

Cross-species models of attention-deficit/hyperactivity disorder and autism spectrum disorder: lessons from *CNTNAP2*, *ADGRL3*, and *PARK2*

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Animal and cellular models are essential tools for all areas of biological research including neuroscience. Model systems can also be used to investigate the pathophysiology of psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). In this review, we provide a summary of animal and cellular models for three genes linked to ADHD and ASD in human patients – *CNTNAP2*, *ADGRL3*, and *PARK2*. We also highlight the strengths and weaknesses of each model system. By bringing together behavioral and neurobiological data, we demonstrate how a cross-species approach can provide integrated insights into gene function and the pathogenesis of ADHD and ASD. The knowledge gained from transgenic models will be essential to discover and validate new treatment targets for these disorders. *Psychiatr Genet* 29:1–17 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Psychiatric disorders

Psychiatric disorders can affect any aspect of mental health, including cognition, emotionality, and sociability. They are a leading cause of disability worldwide and represent a significant economic burden to the society (Bloom *et al.*, 2012; Vigo *et al.*, 2016). The aim of this research using animal and cellular models is to better understand the neurobiology of psychiatric disorders to develop new treatments for the individuals that experience them. In this review, we discussed models for two common overlapping psychiatric disorders: attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD).

Attention-deficit/hyperactivity disorder and autism spectrum disorder

ADHD and ASD are two of the most widely studied neurodevelopmental disorders. According to the *Diagnostic*

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and *Statistical Manual of Mental Disorders*, 5th ed. (DSM-5), ADHD is a clinically heterogeneous disorder characterized by inattention, hyperactivity, and increased impulsivity (American Psychiatric Association, 2013). Approximately 2–10% of school-age children are affected by ADHD (Hawi *et al.*, 2015), and males are more likely to be affected than females (Polanczyk *et al.*, 2007; Nussbaum, 2012). Despite traditionally being regarded as a childhood disorder, a number of longitudinal-based and population-based epidemiological studies have identified an adult form of ADHD (Franke *et al.*, 2012, 2018). ASD is a group of neurodevelopmental disorders characterized by impairments in learning, sensory filtering, communication, and social interactions together with restricted, repetitive, and stereotypical behaviors and interests. The prevalence of ASD in the population is estimated at ~1%, with three to four times as many males as females affected (Baird *et al.*, 2006). There are currently no objective laboratory-based tests to diagnose ADHD or ASD. The latest version of the DSM acknowledges the high rate of comorbidity for ASD and ADHD, allowing their simultaneous diagnosis for the first time (American Psychiatric Association, 2013). The symptoms of ADHD and ASD often co-exist. Overall, 20–50% of children with ADHD also meet

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the criteria for ASD (Rommelse *et al.*, 2011). Several studies have shown social deficits, peer relationship deficits, and empathy problems in children with ADHD, and the DSM-5 allows a comorbid diagnosis of both disorders. Although ADHD and ASD comorbidity has mainly been studied in children, there is also evidence that it occurs in adults (Hartman *et al.*, 2016). The drugs most commonly used to treat ADHD are stimulants such as methylphenidate (MPH). Although effective in controlling some symptoms, MPH treatment shows a large degree of variability across patients (Wolraich and Doffing, 2004). There are currently no pharmacological treatments for the core symptoms of ASD. Behavioral therapy can improve the quality of life for patients with ASD, and pharmacological treatments may reduce secondary symptoms such as hyperactivity (LeClerc and Easley, 2015).

Genetic and environmental factors for autism spectrum disorder and attention-deficit/hyperactivity disorder

A combination of both environmental factors and genetic variants are known to influence the development and expression of ADHD and ASD (Sánchez-Mora *et al.*, 2015; Sjaarda *et al.*, 2017). Meta-analysis of multiple large-scale twin studies estimated the heritability of childhood and adolescent ADHD at between 0.7 and 0.8 (Nikolas and Burt, 2010). The heritability of adult ADHD is reported to be lower, at between 0.3 and 0.4, although this may be an underestimation owing to the self-rating system commonly used in these studies (Franke *et al.*, 2018). The true heritability could be as high as 0.7–0.8 (Brikell *et al.*, 2015; Tick *et al.*, 2015). ASD heritability estimates are similarly high at 0.64–0.91 (Tick *et al.*, 2015). Familial co-aggregation of ADHD and ASD is strongly indicative of an overlap in genetic factors across disorders (Ghirardi *et al.*, 2018). Furthermore, a number of studies have identified genes that are associated with both ADHD and ASD, including contactin-associated protein-like 2 (*CNTNAP2*) (Strauss *et al.*, 2006; Elia *et al.*, 2010; Poot *et al.*, 2010; Rodenas-Cuadrado *et al.*, 2014).

The genetic basis of ADHD and ASD can be explained by the common disease common variant and common disease rare variant (CDRV) hypotheses (Hawi *et al.*, 2015). However, there is a growing recognition that most individuals with ADHD or ASD carry both common and rare variants. The common disease common variant hypothesis focuses on multiple common polymorphisms, with a frequency greater than 5%. Each polymorphism has a low level of penetrance, but when combined they can increase the risk of developing ADHD or ASD. This hypothesis has formed the basis of molecular genetic research over the past two decades starting with the investigation of preselected candidate genes in relatively small studies (Li *et al.*, 2014). With the advent of high-throughput genetic screening, researchers could conduct hypothesis-free experiments in large samples of patients

and controls. These genome-wide association studies have identified a number of polymorphisms, which confer a slightly increased risk of developing a psychiatric disorder. Common variants have been estimated to explain 22% of ADHD heritability and 32% of ASD heritability (Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium, 2017; Demontis *et al.*, 2017). Some of the missing heritability can be explained by the CDRV hypothesis. ADHD and ASD might be caused by genetic variants that are rare in the overall population but have high penetrance in individuals. Rare variants such as monogenic or chromosomal abnormalities are a common cause of syndromic ASD, and in some cases, these abnormalities can be identified by genetic testing (Fernandez and Scherer, 2017). Nonsyndromic ASD might also be caused by monogenic mutations in genes such as Neurexin 1 (*NXRN1*) and SH3 and multiple ankyrin repeat domains (*SHANK3*) (Caglayan, 2010). Another type of rare variant that has been intensely studied is copy-number variations (CNVs). CNVs are chromosomal deletions or duplications that can span several megabases and which alter normal gene expression (Redon *et al.*, 2006). The relevance of CNVs to a particular phenotype can be linked to their position, length, and heterozygosity. There is increasing evidence that CNVs play a role in the pathogenesis of several neurodevelopmental disorders, and it has been demonstrated that patients with ASD have a higher burden of CNVs compared with healthy controls (Shishido *et al.*, 2013). Testing for CNVs in patients with ADHD has identified a number of genes that had previously been implicated in ASD, demonstrating the shared genetic risk for these disorders (Lionel *et al.*, 2011). Other studies have also reported a greater burden of CNVs in patients with ADHD without reporting comorbidity with ASD (Williams *et al.*, 2010, 2012). These CNVs include the parkin 2 (*Park2*) and neuropeptide Y loci (Lesch *et al.*, 2011; Jarick *et al.*, 2014) and genes in the metabotropic glutamate receptor family (Elia *et al.*, 2012). There is some evidence that CNVs may contribute to adult ADHD as well (Ramos-Quiroga *et al.*, 2014), although different genes may be affected. The growing consensus among researchers is that ASD and ADHD are caused by a number of synergistic factors including CNVs, rare variants, and common polymorphisms (Kiser *et al.*, 2015).

