachromosomal marker element rearrangement. Due to mosaicism in the patient derived fibroblasts, conversion to pluripotency serendipitously generated non-carrier isogenic controls (Tcw et al., 2017). Therefore, investigation in hiPSCs necessitates karyotyping to ensure absence of chromosomal abnormalities.

Lastly, variability in hiPSC derived neurons even amongst control subjects can be a concern for disease modeling (Choi et al., 2015; Kilpinen et al., 2017; Kytälä et al., 2016). For example, a recent study exhaustively characterized differentiation capacity, cellular morphology, and copy number alterations through genome wide profiling of 711 hiPSC lines from 301 healthy individuals revealing that most variation results from differences between individuals (Kilpinen et al., 2017). Designing a hiPSC study with sufficient statistical power would require large samples sizes, which is impractical both in terms of cost and lack of techniques for high-throughput functional analyses. However, implementation of genetically-engineered hiPSCs can provide a reasonable solution to this limitation (see above).

CONCLUDING REMARKS

The complex genetic architecture of alcohol use and dependence results in profound clinical heterogeneity and difficulty in developing effective treatments. Animal models fail to recapitulate cellular and molecular complexities of human neurons, astrocytes, microglia, and blood brain barrier and accordingly do not capture all disease features, particularly those linked with noncoding genome sequences. Understanding precise molecular events underlying the disease and how an individual's genetic background contributes to the disease state is essential to move into the age of personalized medicine for neuropsychiatric disorders. Despite their fairly recent development, patient derived hiPSCs have already been utilized to model a variety of psychiatric and neurodegenerative disorders. In addition to the key advantage of being human and patient specific, the ability to differentiate hiPSC into any cell type enables probing differences in cellular function and modeling of cellular interactions. In this review, we have focused on CNS cell types, but hiPSCs have also been reprogrammed into hepatocytes and adipocytes for investigation of liver function (Pashos et al., 2017; Warren et al., 2017), which is key for studying AUD. Furthermore, recent developments in genome editing techniques provide a unique opportunity to manipulate human genes in human cells and to tease apart complex genetic interactions contributing to disease phenotypes.

In conclusion, collaborative studies for understanding genetic risk of alcohol abuse disorders such as COGA are in a prime position to use hiPSCs to study AUD. These genetic studies have collected large numbers of carefully characterized and individually genotyped cells in

repositories, suitable for hiPSCs derivation. The combination of thoroughly characterized clinical phenotypes, risk association genotypes, and functional investigations in patient cells, including gene editing and drug screening, will provide an unprecedented level of individual- and population-based resolution of polygenic disease mechanisms. Future functional studies will need to adapt hiPSC-derived neurons to more high-throughput analyses, such as with 384-well plate imaging or electrophysiological instruments, to greatly increase the number of control and AUD subjects studied. Such multi-level disease characterization will help identify novel therapeutic targets and bring the age of personalized medicine for psychiatric disease to fruition.

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Figure 1. Flow-chart of sample collection and processing pipeline

A diverse patient cohort is clinically evaluated, at which time behavioral or EEG endophenotypes may be measured. Skin or blood samples are collected from patients, part of which is subject to genetic sequencing or fingerprinting for GWAS analysis and part of which may be reprogrammed into hiPSCs. GWAS analysis yields a list of significantly associated loci labelled with the nearest gene, which are then investigated through functional studies.

Figure 2. Flow-chart of functional follow-up of GWAS findings using hiPSCs

After clinical and genetic evaluation patient cohorts can be divided by phenotype or by significantly associated genes. Patient hiPSCs may be genetically manipulated prior to differentiation into cell types of interest. These cells are then characterized through omics (e.g., RNA, protein, epigenetic for directly induced cell types) and functional assays. Additionally, they may be used as a platform for drug screening, either for investigating effects of therapeutics or drugs of abuse.

