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PRIORITIZING THE DEVELOPMENT OF MOUSE MODELS FOR CHILDHOOD BRAIN DISORDERS

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Abstract

Mutations in hundreds of genes contribute to cognitive and behavioral dysfunction associated with developmental brain disorders (DBDs). Due to the sheer number of risk factors available for study combined with the cost of developing new animal models, it remains an open question how genes should be prioritized for in-depth neurobiological investigations. Recent reviews have argued that priority should be given to frequently mutated genes commonly found in sporadic DBD patients. Intrigued by this idea, we explored to what extent “high priority” risk factors have been studied in animals in an effort to assess their potential for generating valuable preclinical models capable of advancing the neurobiological understanding of DBDs. We found that in-depth whole animal studies are lacking for many high priority genes, with relatively few neurobiological studies performed in construct valid animal models aimed at understanding the pathological substrates associated with disease phenotypes. However, some high priority risk factors have been extensively studied in animal models and they have generated novel insights into DBD patho-neurobiology while also advancing early pre-clinical therapeutic treatment strategies. We suggest that prioritizing model development toward genes frequently mutated in non-specific DBD populations will accelerate the understanding of DBD patho-neurobiology and drive novel therapeutic strategies.

Keywords

Autism; Intellectual disability; Epilepsy; Schizophrenia; Syngap1; synapse; mouse model; development

1. Introduction

Developmental brain disorders (DBDs) can be broadly defined as diagnosable neuropsychiatric conditions resulting from abnormal brain function occurring in early life. Common DBDs include intellectual disability (ID), autism spectrum disorder (ASD), severe childhood epilepsies, and schizophrenia (SCZ). There are few if any effective treatments for the most serious symptoms associated with these disorders. This is particular true for

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cognitive deficits, which underlie poor learning and decision-making, and are one of the many driving factors leading to poor societal integration and productivity of affected individuals. Discovery and basic understanding of common disease-related substrates requires a major, concerted effort shared among clinician-scientists, neurobiologists and funding agencies worldwide. In order to improve the lives of affected individuals, it is essential to first understand the neurobiological substrates that drive reduced cognitive ability and then develop novel therapeutic strategies based on these basic preclinical advances.

DBDs have significant symptomatic overlap (Maski et al., 2011), particularly in the cognitive domain, including poor learning and memory, reasoning, and decision-making skills, suggesting that these abnormalities are caused to some extent by common patho-neurobiological substrates. Recent genomic sequencing studies from genetically undefined DBD patient cohorts have uncovered a subset of loci that are both recurrently mutated and causally-associated with a range of low-functioning patients with developmental delay and/or reduced cognitive ability (de Ligt et al., 2012; Deciphering Developmental Disorders, 2015; Epi et al., 2013; Hoischen et al., 2014; Iossifov et al., 2014; Krumm et al., 2014; Zhu et al., 2014). These clinical findings have shown that damaging mutations in the same gene can lead to distinct neurological or psychiatric disorders. Therefore, development and subsequent in-depth disease-focused neurobiological studies in animal models based on these highly-penetrant genetic loci present an exciting opportunity to identify critical neurobiological substrates that drive cognitive abnormalities commonly found in DBD populations.

2. Moving beyond traditional models of DBDs

Genetic mutations play a significant role in the etiology of cognitive abnormalities observed in DBDs (Mitchell, 2011; Ropers, 2008). For instance, some of the most common causes of ID and ASD are caused by either inherited *or de novo* mutations in a variety of genetic loci that result in a strong phenotype, which in-turn results in a clinically identifiable syndrome. Common syndromic single-gene DBDs includes Fragile X syndrome, Rett syndrome and neurofibromatosis type 1. These syndromes are usually first identified by morphological characteristics with or without some form of cognitive or behavioral phenotype. However, these syndromes with broad phenotypic features are associated with high rates of ID, ASD and epilepsy. Over the last two decades, there has been a concerted effort by the research community to understand the patho-neurobiology of *FMRI*, *MECP2* and *NF1* mutant animals, which model Fragile X, Rett and neurofibromatosis syndromes, respectively. Preclinical studies in construct valid animal models of these strong syndromic disorders have dramatically advanced our understanding of the neurobiological defects caused by gene disruptions associated with syndromic DBDs (Bear et al., 2004; Ehninger et al., 2008; Zoghbi and Bear, 2012). Indeed, in-depth modeling of these syndromic disorders in both invertebrate and vertebrate species has uncovered robust patho-neurobiological abnormalities at the molecular, cellular and circuit level. Commonly reported abnormalities in these animal models include disrupted protein translation, abnormal synaptic plasticity, E/I imbalances, and dendritic spine pathologies. These neurobiological abnormalities have been hypothesized as possible substrates that generally contribute to reduced cognitive

ability, social deficits, and behavioral abnormalities across the spectrum of DBDs. A discussion of the literature identifying these substrates in DBD animal models is beyond the scope of this review as this topic has been discussed extensively elsewhere (Castro et al., 2013; Ebrahimi-Fakhari and Sahin, 2015; Krueger and Bear, 2011; Smith and Ehlers, 2012; Volk et al., 2015; Willsey and State, 2015; Zoghbi and Bear, 2012).

There is a strong correlation between pharmacological or genetic improvement of these cellular and molecular substrates and improvement in behavioral measures of sociability and cognition in mouse models (Clement et al., 2012; Deidda et al., 2015; Ehninger et al., 2008; Guy et al., 2007; Molosh et al., 2014; Ozkan et al., 2014). These past findings demonstrate that mouse modeling based on genetic risk factors is a robust approach for understanding the basic neurobiology of developmental brain disorders. Discovery biology elucidating the etiology of ID and ASD has largely been driven by studies in these above-mentioned syndromic DBD models. However, the syndromes modeled by these mutant animals account for fewer than 10% of all ID or ASD cases (even fewer cases of epilepsy or SCZ). In addition, the level of cognitive and behavioral disruptions in patients with these syndromes span a wide spectrum, with some patients severely affected while others exhibiting relatively mild cognitive or behavioral alterations. Thus, it remains an open question to what extent the substrates uncovered in these popular mouse models of these syndromes apply to non-specific DBDs, which account for nearly half of all cases. Therefore, continued model development is needed in order to further test existing preclinical hypotheses of DBD neurobiology. New animal model development may also reveal unexpected cell, molecular and circuit-based mechanisms associated with cognitive and behavioral disruptions commonly observed in DBDs.

Nearly half of all ASD and ID cases are non-specific (Mefford et al., 2012). These children with developmental delay lack strong chromosomal, metabolic or phenotypic features that would enable a syndromic diagnosis. It is now believed that rare *de novo* mutations in autosomal genes cause a substantial fraction of non-specific DBD cases (de Ligt et al., 2012; Epi et al., 2013; Iossifov et al., 2014; Kenny et al., 2014; Krumm et al., 2014; O'Roak et al., 2011; O'Roak et al., 2014; O'Roak et al., 2012; Rauch et al., 2012; Samocha et al., 2014). Indeed, genome-wide association studies combined with a recent push to apply modern DNA sequencing technology to patients suggests that there are more than 500 genetic risk factors believed to contribute risk to a DBD diagnosis (Hoischen et al., 2014; Krumm et al., 2014; Willsey and State, 2015; Zhu et al., 2014). Genetic damage within these loci is believed to disrupt neurodevelopmental processes leading to cognitive and behavioral phenotypes commonly observed in patients. With the identification of so many genetic risk factors contributing to DBDs, it can be reasonably argued that there have been a disproportionately large number of disease-oriented preclinical studies in a relatively small sample of construct valid animal models. To this end, developing new models based on clinically-relevant genetic risk factors will likely lead to a better understanding of the patho-neurobiology underlying DBDs.