Environmental factors including epigenetic modifications have also been linked to these disorders. The prenatal and perinatal stages appear to be particularly susceptible to environmental stimuli. Both premature birth and maternal smoking during pregnancy have been linked to an increased risk of developing ADHD (Halmøy *et al.*, 2012; Zhu *et al.*, 2014). However, when genetic and familial confounders are included, the strength of these associations decreases, calling into question any causal relationship (Sciberras *et al.*, 2017). A further meta-analysis of

15 studies failed to find an association between maternal smoking and ASD (Rosen *et al.*, 2014). Even within the prenatal period, the effect of environmental stimuli depends upon the trimester during which exposure occurs. Exposure to antidepressants during the second and third trimesters has been associated with an increased risk of developing ASD (Boukhris *et al.*, 2016). However, a cohort study of more than 1.5 million Swedish children failed to find an association between maternal antidepressant use during the first trimester and risk of either disease (Sujan *et al.*, 2017). In the perinatal period, strong associations between ASD and birth complications leading to trauma, ischemia or hypoxia have been reported (Modabbernia *et al.*, 2017). Both prenatal and postnatal exposures to abnormal levels of metal toxicants and essential elements have been associated with ASD (Arora *et al.*, 2017). After the antenatal period, a number of early life factors have been linked to these disorders. These include an association between maternal anxiety, depression, and ADHD in 3-year olds (Meadows *et al.*, 2007) and increased parental age and ASD risk (Wu *et al.*, 2016). However, whether causality between environmental factors and neurodevelopmental disorders exists can be hard to determine, and further research, including the use of animal models, is required to investigate this issue.

Cross-species models

The use of animal and cellular models to understand the pathogenesis of human disease and develop treatments has greatly contributed to our understanding of several psychiatric disorders. These models are suitable for large-scale trials that can be translated into human clinical research (Crawley, 2012; van der Voet *et al.*, 2014; Falk *et al.*, 2016; Kim *et al.*, 2016; Ardhanareeswaran *et al.*, 2017).

Mouse (*Mus musculus*)

The mouse is the most commonly used model organism in biomedicine. Their short generation time, small size, and social nature allow a large number of animals to be housed in a small facility. The sequencing of the mouse genome combined with the tools to manipulate it in a targeted manner has revolutionized biomedical research (Hara and Takada, 2017). As mice and humans are very similar genetically (~99% homology), it is possible to investigate genes identified in human studies in mice. Following genetic manipulation, a combination of highly standardized behavioral and neurobiological techniques can be performed. This can provide insights into how polymorphisms or mutations can lead to the pathology of psychiatric disorders (Arguello and Gogos, 2006). Of particular relevance to psychiatric disorders, mice exhibit a range of complex behaviors including reciprocal social interactions (McCammon and Sive, 2015). Mice are often the first step in testing a new treatment for toxicity and efficacy (Vandamme, 2014).

Zebrafish (*Danio rerio*)

The zebrafish is an emerging model organism for biomedical research thanks to their short generation time, small size, and genetic tractability. Zebrafish are particularly useful for developmental and live imaging studies, as they develop externally and are transparent at larval stages. Almost 70% of human genes have at least one zebrafish orthologue (Howe *et al.*, 2013). Transient knockdown of genes in zebrafish was initially achieved by injecting morpholino oligonucleotides (Blum *et al.*, 2015). Recently, stable mutant lines have been created using CRISPR/Cas9 genome editing (Schmid and Haass, 2013). However, modelling human disorders in zebrafish is complicated by an additional whole-genome duplication which occurred ~440 million years ago (Amores *et al.*, 2011). Despite this issue, zebrafish are uniquely suited for high-throughput pharmacological studies, as larvae can be generated in large numbers and drugs can be applied by dilution in the embryo medium (Rihel *et al.*, 2010; Norton, 2013; Gutiérrez *et al.*, 2018). Zebrafish can be used to study ADHD and ASD thanks to their stereotypical behavior and social interactions. It is also possible to test cognitive deficits in this species. Spatial working memory can be measured in the habituation to novelty test, and various forms of learning can be assessed using robust behavioral paradigms (Stewart *et al.*, 2014).

Fruit fly (*Drosophila melanogaster*)

The fruit fly is a widely used model system that has greatly contributed to neuroscience research (Bier, 2005). Fruit flies are small and easy to maintain with a very short generation time, which allows large-scale low-cost experiments. Although the fruit fly brain is relatively small, it is still complex enough to model some aspects of psychiatric disorders (Bellen *et al.*, 2010). Signalling pathways and regulatory molecular networks are also well conserved. Overall, 75% of human disease genes have related sequences in the fly (Bier, 2005). Furthermore, the temporally inducible UAS-GAL4 system can be used to target specific cell types in *Drosophila* (Brand and Perrimon, 1993). When this system is coupled to the expression of RNA interference molecules, it allows reversible genetic manipulation. Transgenic flies can also be generated using the P-element system (Rubin and Spradling, 1982), and CRISPR/CAS9 technology now permits ubiquitous or tissue-specific genome editing (Port *et al.*, 2014). *Drosophila* display a behavioral repertoire that varies from simple aggregation to more complex behaviors such as aggression and courtship (O'Kane, 2011). Therefore, even though the nervous system and social behavior of *Drosophila* are not that similar to vertebrates, the fruit fly presents a valuable model to characterize mechanisms underlying disease-related behaviors (van Alphen and van Swinderen, 2013; van der Voet *et al.*, 2014).

Human induced pluripotent stem cells

The recent development and improvement of human induced pluripotent stem cell (hiPSC) technology permits the conversion of tissue into pluripotent cells that can be redifferentiated into neurons in a noninvasive and ethical manner (Takahashi *et al.*, 2007). This technique offers the possibility to use a range of different samples including skin biopsies, urine samples, and hair follicles. A great advantage of this method is that the genetic makeup of patients is included in the experimental model rather than investigating the influence of single genes. This may be particularly important for multifactorial diseases such as ADHD and ASD. hiPSCs also represent an innovative tool for large-scale drug screening. Moreover, neurons obtained from hiPSCs can be cultured in two dimensions, for single neuron analysis, or in three-dimensional aggregates (brain organoids), allowing evaluation of network-level effects (Falk *et al.*, 2016; Frega *et al.*, 2017; Prytkova and Brennand, 2017). In recent years, hiPSCs have been used as a tool to model psychiatric disorders such as ASD, ADHD, bipolar disorder, Schizophrenia, and the response to lithium at a cellular level (Prilutsky *et al.*, 2014; Ardhanareeswaran *et al.*, 2015; Lim *et al.*, 2015; Soliman *et al.*, 2017; Hoffman *et al.*, 2018; Jansch *et al.*, 2018).