Table 1

Abbreviations: DG- dentate gyrus; ELISA- enzyme-linked immunosorbent assay; HPLC- high performance liquid chromatography; MGE- medial ganglionic eminence; VNTR-variable number tandem repeat polymorphisms; VTA – ventral tegmental area

Publication	Disease	Gene/SNP	Cell Type	Assays	Treatment	Main findings
Lieberman et al., 2012	Alcohol Use Disorder	-	Mixed population forebrain lineage neurons and astrocytes	- qPCR - Patch-clamp	Acute and 7-day alcohol exposure	- Attenuation of NMDA response to alcohol decreased after chronic exposure in non-AUD neurons - Chronic ethanol exposure upregulated NMDA-R subunits
Lieberman et al., 2015	Alcohol Use Disorder	<i>GABRA2</i> (rs279858*C)	Mixed population forebrain lineage neurons and astrocytes	- RT-qPCR - Next generation RNA sequencing	-	- rs279858*C associated with reduced <i>GABRA2</i> expression as well as other <i>GABA_A</i> subunits on chromosome 4p12
De Filippis et al., 2016	Alcohol Use Disorder	-	NPCs	- Patch-clamp - Immunocytochemistry - Western Blot	Chronic alcohol exposure	- Inflammasome pathway activation and reduction in numbers of mature neurons
Lieberman et al., 2018	Alcohol Use Disorder	-	Forebrain-type excitatory glutamate neurons	- Immunocytochemistry - RT-qPCR - Patch-clamp	21 day alcohol exposure	- GABRD increased in AUD-derived cells - No effect of acute or chronic EtOH on GABA-evoked currents
Oni et al 2015	Nicotine Dependence	<i>CHRNA5</i> (rs16969968)	Dopaminergic and glutamatergic neurons	- RT-qPCR - RNAseq - Immunocytochemistry - Patch-clamp	Acute nicotine	- Asn398 neurons respond to nicotine with increased EPSC - Faster desensitization in glutamatergic but not dopaminergic neurons
Deflorio et al., 2017	Nicotine Dependence	<i>CHRNA5</i> (rs16969968)	Dopaminergic neurons	- Immunocytochemistry - RT-PCR - Single cell RT-qPCR - Patch-clamp	Acute nicotine & Acetylcholine	- Decreased sensitivity to nicotinic receptor agonists in with Asn398

Publication	Disease	Gene/SNP	Cell Type	Assays	Treatment	Main findings
Mertens et al., 2015b	Bipolar Disorder		Hippocampal DG-like neurons	<ul style="list-style-type: none"> - Immunocytochemistry - RNA-seq - RT-qPCR - Patch-clamp - Flow cytometry mitochondrial analysis 	Lithium	<ul style="list-style-type: none"> - Enhanced mitochondrial function in bipolar patient derived neurons; - Hyperexcitability in bipolar patient-derived neurons restored by lithium treatment in cells from lithium-responders
Stern et al., 2017	Bipolar Disorder	-	Hippocampal DG-like neurons	<ul style="list-style-type: none"> - Immunocytochemistry - Patch-clamp 	Lithium	<ul style="list-style-type: none"> - Treatment with lithium reduced hyper excitability in Li-responder group
Brennand et al., 2011	Schizophrenia	-	Mixed population of forebrain lineage neurons	<ul style="list-style-type: none"> - Immunocytochemistry - Trans-neuronal rabies virus tracing - RT-qPCR - CNV analysis 	Loxapine	<ul style="list-style-type: none"> - Differential expression of 596 unique genes - deficits in synaptic connectivity partially ameliorated by Loxapine
Toyoshima et al., 2016	Schizophrenia	22q11.2; <i>DCCGR8</i>	Forebrain-like neurospheres	<ul style="list-style-type: none"> - miRNA array - RT-qPCR - Immunocytochemistry - Western Blot - Neurite outgrowth / cell migration 	-	<ul style="list-style-type: none"> - Dysregulated balance of glio/neurogenic competence observed in schizophrenia-patient-derived neurospheres
Lin et al., 2016	Schizophrenia	22q11.2	Mixed population of glutamatergic and GABAergic neurons	<ul style="list-style-type: none"> - RNAseq - RT-qPCR 	-	<ul style="list-style-type: none"> - Subnetworks disrupted by 22q11.2 deletions identified: CD45-mediated (embryonic development) and PRODH-mediated (adolescence)
Liao et al., 2016	Alzheimer's Disease	<i>APP</i>	Mixed population forebrain lineage neurons	<ul style="list-style-type: none"> - RNAseq - Live marker staining in microengraved nanowells - secreted sAPPα and Aβ detection 	-	<ul style="list-style-type: none"> - Identified cells secreting sAPPα but not Aβ, GABAergic enrichment of cells secreting high levels of both, and significant competence of astrocytes to secrete Aβ