3. An approach to identify high-priority genetic risk factors for new model development

The sheer number of risk factors available for in-depth study represents an exciting opportunity for the community to better understand the relationship between genetics and the emergence of cognition and behavior by studying these risk factors in animal models. However, it is expensive and time consuming to perform animal-level studies, particularly in rodents, meaning that there are not enough resources to perform in-depth, animal-based studies for all risk factors. In order to deal with the limited research resources available, there must be priority assigned to a subset of clinically relevant risk factors. How can one prioritize the development of new disease models in order to determine, in an unbiased way, the most promising patho-neurobiological substrates contributing cognitive deficits seen in DBDs? We propose that new DBD model development would ideally be focused on risk loci that are: 1) causally-linked to severe, non-specific, and sporadic DBDs; 2) frequently found in these patient cohorts; 3) causally-linked to several types of DBDs; 4) not commonly found in healthy individuals; and 5) have frequent, but distinct, mutations that cause common disruptions to gene function (e.g. nonsense truncating mutations and missense mutations that clearly disrupt core protein functions). That last point is especially salient because it directly addresses the costs associated with modeling disease-linked genes in higher vertebrates, such as mice. Indeed, it would be very costly to introduce every single disease-associated point mutation for a given frequently mutated gene into a mouse model because each mouse line requires substantial resources for proper characterization. Thus, genes with a frequent number of nonsense mutations or clear missense mutations that disrupt key functional domains could all be modeled with construct validity by using a conventional or conditional knockout (KO) mouse; heterozygous KO mice could be used in the case of autosomal genes. The above-mentioned conditions are met by only a handful of DBD risk factors. These so called “high priority” candidate genes have garnered significant attention lately and it was argued in a series of recent review articles that genes fitting similar criteria should be prioritized for further clinical study because they represent loci that have profound effects on human brain function (Hoischen et al., 2014; Zhu et al., 2014).

An increasing number of non-specific DBD patients are being genetically screened in an attempt to improve diagnostic yield (de Ligt et al., 2012; Deciphering Developmental Disorders, 2015; Epi et al., 2013; Redin et al., 2014). As a result, there have now been thousands of patients (plus parents and siblings) screened across different continents. In order to generate an updated list of high priority DBD risk factors for disease modeling at the preclinical level, we identified commonly mutated genes that appeared in four large-scale studies or databases. In order to be included in this updated list, a gene must have appeared in at least two of the four sources, with the basic assumption that the most penetrant disease-linked genes would commonly appear in multiple large cohort diagnostic screening studies. The genes on this priority list (Table 1) are ranked based on the number of overlapping sources, with a gene being ranked the highest if it appeared in all four sources (e.g. *SCN2A*, *SYNGAP1*). Source 1 combines gene- and variant-level prioritization scores to test for the presence of *de novo* mutations that confers risk in patient genomes. They found that the genes that are intolerant to functional genetic variation are much more likely to

cause neurodevelopmental disorders (Zhu et al., 2014). The Simons Foundation Autism Research Initiative (SFARI) Gene database (Source 2) also developed a gene scoring system specifically to prioritize gene that are implicated in ASDs. They use a rigorous statistical comparison between cases and controls, yielding genome-wide statistical significance, with independent replication to be the strongest possible evidence for a gene that is classified as either category 1 or category 2 (<https://gene.sfari.org>). The Deciphering Developmental Disorders Study (Source 3) is a large consortium collecting DNA and clinical information from over 12,000 undiagnosed children and adults in the UK with developmental disorders and their parents (Deciphering Developmental Disorders, 2015). Their initial results with 1,133 children with severe, genetically undefined developmental disorders identified several genes that are recurrently mutated in ID and severe epilepsy. The final source, Source 4, includes results from Eichler and colleagues (Hoischen et al., 2014), who focused on 2,368 *de novo* mutations from a total of 2,358 probands and 600 *de novo* mutations from 731 controls, and they identified 8 genes that are recurrently and commonly mutated in neurodevelopmental disorders. In each of these studies, patients screened were principally diagnosed with non-specific developmental delay, with varying diagnoses of ID, ASD and/or severe forms of childhood epilepsy.

We chose these four sources because they largely examined the role of rare *de novo* mutations in sporadic cases (e.g. mutations not present in parents and/or siblings). It has now been established through extensive replication sequencing studies in distinct patient cohorts diagnosed with various forms of non-specific DBDs that rare, pathogenic *de novo* mutations account for a substantial portion of these cases, such as ASD, ID and SCZ, and severe childhood epilepsies (de Ligt et al., 2012; Epi et al., 2013; Fromer et al., 2014; Iossifov et al., 2014; Kenny et al., 2014; Krumm et al., 2014; O'Roak et al., 2011; O'Roak et al., 2014; O'Roak et al., 2012; Purcell et al., 2014; Rauch et al., 2012; Samocha et al., 2014). Thus, this priority list represents a subset of highly penetrant genes that are expected to dramatically influence brain development and function. Importantly, a priority list based on variants identified from these sources weighs the power of a given risk factor gene to contribute to disease based primarily on its clinical impact rather than its known gene function. Therefore, this strategy for prioritizing genes for pre-clinical modeling studies in animals will provide unbiased investigations into the patho-neurobiology of DBDs. Such unbiased investigations will be important to determine how previously identified disease-associated substrates are affected in animals models based on high priority risk factors, which will afford better understanding of the cellular and molecular convergence among DBDs with cognitive deficits. It will be of equal importance to understand how genetic variants differentially impact neurobiological substrates and brain function. It is likely that the most effective treatment strategies for spectrum disorders will be to employ a combination of therapies that target both commonly affected substrates, as well as unique substrates known to be influenced by a particular genetic variant.

4. High priority loci are relatively understudied at the whole-animal level

Our analysis resulted in 21 high-impact risk factors (Table 1) and they are ranked based on the number of overlapping sources and include the types of neuropsychiatric disorders associated with each gene. This list includes genes whose function is critical for synapse

regulation (*STXBP1*, *SYNGAP1*, *NRXN1*, *GRIN2B*, *GABRB3*), voltage-gated ion channels (*SCN2A*, *SCN1A*, *SCN8A*) and chromatin modifiers (*ARID1B*, *CHD2*, *SUV420H1*). Interestingly, there were a few genes whose function is not well understood, such as *POGZ*, *SETD5*, and *PTCHD1*. For all but one of these genes, *POGZ*, a KO mouse has been made. Of the 20 genes with a KO mouse made, 16 (80 %) are homozygous lethal. In hindsight, this observation is not surprising considering that recent clinical genetic findings indicating that these genes are casually linked to severe disorders of human brain development and function (Deciphering Developmental Disorders, 2015; Hoischen et al., 2014; Zhu et al., 2014). It is important to note that certain known high risk factors are absent from our priority list. The sources that we used for this analysis focused on children with non-specific developmental delay and at the time of screening. Thus, all children lacked a specific diagnosis. As a result, by definition, children with strong clinically identifiable syndromes were usually excluded from these patient cohorts. Therefore, our priority list is enriched for genetic mutations that cause relatively non-specific morphological disruptions, such as subtle or inconsistent dysmorphic features, but highly specific disruptions to cognition and behavior.