A single model cannot capture all the traits of a complex brain disorder such as ASD and ADHD. As specific behavioral and neurobiological features may be more easily studied in certain organisms, a comparative study across models should be performed whenever possible (Stewart *et al.*, 2015; Wong and Josselyn, 2016).

Contactin-associated protein-like 2, adhesion G protein-coupled receptor L3, and parkin 2

Human genetic studies have identified a large number of genes linked to ADHD and ASD. There are currently 359 genes implicated in ADHD according to the ADHDGene database, whereas 1007 genes are included in the SFARI Gene database for ASD (Zhang *et al.*, 2011; Abrahams *et al.*, 2013). In this review, we have chosen to focus on three candidate genes for ADHD and ASD: *CNTNAP2*, adhesion G protein-coupled receptor L3 (*ADGRL3*), and *PARK2*. As well as being involved in diverse biological processes, the types of variants linking these genes to disease are also different. *CNTNAP2* was initially implicated in ASD through a recessive single base pair deletion in the coding region. In contrast, the association between *ADGRL3* and ADHD comes from noncoding single nucleotide polymorphisms (SNPs), which may affect gene expression levels (Strauss *et al.*, 2006; Martinez *et al.*, 2016). Larger scale CNVs in *PARK2* are associated with ASD (Glessner *et al.*, 2009; Scheuerle and Wilson, 2011). *CNTNAP2* and *PARK2* are primarily implicated in ASD but have also been linked with ADHD. *ADGRL3* is linked with ADHD and substance use disorders (SUD), but there is currently no strong

connection to ASD (Gau *et al.*, 2012). Together this review provides an overview of how genes with different biological functions can be studied in model systems to understand the comorbidity of ADHD and ASD.

Contactin-associated protein-like 2

Biological and molecular description

CNTNAP2 is one of the largest human genes. It spans 2.3 Mb and is located in chromosomal region 7q35. *CNTNAP2* encodes the neuronal transmembrane protein CASPR2, a member of the neurexin (NRXN) superfamily of single-pass transmembrane proteins. CASPR2 is composed of 1331 amino-acid residues organized into eight extracellular domains, two C-terminal intracellular domains, and one transmembrane domain (Poot, 2015). The intracellular domain of CASPR2 may be involved in protein–protein interactions, whereas the extracellular domains mediate cell–cell interactions and bind ligands, receptors, and extracellular matrix components (Poot, 2015; Baig *et al.*, 2017). *CNTNAP2* forms a neuron–glia adhesion complex with contactin 2 that localizes voltage-gated potassium channels to the juxtaparanodal region of myelinated axons (Poliak *et al.*, 1999, 2003). The first link between *CNTNAP2* and ASD was a recessive mutation found in an Amish family affected by a syndromic form of ASD called cortical dysplasia-focal epilepsy syndrome (Strauss *et al.*, 2006). Several more studies have provided convergent evidence that rare and common variations in *CNTNAP2* confer risk for ASD-related endophenotypes such as developmental language disorders and ASD itself (Peñagarikano and Geschwind, 2012). *CNTNAP2* has also been linked to schizophrenia, epilepsy, intellectual disability, learning disability, and ADHD (Rodenas-Cuadrado *et al.*, 2014). However, most studies have focused on the role of *CNTNAP2* in ASD.

In this review, we have chosen to focus on these three candidate genes because they are linked to either ADHD (*ADGRL3*), ASD (*CNTNAP2*), or both disorders (*PARK2*). Each gene has been modelled in a number of different species, making these candidates ideal to demonstrate the power of comparative studies to help translate findings to human patients.

Models

Mouse

The first *Cntnap2* mutant mouse was generated in 2003 (Poliak *et al.*, 2003). After the identification of *CNTNAP2* as a candidate gene for ASD, the original strain was backcrossed onto the C57BL/6J background, which is more suitable for behavioral characterization (Crawley *et al.*, 1997; Penagarikano *et al.*, 2011). *Cntnap2* KO mice display deficits in core behavioral features of ASD: social deficits, restrictive and repetitive behaviors, and reduced vocal communication (Table 1). When compared with wild-type littermates, these mice appear normal, and there are no differences in growth rate and final size.

Table 1 Mouse models

	Mutation	General appearance	Neuronal activity	Histological analyses	Locomotor activity	Memory/cognitive function	Anxiety and depression-like behaviors	Stereotypic behaviors	Social behavior	Drug treatment	References
<i>CNTNAP2</i> knockout	<i>Cntnap2</i> ^{-/-} mice on the C57BL/6J background	No gross morphological deformity or differences in weight or growth rate reported Viable and fertile offspring	Presence of spontaneous seizures in mouse older than 6 months Abnormal electroencephalogram pattern during slow wave sleep in mouse older than 8 months defects in the migration of cortical projection neurons Impairment in synaptic inhibition specifically onto the perisomatic compartment of CA1 pyramidal cells	↓ GABAergic interneurons in all laminae ↓ Parvalbumin + interneurons in hippocampus Asynchronous neuronal firing pattern Presence of ectopic neurons in the corpus callosum at postnatal day 14 (P14)	↑ Locomotor activity	No difference in the spatial learning and memory test	No significant difference between genotypes	Presence of stereotypic motor movements Behavioral inflexibility	↓ Social behavior ↓ Ultrasonic calls	Risperidone treatment: ↓ locomotor activity ↑ nest-building score ↓ grooming behavior and perseveration in the T-maze -No improvement with social interaction nor in sensory hypersensitivity	Penagarikano <i>et al.</i> (2011), Jurgensen and Castillo (2015)
<i>ADGRL3</i> knockout	<i>Adgrl3</i> ^{-/-} mice on a 129S4/Sylva and C57 mixed background	No gross morphological deformity or differences in weight or growth rate reported Viable and fertile offspring	↑ Level of DA ↑ level of 5-HT Disruption of neurotransmitter and ADHD candidate genes at P0 Nonsignificant enrichment of relevant gene sets at P4, P28 & P180 in transcriptomic study	-	↑ Locomotor activity. No movement impairment in rotarod test	↑ Motivation for food	↑ Activity in forced swim test No differences in the time spent in the center of the open field	-	-	↑ Hyperactive response to cocaine	Wallis <i>et al.</i> (2012), Orsini <i>et al.</i> (2016)
<i>PARK2</i> knockout	<i>Parkin</i> ^{-/-} mice on the C57BL/6J background	No gross morphological deformity or differences in weight or growth rate reported Viable and fertile offspring	↑ Basal release of DA from the nerve terminals ↓ LTP in the Hippocampus Normal LTP ↑ PPF and EPSP after low-frequency stimulation ↓ Synaptic excitability on medium-sized striatal spiny neurons Normal number of dopaminergic neurons in the substantia nigra	No SNCA accumulation ↑ DAT VMAT2 protein ↓ TH+ neurons in locus coeruleus ↓ NE in spinal cord and olfactory bulb ↑ DA metabolism and extracellular concentrations in the striatum In other regions: normal DA and TH levels Severe mitochondrial damage	No significant differences in rotarod, general locomotor activity, beam transversal task, footprint analysis	↓ Exploration and habituation to a novel environment ↓ object location on Y maze ↓ Spontaneous alternation in T-maze (not replicated) No significant differences on Novel object recognition test, Morris water maze, T maze ↑ Learning in passive avoidance ↓ Acoustic startle response Impairment in working memory	No significant differences on elevated plus maze, light-dark exploration test No significant differences forced swim test ↑ Immobility at one time point (tail suspension test) No strong phenotype for depression-like traits	-	-	-	Rial <i>et al.</i> , (2014), Kurtenbach <i>et al.</i> (2013), Zhu <i>et al.</i> (2007), Stichel <i>et al.</i> (2007), Itier (2003), Goldberg <i>et al.</i> (2003), Von Coelln <i>et al.</i> (2004), Perez and Palmiter (2005)