Publication	Disease	Gene/SNP	Cell Type	Assays	Treatment	Main findings
Raja et al., 2016	Alzheimer's Disease	<i>APP; PSEN1</i>	Cortical organoids	<ul style="list-style-type: none"> - Immunocytochemistry - ELISA 	<ul style="list-style-type: none"> β and γ secretase inhibitors 	<ul style="list-style-type: none"> - Treatment with β and γ secretase inhibitors reduced amyloid and tau pathology
Huang et al., 2017	Alzheimer's Disease	<i>APOE</i>	Mixed population forebrain lineage neurons	<ul style="list-style-type: none"> - Western Blot - Immunocytochemistry - Immunosorbent Aβ assay 	<ul style="list-style-type: none"> suppression of gene expression with CRISPR 	<ul style="list-style-type: none"> - APOE secreted by glia stimulated neuronal Aβ production, with E2 isoform having the least potency - Identified pathway of ApoE receptor binding regulating APP transcription
Wang et al. 2018	Alzheimer's Disease	<i>APOE</i>	<ul style="list-style-type: none"> - Mixed population forebrain lineage neurons - MGE-like GABAergic neurons - Astrocytes 	<ul style="list-style-type: none"> - Flow cytometry - Immunocytochemistry - Aβ_{40/42} secretion measurement 	<ul style="list-style-type: none"> - Zinc-finger editing to generate isogenic lines - PH002 drug 	<ul style="list-style-type: none"> - Toxic gain of function of APOE4 corrected by small molecule PH002

Table 2

Abbreviations: CGE-caudal ganglionic eminence; DG- dentate gyrus; DRN- dorsal raphe nuclei; EB- embryonic body; FACS- fluorescence activated cell sorting; HC- hippocampus; hESC- human embryonic stem cell; HPLC-high performance liquid chromatography; ICC- immunocytochemistry; MGE-medial ganglionic eminence; NPCs-neural progenitor cells; OPC- oligodendrocyte progenitor cell; UPLC-ESI-MS/MS- ultra-performance liquid chromatography–electrospray ionization–tandem mass spectrometry

Derived cell type	Starting cell type	Reprogramming method	Assays	Publication
MGE-like cells	hESCs	<ul style="list-style-type: none"> - Telencephalic induction: dual SMAD and Wnt inhibition - Ventralization: rmShh and Purmorphamine - interneuron differentiation: neurobasal and neurotrophic factors 	<ul style="list-style-type: none"> - Patch-clamp electrophysiology - Population and single cell RNA seq 	Close et al. 2017
Excitatory forebrain neurons	hiPSC-derived NPCs	<ul style="list-style-type: none"> - retroviral doxycycline-inducible vector with NGN2 	<ul style="list-style-type: none"> - ICC - Patch-clamp electrophysiology - qPCR - Axion multielectrode array 	Ho et al., 2016b
Neurons of different neurotransmitter phenotypes	Human fibroblasts	<ul style="list-style-type: none"> - retroviral doxycycline-inducible vector with NGN2 and ASCL1 	<ul style="list-style-type: none"> - ICC - Whole-cell electrophysiology - Whole genome expression array (Illumina) 	Ladewig et al. 2012
MGE- and CGE-like cells	hiPSCs	<ul style="list-style-type: none"> - Telencephalic induction: IWP-2 - Ventralization: SHH activation and Wnt inhibition - Rostralization and caudalization: FGF8 and FGF19, respectively 	<ul style="list-style-type: none"> - FACS of fixed cells - RT-PCR throughout development - ICC after differentiation and after transplantation into mouse HC 	Anh et al. 2016
Serotonin neurons (rhombomeric segments 2-3 of DRN)	hiPSCs	<ul style="list-style-type: none"> - Transformation to neural stem cells: dual SMAD and GSK3-β inhibition - Transformation to serotonergic fate: Wnt and SHH activation 	<ul style="list-style-type: none"> - qRT-PCR transcriptional profiling - Whole cell electrophysiology - HPLC/UPLC-ESI-MS/MS to measure serotonin release/uptake 	Lu et al. 2016
Serotonin neurons	Human fibroblasts	<ul style="list-style-type: none"> - Lentiviral particles with ASCL1/NGN2 and NKX2.2/FEV/GATA2/LMX1N 	<ul style="list-style-type: none"> - ICC - Western Blot - Whole transcriptome RNAseq - Whole cell electrophysiology 	Vadodaria et al. 2016