We then surveyed the literature to gain a better idea of what is known with respect to how these genes regulate neurobiological processes at the whole animal level. In particular, we focused on in-depth neurobiological studies in construct valid animal models harboring disease-linked mutations from genes on this list. These genes are surprisingly understudied at the whole animal level relative to the more established syndromic DBD genes, such as *FMRI*, *MECP2* and *NF1*. As shown in Table 2, there are at least four genes (*POGZ*, *SUV420H1*, *ARID1B*, *PTCHD1*) where there is essentially nothing known about how a damaging mutation might impact brain function or behavior. For genes where some work has been performed in mice, it is unclear how learning, synaptic function or neural anatomy is impacted by construct valid mutations. These basic studies must first be performed before more disease-relevant and pharmacologically targetable substrates are explored. Therefore, it will be of considerable interest to understand how loss-of-function mutations in these genes affect known DBD substrates, such as learning and memory, social interaction, excitation-inhibition balance, dendritic spine morphogenesis, synapse plasticity, and protein translation. Models that demonstrate large effect sizes in these and similar measures have the potential to evolve into robust neurobiological tools to better understand the neurobiology of DBDs and to then test novel treatment strategies to improve cognition and behavior.

4.1 STXBP1

Most genes on this priority list have been studied to some extent at the whole animal level, but face validity is sparse for most of these mutant mice due to the lack of focus on neurobiological and behavioral studies in construct valid models. Thus, a clear understanding of how many of these genetic mutations impact neurobiology and behavior is lacking. This knowledge gap represents an exciting opportunity to gain further insight into DBD disease processes by studying highly penetrant disease-linked genes. *STXBP1* is one particularly striking example because the function of this gene is well understood at the molecular and cellular levels, yet it remains unknown how haploinsufficiency of this gene impacts circuit function and behavior. *STXBP1* is one of a handful of genes that is frequently

mutated across distinct DBD patient populations (Hoischen et al., 2014; Zhu et al., 2014). Early studies that investigated the contribution of autosomal dominant *de novo* mutations to sporadic ID unexpectedly found that *STXBPI* is one of the most commonly mutated genes in these patients (Hamdan et al., 2011b; Hamdan et al., 2009c; Rauch et al., 2012). Autosomal dominant *de novo* *STXBPI* mutations are perhaps even more common in severe childhood epilepsy syndromes that result in developmental delay or regression. *STXBPI* haploinsufficiency causes Ohtahara syndrome (Saito et al., 2008), a severe form of epileptic encephalopathy, as well as Dravet syndrome (Carvill et al., 2014), which is a distinct form of severe early-onset epilepsy with a high frequency of sudden death. The surprising frequency of *STXBPI* mutations in sporadic DBDs was confirmed recently by a large-scale study (Deciphering Developmental Disorders, 2015). In this large cohort of non-specific patients with developmental delay, *STXBPI* mutations accounted for ~0.5% of all undiagnosed cases, though frequency was far higher among patients with some form of epilepsy (~3%).

STXBPI encodes a fundamental protein, Munc18-1, which is necessary for neurotransmitter release at all central synapses. Munc18-1, or syntaxin-binding protein 1, was originally discovered for its essential contribution to neurotransmitter release through its binding to syntaxin (Garcia et al., 1994; Hata et al., 1993; Pevsner et al., 1994). The essential role of Munc18-1 for release of all neurotransmitter throughout the central nervous system was discovered when *Stxbp1* homozygous KO mice died immediately after birth, likely due to paralysis of the diaphragm, and synapses in the brain and neuromuscular junction showed no neurotransmission despite functional postsynaptic receptors (Verhage et al., 2000). Subsequent studies investigated the role of Munc18-1 in synaptic transmission in neurons cultured from *Stxbp1* heterozygous KO mice (Toonen et al., 2006) or in tissue isolated from embryonic *Stxbp1* homozygous KO mice (Bouwman et al., 2004; Voets et al., 2001). The heterozygous KO mouse, however, was not directly the subject of thorough investigation. Given that there are gene dosage effects of *Stxbp1* on synaptic transmission (Toonen et al., 2006) and that neurons lacking Munc18-1 die even when surrounding neurons are wild type (Dudok et al., 2011; Heeroma et al., 2004), *Stxbp1* heterozygous KO mice are likely to show disease-related phenotypes and in-depth studies could reveal important neurobiological substrates, some of which may be unrelated to the established role of Munc18-1 in neurotransmitter release. Indeed, it remains completely unknown how genetic haploinsufficiency of an essential synaptic release protein results in network instability leading to severe and sometimes fatal seizures. Furthermore, it remains unknown how similar damaging mutations in this gene can lead to ID with or without severe epilepsy. Here, in-depth studies of *Stxbp1* heterozygous KO mice could provide some much needed insight into the pathophysiology in humans with similar mutations. By using modern genetic tools in animal models of DBDs, in-depth experiments can dissociate the molecular, cellular and circuit-based mechanisms that distinguish hyperexcitability/seizure phenotypes from those more directly associated with cognitive processes (Ozkan et al., 2014). These types of studies are informative because epilepsy, ID and ASD are highly comorbid, though it remains unclear whether similar or distinct neurobiological substrates lead to these symptoms.

4.2 GRIN2B

GRIN2B is another example of a fundamental brain-expressed gene causally-linked to DBDs that has been understudied with respect to disease etiology. *GRIN2B* mutations were first found in patients with moderate to severe ID (Endele et al., 2010). Two patients were identified with *de novo* translocation breakpoints disrupting *GRIN2B*. This discovery motivated the targeted sequencing of *GRIN2B* in two ID patient cohorts, totaling 468 individuals. Four *de novo* mutations were found: two splice-site mutations, a missense mutation, and a frameshift mutations. All of these mutations were absent in 360+ controls. A follow-up study identified an additional patient with ID and ADHD harboring a *GRIN2B* missense mutation (Freunscht et al., 2013). In studies aimed at identifying rare *de novo* mutations in SCZ and ASD, *GRIN2B* was found among genes with an excess of rare missense mutations (Myers et al., 2011; Tarabeux et al., 2011). These findings were later corroborated by an independent targeted sequencing study with the goal of identifying rare risk mutations for ASD and SCZ, which found a *de novo* nonsense *GRIN2B* mutation in a patient with ASD out of 147 ASD cases and 273 SCZ cases (Kenny et al., 2014). Two large exome sequencing studies of sporadic ASD or ID cases found a total of 2 missense mutations, 1 insertion, 1 nonsense mutation, and 1 splice site mutation in *GRIN2B* (de Ligt et al., 2012; O'Roak et al., 2012). *GRIN2B* mutations can also cause different forms of epileptic encephalopathies, as one missense mutation was found in a patient with Lennox-Gastaut syndrome (Epi et al., 2013), and two missense mutations were found in two West syndrome patients (Lemke et al., 2014). As with *STXBPI*, the frequency of *GRIN2B* mutations was supported by the Deciphering Developmental Disorders study (Deciphering Developmental Disorders, 2015), which found a *de novo* missense mutation in a patient with global developmental delay, neonatal hypotonia, and delayed CNS myelination. In all, over 37 mutations have been discovered in *GRIN2B* from sporadic ASD, SCZ, ID, and epilepsy patients (Burnashev and Szepietowski, 2015; Yuan et al., 2015).