ADHD, attention-deficit/hyperactivity disorder; DA, dopaminergic; SNCA, α-synuclein; TH, thyroid hormone.

Interestingly, after six months, *Cntnap2*^{-/-} mice develop epileptic seizures and their electroencephalogram has an abnormal pattern during slow wave sleep. In addition, knockout mice are hyperactive (Penagarikano *et al.*, 2011). *Cntnap2*^{-/-} mice show neuronal migration abnormalities that reveal the fundamental role of this gene in cortical projection neuron migration (Penagarikano *et al.*, 2011). Interestingly, it has been found that *Cntnap2*^{-/-} mice display a reduced number of GABAergic interneurons (Penagarikano *et al.*, 2011). Pharmacological treatment with risperidone rescued the stereotypic behavior of *Cntnap2*^{-/-} mice, whereas social deficits were unaffected, similar to the pattern of improvements seen in human patients (Penagarikano *et al.*, 2011). Overall, this model recapitulates certain features of patients with *CNTNAP2* mutations, and this is one of the best studied genes for this disorder (Poot, 2015).

Zebrafish

A zebrafish *cntnap2* mutant model was recently created (Table 2). *Cntnap2a*^{-/-} and *cntnap2b*^{-/-} double mutants (*cntnap2ab*) harbor homozygous loss-of-function mutations in both genes resulting in a complete loss of the protein (Hoffman *et al.*, 2016). Hoffman *et al.* (2016) analyzed excitatory and inhibitory neuron populations in *cntnap2ab* during early development, as an imbalance in inhibitory and excitatory signalling could be involved in ASD (Rubenstein and Merzenich, 2003). They observed that mutants have a smaller head than wild type. Moreover, mutants show a decrease in GABAergic cell count; however, there were no prominent deficits in glutamatergic neurons (Hoffman *et al.*, 2016). Loss of inhibitory neurons can increase seizure susceptibility (Cobos *et al.*, 2005). Zebrafish *cntnap2ab* mutants are more sensitive to drug-induced seizures and show night-time hyperactivity, another phenotype linked to the imbalance between excitatory and inhibitory neurotransmission. The night-time hyperactivity phenotype can be rescued by estrogens (Hoffman *et al.*, 2016). This finding has reinforced the use of whole organism drug screening as an effective system to identify compounds that can be developed into pharmaceutical treatments.

Fruit fly

CNTNAP2 and *NRXN1* are members of the neurexin superfamily. SNPs and CNVs in both genes have been associated with disorders including ASD. Zweier *et al.* (2009) have used *Drosophila* to examine a common synaptic link between these two genes and neuropsychiatric disorders (Table 3). This group studied both *Nrx-I* and *Nrx-IV*, the *Drosophila* orthologs of *NRXN1* and *CNTNAP2*. Knockdown (KO) of *Nrx-IV*, either ubiquitously or only in neurons, led to late embryonic lethality. Immunostaining of *Nrx-IV* KO embryos showed an overall reduction in staining for the presynaptic protein bruchpilot that maintains the structural integrity of synaptic active zones (Wagh *et al.*, 2006). They also found a dosage-dependent increase of

bruchpilot staining when *Nrx-IV* was overexpressed in larval neurons. bruchpilot and Nrx-I colocalize at presynaptic active zones (Li *et al.*, 2007). In conclusion, *CNTNAP2* and *NRXN1* may be involved in a common synaptic mechanism, contributing to the etiology of neurodevelopmental disorders together with bruchpilot (Zweier *et al.*, 2009). Further studies are required to determine the mechanism by which Nrx-I and Nrx-IV control bruchpilot levels.

Human induced pluripotent stem cells

hiPSCs carrying a heterozygous deletion in *CNTNAP2* have been generated to characterize the associated molecular and cellular phenotypes (Table 4). However, they were derived from a female proband who met the DSM-IV criteria for schizo-affective disorder, not ASD or ADHD. hiPSCs were also generated from both parents as well as five unrelated healthy controls (Lee *et al.*, 2015). Neural migration was quantified using a neurosphere assay. Interestingly, hiPSC neural progenitor cells derived from the heterozygous *CNTNAP2* schizo-affective patient showed significantly reduced neural migration relative to controls (Lee *et al.*, 2015). This phenotype is consistent with the neuronal migration abnormalities observed in the *Cntnap2*^{-/-} mouse model of ASD (Penagarikano *et al.*, 2011). Global gene expression and neuronal activity were analyzed in the same cohort. HiPSC-derived neurons from individuals carrying the heterozygous *CNTNAP2* deletion show altered neuronal activity and differential expression of genes involved in synaptic transmission (Flaherty *et al.*, 2017).

Adhesion G protein-coupled receptor L3

Biological and molecular description

The latrophilin family of proteins was initially discovered owing their ability to bind α -latrotoxin, a component of black widow spider venom (Davletov *et al.*, 1996). The ADGRL3 (previously known as LPHN3) is of particular interest in relation to neurodevelopmental disorders. Multiple polymorphisms in *ADGRL3* have been linked to an increased risk of ADHD and SUD (Arcos-Burgos *et al.*, 2010; Ribasés *et al.*, 2010). ADGRL3 forms a trimeric complex with FLRT3 and UNC5, which supports transcellular adhesion and glutamatergic synapse development (Jackson *et al.*, 2015). The identification of *ADGRL3* in human genetic studies, coupled with its putative roles in neuronal migration and synapse development, provides strong support for *ADGRL3* polymorphisms in the development of ADHD (O'Sullivan *et al.*, 2014). The lack of naturally occurring coding variants in humans greatly limits our ability to investigate *ADGRL3* (Orsini *et al.*, 2016). Coding variants may not affect the genes that they are located in, but may rather have effects elsewhere in the genome. However, there is functional evidence that the noncoding variant rs2271338 affects *ADGRL3* expression, further linking this gene to ADHD (Martinez *et al.*, 2016).