Derived cell type	Starting cell type	Reprogramming method	Assays	Publication
			- Ca ²⁺ imaging with Fluo-4	
Dopamine neurons	hiPSCs	<ul style="list-style-type: none"> - Culturing of EBs in neural induction medium to generate neural stem cells - Dopaminergic directed differentiation in defined media containing Shh and FGF8 - Shh and FGF8 withdrawal after 10 days, supplementation with BDNF and GDNF 	<ul style="list-style-type: none"> - ICC - Microarray - PCR - Transplantation into Parkinson's rat model 	Swistowski et al. 2010
Dopamine neurons	Human fibroblasts	<ul style="list-style-type: none"> - Doxycycline inducible vector with NURR1/MASH1/LMX1A 	<ul style="list-style-type: none"> - ICC - qRT-PCR - Patch clamp 	Caiazzo et al. 2011
Astrocytes	hiPSCs-derived NPCs	<ul style="list-style-type: none"> - 30-day exposure to commercial astrocyte media (ScienCell and Lonza) 	<ul style="list-style-type: none"> - ICC - FACS - qRT-PCR transcriptional profiling - Protein array (ELISA) - Ca²⁺ imaging with Fluo-4AM - Phagocytosis assay 	Tcw et al., 2017
Hippocampal DG neurons	hiPSC-derived EBs and NPCs	<ul style="list-style-type: none"> - Telencephalic induction: inhibition of Wnt, BMP, and TGF-β - Dorsal enrichment: inhibition of SHH - DG differentiation: Wnt3a and BDNF 	<ul style="list-style-type: none"> - ICC - qRT-PCR - Whole cell electrophysiology - Ca²⁺ imaging with Fluo4-A< and Synapsin-DsRed LV vector - In vivo transplantation 	Yu et al. 2014
Microglia-like cells	hiPSCs, with EB/NPC intermediate	<ul style="list-style-type: none"> - serum free neuroglial differentiation with TGF-β inhibition, CSF1, and IL-34 	<ul style="list-style-type: none"> - Morphology characterization at differential concentrations of CSF1 and IL-34 - Endotoxin challenge cytokine profiling - ICC - qRT-PCR - RNA-seq - Gene ontology 	Muffat et al. 2016
Oligodendrocytes	hiPSCs, with EB/NPC intermediate	<ul style="list-style-type: none"> - Dual SMAD inhibition to generate NPCs from EBs 	<ul style="list-style-type: none"> - ICC - Whole-cell electrophysiology 	Livesey et al. 2016

Derived cell type	Starting cell type	Reprogramming method	Assays	Publication
		<ul style="list-style-type: none"> - Retinoic acid for caudalization - FGF-2 for ventrification - FGF-2 withdrawal for glial differentiation - Isolation of OPCs - Mitogen withdrawal for final oligodendrocyte differentiation 		
Oligodendrocytes	hiPSC-derived NPCs	<ul style="list-style-type: none"> - Lentiviral induction with SOX10, OLIG2, NKX6.2 (SON) 	<ul style="list-style-type: none"> - ICC - Electron microscopy - Flow cytometry - Global gene expression profiling - qRT-PCR - Implantation in vivo 	Ehrlich et al. 2017