While heterozygous loss-of-function *GRIN2B* mutations are commonly found in ID and ASD patients, it is unclear how these mutations result in brain dysfunction even though this gene is relatively well studied at the cellular level. *GRIN2B* encodes the GluN2B subunit of NMDA receptors, which are glutamate receptors essential for selection of appropriate neuronal connections during brain development and for initiating Hebbian forms of synaptic plasticity in mature circuits (Traynelis et al., 2010). Much of what is known about NMDAR function has occurred through selective antagonists or genetic deletion in particular subtypes of neurons at various times in development. However, very few studies have focused on the specific contributions of *GRIN2B* to disease-related phenotypes and neurobiological substrates that are disrupted by *GRIN2B* haploinsufficiency. Additionally, *Grin2b* homozygous KO mice die perinatal because they don't learn to suckle (Kutsuwada et al., 1996), and this observation has purportedly hampered many investigations into GluN2B function. To circumvent the perinatal lethality many clever approaches have been used, including culturing neurons from embryonic mice (Tovar et al., 2000), genetically replacing GluN2B with GluN2A (Wang et al., 2011), or using conditional *Grin2b* KO mice crossed with Cre driver mice to selectively ablate GluN2B in defined cell types (Badanich et al., 2011; von Engelhardt et al., 2008). However, regardless of the approach to circumvent the perinatal lethality, heterozygous KO of *Grin2b* has been largely ignored, which is

unfortunate because these mice represent a construct valid model for a subset of patients with severe cognitive and behavioral abnormalities. Given that overexpressing *GluN2b* in mice enhances learning and memory, there is likely a gene dosage effect of GluN2B, and the heterozygous KO mice will probably show interesting disease-related phenotypes. Indeed, *Grin2b* heterozygous KO mice show increased sensitivity to electrical, thermal, and mechanical noxious stimuli (Wainai et al., 2001), elevated acoustic startle (Takeuchi et al., 2001), and failed to learn in delay and trace associative eye-blink conditioning (Takehara et al., 2004). However, there are few studies that have addressed the cellular and molecular consequences of *GRIN2B* haploinsufficiency on mouse brain development and how these alterations may drive cognitive and behavioral abnormalities. Considering the impact that NMDAR function has on synapse biology and brain development, in-depth neurobiological studies in this model will likely greatly advance our understanding of how synapse dysfunction contributes to cognitive and behavioral abnormalities associated with childhood brain disorders.

5. Disease-oriented studies in animal models with mutations in high priority risk factors

While a surprising number of high priority DBD genes are relatively understudied, at least two genes on this list, such as *SYNGAP1* and *SCN1A*, have been extensively studied through the development of construct valid animal models. These animal studies were designed specifically to better understand how pathogenic mutations in these genes could impact brain development and function. These models of rare monogenic DBDs display very strong molecular, cellular and behavioral phenotypes that have contributed to the neurobiological understanding of the DBD disease process (*discussed in Sections 5.1 and 5.2*). In addition, the strong face valid phenotypes exhibited by mice with disease-relevant mutations suggest that these animals will serve as valuable translation tools to test novel therapeutic hypotheses related to DBDs. These “success stories” suggest that further in-depth studies in other high priority genetic risk factors will result in similarly strong phenotypes when modeled in mice or related species, which should further accelerate the neurobiological understanding of DBDs.

5.1: Mouse Modeling of SYNGAP1-related DBDs

SYNGAP1 is one of the more heavily studied genes on this priority list, particularly with respect to focused, disease-oriented studies that seek a neurobiological understanding of how autosomal dominant genetic haploinsufficiency can disrupt cognitive maturation during early postnatal periods of brain development. Damaging *SYNGAP1* mutations are commonly found in patients with DBDs ranging from severe ID to SCZ. *De novo* truncating mutations in *SYNGAP1* leading to genetic haploinsufficiency were initially discovered in relatively small cohorts (~200 patients) of genetically undefined intellectual disability patients (Berryer et al., 2013; Hamdan et al., 2011a; Hamdan et al., 2009a). These initial studies suggested that *SYNGAP1* mutations account for ~2–8% of unexplained ID cases. Importantly, other groups using similarly sized ID patient cohorts observed very similar results, with truncating *SYNGAP1* mutations present in ~2% of patients (de Ligt et al., 2012; Rauch et al., 2012), suggesting that truncating *SYNGAP1* mutations are one the more

common causes of non-specific forms of ID. Consistent with the relatively high frequency of *SYNGAP1* disruptions in these ID patients, there are now frequent reports of *SYNGAP1* mutations in patients with unexplained moderate-to-severe ID (Klitten et al., 2011; Krepischi et al., 2010; Redin et al., 2014; Samocha et al., 2014; Writzl and Knecht, 2013). The general prevalence of pathogenic *SYNGAP1* mutations in patients with a developmental disorder was recently supported by a large-scale UK study seeking genetic causes of unexplained DBD cases (Deciphering Developmental Disorders, 2015). This study identified *SYNGAP1* as one of the top 5 recurrently mutated genes in patients with genetically undefined DBDs, with 7 confirmed cases out of ~1000 subjects, and no pathogenic *SYNGAP1* mutations in >1000 controls. Damaging *SYNGAP1* mutations are also causally associated with other neuropsychiatric disorders. Several groups have found damaging *SYNGAP1* mutations or copy number variations in ASD patients (De Rubeis et al., 2014; Iossifov et al., 2014; O'Roak et al., 2014; Pinto et al., 2010). Based on these studies, and along with other lines of evidence, SFARI has listed *SYNGAP1* as a Type 1 ASD risk factor. Damaging *de novo* *SYNGAP1* mutations were also recently found in a very large cohort of SCZ patients (Purcell et al., 2014). Finally, several patients with idiopathic epileptic encephalopathy, which is a devastating form of infantile epilepsy, were found to have *de novo* truncating *SYNGAP1* mutations (Carvill et al., 2013).