Table 2 Fish models

	Mutation	General appearance	Neuronal activity	Histological analyses	Locomotor activity	Excitatory/inibithory balance	DRUGS treatment	References
<i>CNTNAP2</i>	Double mutant: <i>cntnap2a</i> ^{Δ121/Δ121} <i>cntnap2b</i> ^{31i/31i} and <i>cntnap2a</i> ^{Δ25/Δ25} <i>cntnap2b</i> ^{Δ7/Δ7}	No gross morphological deformity in the axon scaffold at embryonic stages ↓ Head size	↑ Increased sensitivity to pentylentetrazol induced seizures at embryonic stages	↓ GABAergic cells in the cerebellum and forebrain at 4 days postfertilization Normal glutamatergic neurons	↑ Night-time hyperactivity	Dysfunction in both glutamatergic and GABAergic pathways	Estrogen treatment: (Biochanin A and β-estradiol 17-cypionate) suppress the mutant nighttime hyperactivity phenotype	Hoffman <i>et al.</i> (2016)
<i>ADGRL3</i>	ADGRL3.1 Morphant Zebrafish	No gross morphological deformity reported	–	↓ Dopaminergic cells at 3 and 6 days postfertilization in the posterior tuberculum Normal noradrenergic and serotonergic systems	↑ Hyperactivity ↑ Acceleration	–	Hyposensitive locomotive response to DA manipulation Methylphenidate and atomoxetine reduces hyperactivity	Lange <i>et al.</i> (2012), Lange <i>et al.</i> (2018)
<i>PARK2</i>	PARK2 knockdown (Morpholino)	Protein aggregation in T-tubules (Ca ⁺⁺ homeostasis)	↑ DA neuron lost	Normal mitochondria ↓ Complex I activity	No effects in larvae swimming behavior	–	–	Flinn <i>et al.</i> (2009)
	Transient antisense PARK2 knockdown (53% parkin mRNA reduction)	↑ Susceptibility to cellular stress	No effects	No effects	No effects	–	–	Fett <i>et al.</i> (2010)
	Human PARK2 overexpression (Gal4/UAS-based bidirectional expression system)	Protective effect against stress	–	–	–	–	–	Fett <i>et al.</i> (2010)

DA, dopaminergic.

Table 3 Fruit fly

	Mutation	General appearance	Neuronal activity	Histological analyses	Locomotor activity	Memory cognitive function	Drug treatment	References
<i>CNTNAP2</i>	Knockdown of the orthologues Nr _x -IV either ubiquitously (actin-Gal4 drivers) or specifically in neurons (elav-Gal4 driver)	Late embryonic lethality	–	↓ Presynaptic protein bruchpilot	–	–	–	Zweier <i>et al.</i> (2009)
	Inducible Nr _x -IV overexpression line P (EP)Nr _x -IV ^{EP604}	–	–	↑ Presynaptic protein bruchpilot	–	–	–	Zweier <i>et al.</i> (2009)
<i>ADGRL3</i>	Pan-neuronal knockdown with UAS-GAL4 system and RNAi	No gross morphological deformity reported	–	No disruption of dopaminergic neuron count	↑ Hyperactivity Heightened hyperactivity in night-time cycle, which is light dependent	–	Methylphenidate reduces hyperactivity particularly at night	Van der Voet <i>et al.</i> (2015)
<i>PARK2</i>	PARK2 knockout	↓ Life span ↓ Body weight (↓ cell size) Male sterility (caused by mitochondrial dynamics failure during spermatogenesis)	No age-dependent DA neuron loss NO general DA neurons lost In a subset of cerebral DA neurons: degeneration, shrinkage and decreased TH levels	Mitochondrial disintegration and loss of cristae	Unable to jump or fly (indirect flight muscle degeneration and defects of nervous system) weakened circadian rhythms due to altered clock neuron electrophysiology	Memory abnormalities	–	Greene <i>et al.</i> (2003), Whitworth <i>et al.</i> (2005), Cha <i>et al.</i> (2005), Riparbelli and Callaini (2007), Pesah <i>et al.</i> (2005), Vincent <i>et al.</i> (2012), Navarro <i>et al.</i> (2014), Cackovic <i>et al.</i> (2018), Julienne <i>et al.</i> (2017)
	Overexpression of PARK2 mutant forms (Gln311Stop – Q311X and Thr240Arg – T240R)	–	Age dependent DA neuron degeneration	–	Age-dependent motor deficits	–	–	Sang <i>et al.</i> (2007)

DA, dopaminergic; TH, thyroid hormone.

Table 4 Human induced pluripotent stem cells

	Mutation	Neural differentiation	Exon, allele and gene specific expression	Biochemical features	References
<i>CNTNAP2</i>	Four patient-derived lines: (patient carrying a 289 kb heterozygous deletion – exons 14–15) hiPSC neural progenitor cells Two independent hiPSC-derived neurons hiPSC-derived oligodendrocyte precursor cells	↓ Neural migration altered neuronal activity	Differential expression of genes involved in synaptic transmission ↓ Expression of 14–15 exon ↑ Expression of 23-24 exon ↑ Expression of the mutant allele	–	Lee <i>et al.</i> (2015), Flaherty <i>et al.</i> (2017)
<i>PARK2</i>	Two patient-derived lines: Heterozygous deletion exon 3 and exon 5 Homozygous deletion exon 3	↓ Neuronal processes complexity (length, number of terminals, branch points) ↓ Microtubule stability	–	↑ Spontaneous DA release ↓ DA uptake ↓ DAT binding sites ↑ ROS (by increasing MAO transcripts)	Jiang <i>et al.</i> (2012), Ren <i>et al.</i> (2015)
	Four patient-derived lines: Compound heterozygous deletion exon 3 and R42P Compound heterozygous deletion exon 3–4 and 1-BP DEL, 255A R275W R42P Two isogenic lines (PARK ^{-/-} , PARK ^{w+/-}) Two patient-derived lines: homozygous deletion exon 2–4 Homozygous deletion exon 6 and 7	↓ DA differentiation	–	↓ Mitochondrial volume fraction Mitochondrial ultrastructure abnormalities (PARK ^{-/-} line)	Shaltouki <i>et al.</i> (2015)
	Three patient-derived lines: compound heterozygous deletion exon 7 and c.1072delT Compound heterozygous deletion exon 1 deletion and c.924C>T Homozygous deletion c.1072delT One patient-derived line: Heterozygous deletion exon 5	–	–	↑ Oxidative stress and ROS ↑ Nrf2 pathway Abnormal mitochondrial morphology SNCA accumulation in one line ↓ Mitophagy ↓ Complex I activity ↑ Mitochondrial branching	Imaizumi <i>et al.</i> (2012), Suzuki <i>et al.</i> (2017) Zanon <i>et al.</i> (2017)
		–	–	↓ Proteasome activity ↓ Antioxidative pathways	Chang <i>et al.</i> (2016)

DA, dopaminergic; DAT, dopamine transporter; hiPSC, human induced pluripotent stem cell; ROS, reactive oxygen species; SNCA, α -synuclein.

Models

Mouse

ADGRL3^{-/-} mice displayed hyperactivity in the open-field test, (Table 1) but no significant genotype differences were observed in the amount of time spent in the center of the open field, a common test for anxiety. *ADGRL3* null mice also displayed a heightened locomotor response to cocaine. A differential response to cocaine is of interest because of the link between *ADGRL3* polymorphisms and SUD (Arcos-Burgos *et al.*, 2012). In a food reward paradigm, *ADGRL3*^{-/-} mice displayed increased levels of motivation to work for food, whereas motor coordination was unaffected in the rotarod test. In the Porsolt swim test, *ADGRL3*^{-/-} mice spent more time swimming and had increased latency to immobility, possibly owing to reduced depressive behavior or hyperactivity. Whole brains of *ADGRL3*^{-/-} and wild-type P0 pups were used to quantify candidate gene expression by qPCR. The genes chosen were involved in neuronal differentiation and survival as well as dopaminergic (DA) and serotonergic neurotransmission. The following genes were differentially expressed in *ADGRL3*^{-/-}: serotonin transporter (*SLC6A4*), serotonin 2C receptor (*5-Htr2c*), dopamine transporter (*SLC6A3*), dopamine receptor D4 (*Drd4*), neural cell adhesion molecule 1 (*Ncam1*), nuclear receptor subfamily 4 group A member 2 (*Nr4a2*), and tyrosine hydroxylase (*Th*). High-pressure liquid chromatography also revealed an increased level of dopamine and 5-HT in *ADGRL3*^{-/-} mice (Wallis *et al.*, 2012). Transcriptomic analysis was performed on the prefrontal cortex, striatum, and hippocampus of *ADGRL3*^{-/-} and control mice at 4 days, 28 days, and 6 months. Collapsing the RNA-sequencing results across brain regions and time points identified 11 genes that are differentially expressed in *ADGRL3*^{-/-} mice. Two of these genes (*Pcdhgb8*, upregulated and *Pcdhbb9*, downregulated) are members of the protocadherin family of calcium-dependent cell–cell adhesion molecules (Orsini *et al.*, 2016), which have been linked to ADHD and ASD (Rivero *et al.*, 2015). Independent analysis of brain regions and time points revealed a large number of differentially expressed genes including overexpression of *Htr2c* [5-hydroxytryptamine (serotonin) receptor 2c] in the cortex and *SLC6A3* (dopamine transporter) in the hippocampus of 6-month old *ADGRL3*^{-/-} mice. Gene set analysis did not find a statistically significant enrichment of gene sets following adjustment for multiple testing. However, a number of the most enriched pathways were relevant to ADHD and SUD, including synaptic vesicle cycle, DA synapse, glutamatergic synapse, neurotrophin signalling pathway, and both amphetamine and nicotine addiction.

Zebrafish

Zebrafish have two paralogues of human *ADGRL3*: *adgrl3.1* and *adgrl3.2*. *adgrl3.1* shows a more specific expression pattern during embryonic development. *adgrl3.1* morphant larvae displayed hyperactivity under both light and dark

conditions at 6 days after fertilization (Table 2). The increased distance swum was because of greater acceleration and average speed throughout the trial (Lange *et al.*, 2012). In most patients with ADHD, MPH administration results in a reduction in hyperactivity (Ramos-Quiroga *et al.*, 2009). MPH decreased hyperactivity in *adgrl3.1* morphant fish but not in controls. The total distance swam was reduced by decreasing the average speed. Application of another ADHD treatment drug, the selective noradrenaline reuptake inhibitor atomoxetine, reduced the total distance swam by *adgrl3.1* morphant zebrafish by increasing the resting time. The number of DA neurons in the posterior tuberculum of *adgrl3.1* morphants was reduced at 3 and 6 days after fertilization. Subregions of the posterior tuberculum were also disorganised (Lange *et al.*, 2012). Projectome analysis has indicated a functional correlation between the posterior tuberculum and the mammalian A11 group of DA neurons (Tay *et al.*, 2011). Administration of DA receptor agonists and antagonists showed that *adgrl3.1* MO larvae are hyposensitive to DA manipulation compared with *adgrl3.1* CO. A possible explanation for this hyposensitivity is that there could be a heightened level of DA in the synaptic cleft of *adgrl3.1* morphants, which might desensitize both DA-1 and DA-2 like receptors. A heightened level of DA might also explain the hyperactivity observed in these animals (Lange *et al.*, 2018). An evolutionarily conserved enhancer for *adgrl3.1* has been used to express green fluorescent protein in zebrafish. This enhancer was sufficient to produce an expression pattern similar to endogenous *adgrl3.1* in the fore, mid, and hind-brain but not in the telencephalon and retina (Martinez *et al.*, 2016).

Fruit fly

The *Drosophila* orthologue of *ADGRL3* is known as latrophilin. Locomotion activity and sleep have been assessed in latrophilin KO flies using the *Drosophila* Activity Monitor (Table 3). Latrophilin KO flies were found to have heightened locomotor activity and reduced sleep, particularly during the 12-h night period. Testing in constant darkness showed that this hyperactive phenotype was light dependent. As light can buffer the wake-promoting effect of DA (Shang *et al.*, 2011), the presence of a light-sensitive phenotype hints at a disruption of the DAergic system. However, the number and position of DA neurons is normal in latrophilin flies, suggesting that impairment of the DA system may result from changes in downstream signalling. *Drosophila* with a pan-neuronal KO of the dopamine transporter (*DAT*) gene displayed a similar light-sensitive hyperactive phenotype. Furthermore, application of MPH normalized the locomotor and sleep phenotype of latrophilin flies, further linking this gene to DA signalling (van der Voet *et al.*, 2015).

Cellular models

Although no hiPSCs-containing *ADGRL3* polymorphisms are currently available, a number of conventional cellular models

have been used to study this gene. A family-based genetic analysis of 838 individuals identified six evolutionary conserved regions (ECRs) in the ADGRL3 genomic region that contained SNPs or haplotypes linked to ADHD (Martinez *et al.*, 2016). ECR 37 and 47 were the only regions found to drive luciferase expression. The ECR 37 risk variant did not affect luciferase levels whereas the ECR 47 risk haplotype reduced luciferase activity by ~40% in both B35 neuroblastoma and U87 astrocytoma cell lines. Transcription factor binding motif analysis of ECR 47 showed an over-representation of transcription factors associated with brain function. Electromobility shift assays showed that the rs2271338 SNP in ECR 47 prevented binding of the Yin Yang 1 (YY1) transcription factor. However, YY1 interfering small RNA molecules did not reduce ADGRL3 expression in SH-SY5Y neuroblastoma cells, perhaps because of an inability to model this complex interaction outside of its native system. Expression quantitative trait loci analysis of post-mortem human brain tissue has linked the rs2271338 AA risk genotype allele to decreased thalamic expression of ADGRL3 (Martinez *et al.*, 2016).