There have been extensive neurobiological studies carried out in *Syngap1* mutant mice. *Syngap1* homozygous KO mice die shortly after birth (Kim et al., 2003; Knuesel et al., 2005; Komiyama et al., 2002). However, several groups have performed behavioral endophenotyping of *Syngap1* heterozygous KO mice, which offer construct validity for *SYNGAP1*-related ID because this is an autosomal dominant haploinsufficiency disorder (Berryer et al., 2013; Hamdan et al., 2009a; Parker et al., 2015). *Syngap1* heterozygous mice have strong behavioral phenotypes, with deficits in spatial learning, anxiety and risk taking, working memory, stereotypies, horizontal activity, acoustic startle, PPI, social interactions, and emotional learning (Clement et al., 2012; Guo et al., 2009; Komiyama et al., 2002; Muhia et al., 2010). Importantly, various investigators using independently generated mutant lines have replicated the core behavioral phenotypes characteristic of these mice. SynGAP, which is encoded by *SYNGAP1/Syngap1*, is a neuronal RasGAP that is targeted to dendritic spines where it regulates AMPA receptor trafficking and synaptic function. In *Syngap1* heterozygous KO mice, synapses form and mature at abnormal rates and there are significant alterations in both hippocampal and cortical forms of LTP (Clement et al., 2013; Kim et al., 2003; Komiyama et al., 2002). Together with the behavioral abnormalities, these findings demonstrate that *Syngap1* heterozygous KO mice offer significant face validity for the corresponding human disorder. Face validity is further strengthened by the discovery of similar interictal EEG patterns in both *Syngap1* non-syndromic ID patients and *Syngap1* heterozygous KO mice (Berryer et al., 2013; Ozkan et al., 2014) and a zebrafish model of *Syngap1* disruptions also show phenotypes consistent with circuit hyperexcitability (Kozol et al., 2015). Interestingly, direct measurements in construct valid *Syngap1* mice demonstrate robust excitation-inhibition imbalances in various types of forebrain neurons (Clement et al., 2012; Ozkan et al., 2014) and reduced *Syngap1* function also leads to altered neuronal protein synthesis (Wang et al., 2013), which are two common neurobiological substrates believed to generally contribute to DBDs. These findings in construct valid

Syngap1 mutant mice strengthen the idea that substrates first discovered in syndromic DBD models may generally contribute to DBD patho-neurobiology. In addition, due to the strong and diverse phenotypes present in *Syngap1* animals, these results also support the notion that basing DBD model development on the frequency of mutations observed in clinical populations is a promising approach for risk factor prioritization.

Studies in construct valid *Syngap1* mouse models have uncovered unexpected neurobiological defects that may lead to the identification of novel substrates underlying DBDs. The core cognitive and behavioral deficits in *Syngap1* heterozygous KO mice are believed to be driven by widespread disruptions to synapse maturation rates, dendritic spine pruning, and synapse plasticity occurring across different areas of the forebrain at different times in development (Clement et al., 2012; Clement et al., 2013; Ozkan et al., 2015; Ozkan et al., 2014). These findings have implications with respect to the understanding of how neural circuits form, refine, and function during postnatal brain development and how these essential developmental processes instruct cognitive maturation. Recent studies that varied the spatial and temporal induction and rescue of construct valid *Syngap1* mutations revealed that cellular and synaptic deficits that mark a restricted sensitive period of *Syngap1* function in the developing forebrain are causally associated with the onset of persistent cognitive and behavioral abnormalities commonly observed in these mice (Aceti et al., 2015; Clement et al., 2012; Ozkan et al., 2014). Due to the strong linkage of a defined postnatal sensitive period for *Syngap1* function to the emergence of cognitive ability, combined with the relatively strong measurable synaptic phenotypes that occur in these animals, *Syngap1* mutant mouse lines have emerged as a robust model for understanding how critical periods of postnatal development act to sculpt the emergence of cognitive ability as animals mature. Abnormal synapse dynamics during development are believed to be an important substrate for the onset of behavioral and cognitive abnormalities in various neuropsychiatric disorders (Penzes et al., 2011). Other lines of evidence suggest that disruptions to critical periods of brain development contribute importantly to the DBD disease process (LeBlanc and Fagiolini, 2011; Meredith, 2015). Furthermore, abnormal local- and long-distance connectivity is believed to underlie functional deficits in DBDs (Broyd et al., 2009; Just et al., 2004). Therefore, neurobiological discoveries made in *Syngap1* mice, such as the identification of subsets of circuits that are particularly sensitive to postnatal development perturbations, may represent novel, and perhaps common, substrates of brain dysfunction generally related to DBDs.

Findings in construct valid *Syngap1* mice may inform new strategies for treating DBDs. *SYNGAP1*-related disorders are a type of RASopathy, which are human disorders defined by dysregulation of Ras signaling. SynGAP, which is a synapse-targeted RasGAP (Chen et al., 1998; Kim et al., 1998), functions to suppress Ras-ERK signaling generally in neurons (Komiya et al., 2002; Rumbaugh et al., 2006) and specifically in dendritic spines (Ozkan et al., 2014). Interestingly, hippocampal LTP deficits in *Syngap1* heterozygous KO mice are strongly linked with elevated baseline Ras/ERK signaling. Genetic reversal of pathogenic *Syngap1* mutations rescues synaptic plasticity defects while at the same time restoring Ras/ERK signaling in dendritic spines to baseline levels (Ozkan et al., 2014), suggesting that restoring the integrity of this signaling pathway may be therapeutically beneficial in animal

models. Experiments that test the impact of Ras/ERK pathway regulators on cognition and behavior in *Syngap1* heterozygous KO mice have yet to be conducted. However, this strategy has been successful in other mouse models of ID characterized by elevated Ras/ERK signaling in neurons. In pioneering studies by the Silva group, pharmacological regulators of the Ras/ERK pathway, including the FDA-approved statin lovastatin, were able to normalize enhanced ERK signaling while also improving cognitive performance in a mouse model of neurofibromatosis type 1 (Costa et al., 2002; Cui et al., 2008; Li et al., 2005), a RASopathy associated cognitive abnormalities. Similar Ras/ERK-related findings were observed in a mouse model of Noonan syndrome (Lee et al., 2014), which is a distinct RASopathy associated with ID. Statins have been tested in clinical trials for efficacy in children with neurofibromatosis type I (Krab et al., 2008; van der Vaart et al., 2013). While statins did not improve cognitive function in patients in these trials, there were some promising signs with respect to certain secondary endpoints related to cognitive ability in one of the studies (Krab et al., 2008). Interestingly, many trial enrollees were in their teens, suggesting that Ras/ERK-related developmental critical periods may have been closed in some subjects, which could have limited the therapeutic potential of this therapy. *Syngap1* mice have disrupted postnatal critical periods marked by robust and widespread behavioral disruptions, some of which emerge early in development (Clement et al., 2012; Guo et al., 2009). These mice also have developmental disruptions in forebrain synaptic function and connectivity of circuits that emerge over a similar time course of postnatal development (Aceti et al., 2015; Clement et al., 2012; Clement et al., 2013; Ozkan et al., 2014). Therefore, *Syngap1* mice can be viewed as an attractive model to test how pharmacological normalization of Ras/ERK signaling deficits in young mice influence damaged critical periods and altered neuronal connectivity. The hope is that transient pharmacological treatments initiated before the closure of forebrain critical periods of synaptogenesis will lead to a sustained improvement in brain function over the life of *Syngap1* mice.