PARK2

Biological and molecular descriptions

Mutations in *PARK2* were first discovered in a small percentage of patients with Parkinson's disease (Lücking *et al.*, 2000). Subsequent whole-genome studies have linked *PARK2* CNVs with Han Chinese children with ASD (Yin *et al.*, 2016) and European populations (Glessner *et al.*, 2009; Scheuerle and Wilson, 2011). CNVs within the *PARK2* locus have also been found among ADHD patient cohorts (Jarick *et al.*, 2012).

PARK2, also known as *PRKN*, codes for an E3 ubiquitin ligase previously called Parkin. One of the most studied partners of *PARK2* is the serine/threonine protein kinase PINK1. Acting together, *PARK2* and PINK1 constitute an internal sensor system for disparate perturbations to cellular homeostasis (Pickrell and Youle, 2015). In fact, different stimuli can lead to PINK1 accumulation on the outer mitochondrial membrane where it can recruit and activate cytosolic *PARK2*. *PARK2* can then target several mitochondrial proteins for degradation (Hang *et al.*, 2015). The interaction between PINK1 and *PARK2* plays a pivotal role in mitochondria dynamics and quality control, regulating processes such as mitophagy, fusion and fission, biogenesis, and transport (Scarffe *et al.*, 2014). *PARK2* is also linked to apoptotic pathways and could exert a proapoptotic or anti-apoptotic effect in a stressor-dependent and environmental-dependent manner (da Costa *et al.*, 2009; Müller-Rischart *et al.*, 2013; Hollville *et al.*, 2014; Zhang *et al.*, 2014).

PARK2 shows high amino-acid sequence conservation across species, suggesting that it has an evolutionarily conserved function (Pienaar *et al.*, 2010). Orthologues of this gene have been found in most of the model organisms used in translational research, permitting the

molecular function of this gene to be studied in different systems (Pienaar *et al.*, 2010).

Models

Mouse

Surprisingly, *Park2*^{-/-} mice do not represent a robust model for Parkinson's disease (Perez and Palmiter, 2005), but phenotypic characterization of these animals can be used to investigate whether there is a link to neurodevelopmental disorders like ADHD and ASD (Table 1). *Park2*^{-/-} mice did not show impairment of general activity, coordination, and gait performance (Goldberg *et al.*, 2003; Perez and Palmiter, 2005; Rial *et al.*, 2014) or display any olfactory changes (Kurtenbach *et al.*, 2013). Anxiety-related behaviors were shown in one study (Zhu *et al.*, 2007) but were not confirmed by two other groups (Perez and Palmiter, 2005; Rial *et al.*, 2014). Similarly, *Park2*^{-/-} mice did not show a strong phenotype for depression-like traits (Perez and Palmiter, 2005; Rial *et al.*, 2014). Interestingly, most studies have shown effects on cognition and working memory. *Park2*^{-/-} mice have impaired habituation and exploratory activity in a novel environment (Itier, 2003; Zhu *et al.*, 2007) and exhibit worse object location performance in a Y maze (Rial *et al.*, 2014). Additionally, they show less spontaneous alternation in a T-maze. There is no evidence of impaired sensorimotor gating, although one study found a reduced acoustic startle response (von Coelln *et al.*, 2004) concomitant with a selective reduction of Th-positive neurons in the locus coeruleus and noradrenaline-positive neurons in the spinal cord and olfactory bulb. Unfortunately, biochemical and electrophysiological evaluation of these models is not clear. Although no studies have reported DA neuron degeneration, some research has found an increase of DA leakage from nerve terminals (Rial *et al.*, 2014), higher extracellular levels of DA in the striatum, and an increase in *DAT* expression (Itier, 2003). Long-term potentiation was decreased in one study, and a higher rate of paired pulse facilitation and excitatory postsynaptic potentials was found after low-frequency stimulation (Itier, 2003). Proteomic studies highlighted a strong reduction in mitochondrial respiratory chain proteins and stress response proteins (Shin *et al.*, 2011) together with mitochondrial damage (Stichel *et al.*, 2007). Lack of *Park2* also seems to affect mitochondrial morphology (Pinto *et al.*, 2018). The great variability among *Park2*^{-/-} mouse models could be related to the background line and the type of mutation introduced (Perez and Palmiter, 2005). The absence of a strong Parkinsonian phenotype in *Parkin*-deficient mice may be because of the presence of compensatory systems such as multiple redundant E3 ubiquitin ligases. Although *Park2*^{-/-} mice have not been subjected to tests for core features of ADHD, they do show impairments in working memory, a feature shared by both patients with ADHD and those with ASD (Craig *et al.*, 2016). Specifically, the impairments in spontaneous

alternation and exploration of a novel environment (Ttier, 2003) might mirror the restricted interest and cognitive rigidity that are shown by some mouse models of ASD (Kazdoba *et al.*, 2016). A reduced acoustic startle response has been also shown in other mouse ASD models (DeLorey *et al.*, 2011; Wurzman *et al.*, 2015), and might be linked to the inattention that characterizes patients with ADHD. There are no published findings regarding social behavior or impulsivity, making it difficult to further speculate about the similarity of this model to ADHD or ASD phenotypes.

Zebrafish

Park2 protein is extensively expressed in ventral diencephalic DA neurons in zebrafish (Pienaar *et al.*, 2010). No stable *park2* mutants are available in this species. Transient KO zebrafish have been created and these show no motor impairments. Interestingly, one of these models showed a loss of neurons, and despite showing a normal mitochondrial subset, complex I activity was found to be reduced (Table 2; Flinn *et al.*, 2009). Similarly, KO of *park2* increased cellular susceptibility to stress, whereas overexpression of human *PARK2* exerted a general protective effect against the same conditions (Fett *et al.*, 2010).

Fruit fly

PARK2 protein is highly expressed in *Drosophila* and a number of studies have begun to unravel the biological functions of PARK2 in this organism. Generation of PARK2/PINK1 double mutants has been crucial to understand the PARK2 signalling pathway and identify protein substrates (Botella *et al.*, 2009; Pienaar *et al.*, 2010; Guo, 2012). *Park*^{-/-} *Drosophila* have a short life span and a low body weight (Table 3). The most prominent feature of these mutants is mitochondrial disintegration (fragmented mitochondrial networks), advanced aging, and loss of inner cristae (Cackovic *et al.*, 2018). Moreover, failure of mitochondrial dynamics leads to male sterility and most probably to muscle degeneration, leaving the flies unable to jump or fly. Variable results have been reported regarding degeneration of a small subset of DA neurons (Greene *et al.*, 2003; Cha *et al.*, 2005; Pesah *et al.*, 2005; Whitworth *et al.*, 2005; Riparbelli and Callaini, 2007; Vincent *et al.*, 2012; Navarro *et al.*, 2014). Overexpression of mutant isoforms of *PARK2* leads to a more pronounced age-dependent loss of DA neurons (Sang *et al.*, 2007). A recent study reported learning and memory abnormalities in *park*^{-/-} and a weakening of circadian rhythms because of changes in clock neurons (Julienne *et al.*, 2017).

Human induced pluripotent stem cells

At the present time, 12 different hiPSC lines with various CNVs and mutations in the *PARK2* locus have been created (Table 4) (Xu *et al.*, 2016). All the donors were diagnosed with Parkinson's disease, and no hiPSC study has been carried out using ASD or ADHD donors. The

creation of human midbrain DA neurons from these stem cells uncovered an increase of DA release from terminals accompanied by decreased *DAT* expression and lower reuptake (Jiang *et al.*, 2012). The same cells show a diminished degree of neuronal process complexity, measured by different features such as length, number of terminals, and branch points, probably because of microtubule instability (Ren *et al.*, 2015). The rate of DA differentiation is lower in hiPSCs carrying *PARK2* mutations than in controls (Shaltouki *et al.*, 2015). Most studies have found impairment in mitochondrial morphology (Imaizumi *et al.*, 2012; Shaltouki *et al.*, 2015; Zanon *et al.*, 2017) and complex I activity (Zanon *et al.*, 2017) together with an increase in the presence of reactive oxygen species and a decrease in antioxidative pathways (Chang *et al.*, 2016; Suzuki *et al.*, 2017). An impairment in mitophagy has also been demonstrated (Suzuki *et al.*, 2017).

Discussion

The comparison of CNTNAP2, ADGRL3, and PARK2 across species has shown how these models can link genetic variants found in human populations to ADHD and ASD at both behavioral and neurobiological levels (Table 5). Transgenic animal models of *CNTNAP2*, a gene coding for a neuron–glia adhesion protein found in myelinated axons, show that this gene is essential for normal neurodevelopment. Depletion of CNTNAP2 in mice results in epileptic seizures and a reduction in the number of GABAergic interneurons (Crawley *et al.*, 1997; Penagarikano *et al.*, 2011). The same features are found in the equivalent zebrafish model, which shows a smaller head and increased sensitivity to drug-induced seizures (Hoffman *et al.*, 2016). The phenotype is even stronger in *Drosophila* where the absence of the CNTNAP2 orthologues results in late-stage embryonic lethality (Zweier *et al.*, 2009). Finally, neural progenitor cells derived from a heterozygous *CNTNAP2* patient show significantly reduced neural migration (Lee *et al.*, 2015), and hiPSC-derived neurons from carrier individuals show differential expression of genes involved in synaptic transmission and neuronal activity (Flaherty *et al.*, 2017). Interneuron dysfunction has been repeatedly linked with ASD (Takano, 2015). On a behavioral level, some phenotypic features are common across species such as the hyperactivity seen in both mice and zebrafish (Penagarikano *et al.*, 2011; Hoffman *et al.*, 2016). This hyperactivity mirrors one of the most prominent symptoms of ADHD.

The biological role of ADGRL3 is also linked to transcellular adhesion and glutamatergic synapse development (Jackson *et al.*, 2015). Multiple models of *ADGRL3* have displayed alterations in their DA system including increased dopamine levels in ADGRL3 mutant mice and a reduced number of DA neurons in *adgrl3.1* morphant zebrafish (Lange *et al.*, 2012; Orsini *et al.*, 2016). Dysregulation of dopamine has been implicated in the etiology of ADHD

Table 5 Overview of genes linked to attention-deficit/hyperactivity disorder and autism spectrum disorder

Genes	Linked disorder	Gene function	Main phenotype
<i>CNTNAP2</i>	ASD	Neuron–glia adhesion (voltage-gated K channel localization)	Interneurons disfunction
<i>ADGRL3</i>	ADHD	Transcellular adhesion/glutamatergic synapse development	Dopaminergic alteration
<i>PARK2</i>	ASD ADHD	E3 Ub protein ligase (mitochondria quality control)	Mitochondrial impairment Dopaminergic disfunction

ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder.

by both genetic and functional imaging studies (Li *et al.*, 2006; Faraone *et al.*, 2015), and the most effective pharmacological treatments for ADHD are stimulant medications that act on the DA and noradrenergic systems (Engert and Pruessner, 2008). Behavioral analysis of *ADGRL3* transgenic animals has revealed a number of phenotypes, the most prominent of which is the cross-species hyperactivity, which is modulated by light in *Drosophila*. The impairment of DA signalling in *ADGRL3* transgenic animals could provide insights into how genes not directly involved in this neurotransmitter pathway may confer increased risk of ADHD.

Although mutations in the *PARK2* gene are historically linked with Parkinson's disease, mouse and zebrafish do not robustly model this disease (Perez and Palmiter, 2005). Instead, knockout of *Park2* in both mouse and *Drosophila* leads to ADHD-like impairments in working memory. Most *PARK2* models show mitochondrial phenotypes including fragmented mitochondrial networks in *Drosophila* and disrupted mitophagy, mitochondrial morphology, and complex I activity in neurons derived from iPSCs of patients with various genetic abnormalities in *PARK2* (Imaizumi *et al.*, 2012; Shaltouki *et al.*, 2015; Zanon *et al.*, 2017). Different studies have underlined an association between ASD, ADHD, and mitochondrial impairment. General mitochondrial dysfunction is believed to be present in 30–40% of autistic children (Giulivi *et al.*, 2010; Rossignol and Frye, 2011; Morris and Berk, 2015). With regards to ADHD, sporadic mtDNA mutations and increases in oxidative markers have been reported (Marazziti *et al.*, 2011), as well as lower levels of oxygen consumption and ATP production together with increased levels of superoxide radicals (Verma *et al.*, 2016).

PARK2 hiPSC models also indicate an impairment in the DA system. Midbrain DA neurons created from these hiPSC lines showed an increase in DA release from terminals accompanied by lower reuptake of this neurotransmitter (Jiang *et al.*, 2012). The same cells show a diminished degree of neuronal process complexity, probably because of microtubule instability (Ren *et al.*, 2015).

Conclusion

In this review, we highlight the use of animal and cellular models to study neuropsychiatric disorders. By focusing on three genes that are linked to one or more psychiatric disorders, we have shown that comparing phenotypes

across different species can provide more convincing insights into the aetiology of these diseases. Examples of this include the link between *ADGRL3.1* and DA signalling, and *PARK2* and mitochondrial dysfunction. Despite the difficulties in recapitulating complex human diseases, animal models are vital to identify novel treatments. Moreover, the advent of hiPSC models may generate novel insights into pathophysiology as they can mirror the heterogeneous genetic background typical of ADHD and ASD in a manner that is difficult to achieve in animal models. The diversity of genetic variants associated with neurodevelopmental disorders including ASD may be explained by their downstream convergence on biological processes such as synapse development and neurotransmission (Sanders, 2015). In the future, additional hiPSC models and more complex animal models of ASD and ADHD coupled with stringent testing will be necessary to unravel the molecular mechanisms underlying these disorders and to develop novel treatments for them.

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Conflicts of interest

There are no conflicts of interest.

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