5.2: Mouse Modeling of SCN1A-related DBDs

SCN1A is one of the most commonly mutated genes in epilepsy. The link between *SCN1A* and epilepsy was first discovered when two *SCN1A* missense mutations were found in two families with generalized epilepsy with febrile seizures plus (GEFS+) type 2 (Escayg et al., 2000). Subsequently, more than 20 *SCN1A* mutations were found in patients with GEFS+, and *SCN1A* mutations together accounted for about 10% of GEFS+ cases (Claes et al., 2009; Lossin, 2009). Shortly after the discovery of *SCN1A* mutations in GEFS+, *SCN1A* mutations were identified in patients with Dravet Syndrome, or severe myoclonic epilepsy of infancy (Claes et al., 2001). Over the next decade, many targeted sequencing studies of patients with Dravet syndrome revealed that about 80% of Dravet syndrome cases were caused by *SCN1A* mutations (Claes et al., 2009; Lossin, 2009), and unexpectedly, most of these were *de novo*. Additionally, *SCN1A* mutations were discovered in a range of other epilepsies with varying degrees of severity, including febrile seizures, and epileptic encephalopathies (Harkin et al., 2007). As of 2011, over 600 mutations had been reported in the literature (<http://www.molgen.ua.ac.be/SCN1AMutations/home/Default.cfm> and <http://www.scn1a.info/>), mostly from targeted sequencing of patients with GEFS2+ or Dravet syndrome. The clinical relevance of *SCN1A* mutations continued to be uncovered in 11 recent large-scale exome sequencing studies of patients with sporadic developmental brain disorders (reviewed in

(Hoischen et al., 2014; Zhu et al., 2014)). In these studies, 9 *SCN1A* mutations were identified: 1 in ASDs and 8 in epileptic encephalopathies. Further, in the Deciphering Developmental Disorders study (Deciphering Developmental Disorders, 2015), 2 *SCN1A* mutations, one missense and one copy number variation, were found in children with severe, undiagnosed developmental disorders. Altogether, many clinical sequencing studies of diverse cohorts of patients with epilepsy or other DBDs have revealed the clinical importance and relatively frequent occurrences of *SCN1A* mutations.

Several construct valid models for *SCN1A*-related DBDs have been created, including conventional and conditional heterozygous KO mice, and several heterozygous knock-in mice containing mutations found in humans with epilepsy. Conventional homozygous KO mice develop ataxia and die before P15, and conventional heterozygous KO mice spontaneously seized and unexpectedly died started after P21 (Yu et al., 2006). Similarly, homozygous and heterozygous knock-in mice for a loss-of-function nonsense mutation -- R1407X (found in three unrelated Dravet syndrome patients) -- developed seizures in the first postnatal month (Ogiwara et al., 2007). Homozygous knock-in mice with the human GEFS+ mutation *Scn1a*-R1648H spontaneously seize and die between P16–26, and heterozygous R1648H knock-in mice have reduced threshold and accelerated propagation of febrile seizures, and decreased flurothylinduced seizures (Martin et al., 2010). Hence, mice with *Scn1a* missense mutations found in patients and *Scn1a* heterozygous KO mice have similar phenotypes, suggesting that the later may be a model worthy of further in-depth study because these results could be extrapolated to other mutations linked to protein loss of function.

Scn1a mouse models have led to a better understanding of severe epilepsy syndromes while also driving the development of novel treatment strategies. *SCN1A* codes for the neuronal voltage-dependent sodium channel Na_v1.1, and the effects of pathogenic *SCN1A* mutations on sodium channel function were initially studied at the biophysical level, usually in heterologous expression systems (Mulley et al., 2005). Although these studies identified some detailed changes in *Scn1a* function, it was not immediately clear how these changes could lead to hyperexcitability (Mulley et al., 2005). When *Scn1a* heterozygous KO mice were examined, however, a framework emerged in which to interpret the damaging mutations. Specifically, interneuron excitability was disproportionately impaired in *Scn1a* heterozygous KO mice. Sodium currents in inhibitory interneurons from *Scn1a* heterozygous KO mice were reduced, though sodium currents in excitatory pyramidal neurons were not (Martin et al., 2010; Ogiwara et al., 2007; Yu et al., 2006). Mice with homozygous deletion of *Scn1a* restricted to only forebrain GABAergic neurons died prematurely due to tonic-clonic seizures (Cheah et al., 2012), further supporting the importance of inhibitory interneurons to the pathophysiology caused by pathogenic *SCN1A* mutations. Restricting heterozygous KO to forebrain GABAergic neurons was sufficient to cause seizures and phenocopy behavioral deficits observed in conventional heterozygous KO mice (Han et al., 2012). Indeed, restricting haploinsufficiency to GABAergic neurons caused the seizures to be worse than global heterozygous KO, whereas *Scn1a* heterozygous KO in only excitatory cells caused no seizure phenotype (Dutton et al., 2013; Ogiwara et al., 2013). Furthermore, treating adult *Scn1a*+/- mice with the benzodiazepine clonazepam

rescued the social behavior and spatial learning deficits (Han et al., 2012). Together these data provide evidence for the hypothesis that forebrain GABAergic interneurons are the sensitive cell type mediating *Scn1a* pathophysiology and that *Scn1a* functions to maintain circuit function throughout life. Importantly, these studies in construct valid *Scn1a* mouse models have impacted treatment of patients with *SCN1A* mutations (<http://www.ncbi.nlm.nih.gov/books/NBK1318/>). The finding that seizures in *Scn1a* heterozygous KO mice were likely due to impaired sodium channel function in GABAergic interneurons leading to an overall decrease in inhibition, also informed treatment of seizures in people with *SCN1A* mutations. Sodium channel blockers such as phenytoin and carbamazepine would exacerbate hyperexcitability and should therefore be avoided. Studies of the heterozygous KO mice also showed that treatments that directly increase GABA receptor function, such as clonazepam, would be particularly effective in patients with *SCN1A* mutations. Additionally, the robust animal models of *SCN1A*-related DBDs can serve as tools for translational research, as demonstrated recently in experiments with zebrafish in which one copy of the *SCN1A* ortholog was knocked out. A screen of FDA-approved compounds in these *scn1a*-deficient zebrafish found that clemizole, an H1 antihistamine, inhibited behavioral and electrographic seizures and could serve as a potential therapeutic agent in humans with *SCN1A* mutations (Baraban et al., 2013).

6. Conclusions

Large-scale whole-exome sequencing studies in idiopathic and sporadic cases of ASD, ID, epilepsy and SCZ within the past decade have delivered breakthrough discoveries of new genetic risk factors for DBDs. With over 500 loci now reported, these emerging risk factors need to be prioritized for study in order to maximize the possibility of generating robust models of human neurodevelopmental disorders. Such robust models are optimally suited for discovery of shared neurobiology across DBDs, for discovery of potential biomarkers that relate to human phenotypes, and to serve as translational tools to test new therapies. Basing models on highpriority risk factors identified from frequently mutated genes found in the clinic will likely result in high-quality models capable of advancing the neurobiology of DBDs.

While studies using animal models of classically syndromic DBDs have discovered candidate neurobiological substrates underlying cognitive deficits, the extent to which these substrates are shared across DBDs is not well known. Indeed, each genetic cause of DBDs may have unique features that are important for pathophysiology. As a complement to genetic causes of classically syndromic DBDs, we have highlighted 21 high-impact genetic risk factors that are commonly mutated in several DBDs. In-depth studies with construct valid mouse models of high-impact genetic risk factors such as these will likely uncover both convergent and divergent neurobiological mechanisms associated with these disorders. In turn, these principles could inform new therapeutic strategies for DBDs. Moreover, mouse models of genes on this list have the potential for strong face valid phenotypes, which could serve as endpoints for efficacy testing of therapeutics. Progress in understanding the patho-neurobiology underlying DBDs will require a concerted effort by multiple labs, studying these risk factors at multiple levels (molecular, cellular, circuit,

systems). Therefore, development and subsequent in-depth study of mouse models based on high priority risk factors should be an important goal for the neuroscience community.

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Highlights

1. Over 500 genetic risk factors for developmental brain disorders (DBDs) exist
2. Too few risk factors have been studied in-depth using mouse models
3. Resource constraints require prioritizing genes for in-depth study
4. We made a list of high priority genes that can serve as a new generation of models
5. Successes from this list suggest modeling these genes will advance the DBD field

Table 1

Twenty-one genes commonly mutated across DBDs

We compiled a list of genes in which mutations were discovered in patients with diverse DBDs. We tallied genes from four different sources: Hoischen et al. (2014), Petrovski et al. (2014), the SFARI gene database (<https://gene.sfari.org>), and the Deciphering Developmental Disorders Study (2015), and included genes that were found in at least two of these sources.

Number of Overlapping of Sources	Gene Symbol	Location	Zhu et al (Zhu et al., 2014)	SFARI	DD Project (Deciphering Developmental Disorders, 2015)	Hoischen et al, 2014 (Hoischen et al., 2014)	ASD	ID	EE	SC	Gene Function
4	SCN2A	Chr2 q24.3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Alpha II subunit of voltage gated sodium channels. Nav α 1.2
4	SYNGAP1	Chr6 p21.3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Neuronal specific GTPase activating protein that regulates ERK signaling
3	GABRB3	Chr15 q12	Yes	Yes	No	Yes	Yes	No	Yes	No	GABA _A Receptor β 3
3	SCN1A	Chr2 q24.3	Yes	No	Yes	Yes	Yes	Yes	Yes	No	α 1.1 subunit of voltage gated sodium channels, Nav1.1
3	STXBP1	Chr9 q34.1	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Munc18-1, binds and regulates syntaxin in neurotransmitter release.
2	POGZ	Chr1 q21.3	No	Yes	No	Yes	Yes	Yes	No	Yes	No known neuronal function, pogo transposable element with ZNF domain
2	SUV420H1	Chr11 q13.2	Yes	Yes	No	No	Yes	No	No	No	Histone H4 lysine 20 methyltransferase. Roles in myogenesis. Epigenetic regulator. "The function of this gene has not been determined."
2	GRIN2B	Chr12 p12	Yes	Yes	No	No	Yes	No	Yes	No	GluN2B subunit of NMDA receptors
2	SCN8A	Chr12 q13	Yes	No	Yes	No	No	Yes	Yes	No	α 8 subunit of voltage gated sodium channels, Nav1.6
2	CHD2	Chr15 q26	No	Yes	Yes	No	Yes	Yes	No	No	chromodomain helicase DNA binding protein 2, a chromatin remodeller from SNF2 family
2	GNAO1	Chr16 q13	Yes	No	Yes	No	No	Yes	Yes	Yes	Most abundant G protein expressed in brain
2	ANKRD11	Chr16 q24.3	No	Yes	Yes	No	Yes	Yes	No	No	ankryrin repeat domain-containing protein, inhibits ligand-dependent activation of transcription
2	NRXN1	Chr2 p16.3	Yes	Yes	No	No	Yes	Yes	No	Yes	Cell adhesion molecules and receptors in the nervous system
2	DYRK1A	Chr21 q22.13	No	Yes	Yes	No	Yes	Yes	No	No	A member of the Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family

Number of Overlapping of Sources	Gene Symbol	Location	Zhu et al (Zhu et al., 2014)	SFARI	DD Project (Deciphering Developmental Disorders, 2015)	Hoischen et al, 2014 (Hoischen et al., 2014)	ASD	ID	EE	SC	Gene Function
2	MYH9	Chr22 q13.1	Yes	No	No	Yes	Yes	Yes	No	Yes	Encodes heavy chain of non-muscle myosin IIA in neurons as well as in non-neuronal cells
2	SETD5	Chr3 p25.3	Yes	Yes	No	No	Yes	Yes	No	No	No known function
2	TRIO	Chr5 p15.2	Yes	No	No	Yes	Yes	Yes	Yes	No	Rho GEF involved in neurite outgrowth, leukocyte transendothelial migration, and EGF-dependent microtubule plus end tracking
2	ARID1B	Chr6 q25.1	No	Yes	Yes	No	Yes	Yes	No	No	The part of the BAF (Brg1-associated factors) chromatin remodeling enzyme complex
2	RELN	Chr7 q22	Yes	Yes	No	No	Yes	No	No	Yes	Secreted extracellular matrix protein, controls cell-cell interactions during brain development.
2	HUWE1	ChrX p11.22	Yes	No	Yes	No	No	Yes	No	Yes	E3 ubiquitin ligase
2	PTCHD1	ChrX p22.11	No	Yes	Yes	No	Yes	Yes	No	No	encodes a membrane protein with a patched domain, similar to proteins in Drosophila that are receptors for sonic hedgehog

Table 2

Survey of DBD-related phenotypes in mouse models of high priority genetic risk factors

We surveyed studies that used homozygous and heterozygous knockout mice for the mouse orthologs of the human genetic DBD risk factors. We catalogued the DBD-related phenotypes found in these mice, with a focus on phenotypes that were discovered in mouse models of syndromic DBDs.

Gene Symbol	KO	HOMO KO Lethal	Mouse Growth/ Survival	Gross Brain Morphology	Behavioral Endophenotyping KO	Behavioral Endophenotyping g Het	Learning and Memory	Cell Type/ Brain Region Specificity	Seizures	Synaptic Phenotypes KO	Synaptic Phenotypes s Het	Synaptic Plasticity	Developmental Phenotypes	Notes
SCN2A	Yes	Yes(Planells-Cases et al., 2000)	NA	(HOMO) – Normal (HET) - Normal(Planells-Cases et al., 2000)	NA	NA	NA	(WT) Enriched expression in brainstem(Planells-Cases et al., 2000)	None observed	NA	NA	NA	(HET) Well-defined cytoarchitectures from ctx, hip, cbm, brsm (Planells-Cases et al., 2000)	Sodium channel currents attenuated in hip pyr cells (Planells-Cases et al., 2000)
SYNGAP1	Yes	Yes	(HET) Normal	(HET) Normal(Komiyama et al., 2002)	Impairs reversal learning and spatial memory(Muhia et al., 2012)	Well characterized(Guo et al., 2009; Komiyama et al., 2002; Muhia et al., 2010; Ozkan et al., 2014)	Well characterized(Guo et al., 2009; Komiyama et al., 2002; Muhia et al., 2010; Ozkan et al., 2014)	Cell type specific HI in forebrain excitatory neurons recapitulates phenotype(Ozkan et al., 2014)	No seizures, interictal discharges(Clement et al., 2012; Ozkan et al., 2014)	Increased number of synapses (Muhia et al., 2012)	Early synapse maturation, cortical hyperexcitability(Clement et al., 2012; Ozkan et al., 2014)	Reduction in LTP(Komiyama et al., 2002; Ozkan et al., 2014)	(HET)Early maturation of excitatory synapses(Clement et al., 2012)	
GABRB3	Yes	Yes – 90% die within 24 h of birth(Homanics et al., 1997)	(HOMO) Runted until weaning but normal in adult; reduced life span(Homanics et al., 1997)	(HOMO) Normal(Homanics et al., 1997)	Hyperactive and motor impairments(Homanics et al., 1997)	Altered tactile sensitivity, heat sensitivity, sensorimotor and competence PPI(DeLorey et al., 2011)	(HOMO) Contextual fear deficit (DeLorey et al., 1998) (HET) Decreased freezing in contextual fear conditioning (DeLorey et al., 1998)	NA	(HOMO) frequent myoclonus and occasional epileptic seizures(DeLorey et al., 1998; Homanics et al., 1997) increased epileptiform EEG activity and elevated seizure susceptibility(DeLorey et al., 1998; Homanics et al., 1997)	Decreased sIPSC amplitude and frequency; reduced evoked IPSC amplitude (Hunisman et al., 1999)	NA	NA	(HOMO) Cleft palate (Homanics et al., 1997)	
SCN1A	Yes	Yes before P15 (Ogiwara et al., 2007; Yu et al., 2006)	Weight loss(Ogiwara et al., 2007)	Decreased brain size(Ogiwara et al., 2007)	NA	Hyperactivity, stereotypies, social interaction deficits(Han et al., 2012)	(HET) Contextual fear deficit and reduced performance and memory in Barnes maze(Han et al., 2012)	HI in forebrain interneurons is sufficient to cause impairments. (Han et al., 2012) HI in pyramidal neurons ameliorates impairments(Ogiwara et al., 2013)	(HOMO and HET) spontaneous seizures(Ogiwara et al., 2013; Ogiwara et al., 2007; Yu et al., 2006)	NA	Reduced Na ⁺ currents and impaired action potential firing (Yu et al., 2006) reduced sIPSC frequency and increased sEPSC frequency(Han et al., 2012)	NA	Clonazepam, a GABA _A PAM, rescued abnormal social behaviors and deficits in fear memory in adulthood(Han et al., 2012)	

Gene Symbol	KO	HOMO KO Lethal	Mouse Growth/ Survival	Gross Brain Morphology	Behavioral Endophenotyping KO	Behavioral Endophenotyping g Het	Learning and Memory	Cell Type/ Brain Region Specificity	Seizures	Synaptic Phenotypes KO	Synaptic Phenotypes g Het	Synaptic Plasticity	Developmental Phenotypes	Notes
STXBPI	Yes	Yes(Verhage et al., 2000)	NA	(HOMO) Normal(Verhage et al., 2000)	NA	NA	Impaired fear conditioning(Hager et al., 2014)	NA	(HET) Depends on genetic background(Hager et al., 2014)	No synaptic transmission (Verhage et al., 2000)	Impaired synaptic transmission at high firing rates(Toonen et al., 2006)	NA		
POGZ	Cell line only	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA	
SUV420H1	Yes	Yes(Schotta et al., 2008)	(HOMO) Mice are smaller and born at sub-Mendelian ratios (Schotta et al., 2008)	NA	NA	NA		NA	NA	NA	NA	NA	NA	NA
GRIN2B	Yes	Yes(Kutsuwada et al., 1996)	NA	Disorganized barrelettes(Kutsuwada et al., 1996)	NA	Enhanced startle (Takeuchi et al., 2001), increased nociceptive responses to foot shock, tail-flick, tail-pinch, and hotplate(Wainai et al., 2001)		Many cell type-specific effects	NA	Reduced NMDAR-mediated EPSCs(Kutsuwada et al., 1996; Tovar et al., 2000)	NA	(HOMO) Impaired LTD(Kutsuwada et al., 1996)	Role in barrel formation(Yamasaki et al., 2014)	
SCN8A	Yes	Yes, for some mutations(O'Brien and Meisler, 2013)	(HOMO) Decreased body weight and premature death(Kohman et al., 1995)	Enlarged ventricles; hydrocephaly(Buchner et al., 2004)	Muscle weakness, hindlimb paralysis, ataxia, tremor(O'Brien and Meisler, 2013)	Ataxia and motor impairments; Increased avoidance of well lit open environments; decreased startle(McKinney et al., 2008)	(HOMO) impaired Morris water maze(Woodruff-Pak et al., 2006) (HET) greater fear conditioning(McKinney et al., 2008)	Cerebellar purkinje neurons(Woodruff-Pak et al., 2006)	(HETS) Spike-wave discharges(Papale et al., 2009)	Reduced repetitive firing and reduced persistent and resurgent current(O'Brien and Meisler, 2013)	NA	NA	Expression in WT starts P0-P8(Garcia et al., 1998)	
CHD2	Yes	Yes	(HET) Reduced body weight and survival(Marfella et al., 2006)	NA	(HOMO) Increased grip strength (IMPC)	NA	NA	NA	NA	NA	NA	NA	Likely developmental phenotypes(Shen et al., 2015). Spinal cord and spine abnormalities.	
GNAO1	Yes	No	(HOMO) Reduced life span, body weight(Jiang et al., 1998; Valenzuela et al., 1997) (HET) slow growth	NA	Impaired motor control, hyperactivity, turning behavior(Jiang et al., 1998), impaired olfaction(Luo et al., 2002), change in hearing rate(Duan et al., 2007), loss of	Normal activity levels(Jiang et al., 1998), strain dependent impairment in olfaction(Luo et al., 2002)	NA	NA	(HOMO) Occasional seizures(Valenzuela et al., 1997) (HET) Knock-in mice have vomeronasal sensory neurons (Kehrl et al., 2014)	Altered K+ can Ca+2 channel modulation(Greif et al., 2000), reduced field potential in vomeronasal sensory neurons (Chamero et al., 2011)	NA	NA	NA	Natural variation in this gene affects dependence on opioids in mice(Kest et al., 2009),

Gene Symbol	KO	HOMO KO Lethal	Mouse Growth/ Survival	Gross Brain Morphology	Behavioral Endophenotyping KO	Behavioral Endophenotyping g Het	Learning and Memory	Cell Type/ Brain Region Specificity	Seizures	Synaptic Phenotypes KO	Synaptic Phenotypes g Het	Synaptic Plasticity	Developmental Phenotypes	Notes
					aggression(Chamero et al., 2011)	2011								Ogden et al.
ANKRD11	Yes	Yes	(HET)Reduced body size, reduced bone mineral density(Barburic et al., 2008), IMPC	(HET) Unknown, but see development column	NA	Yes, hypoactivity, reduced social interaction, higher grooming(Gallagher et al., 2015), reduced startle (IMPC)	Reduced social memory (Gallagher et al., 2015)	Effects are observed only in excitatory neurons(Gallagher et al., 2015)	NA	NA	NA	NA	(HET)Altered Cortical Precursor Proliferation, Neuronal Numbers, and Neuronal Localization, adult neurogenesis(Gallagher et al., 2015)	HET mice refers to Yod/+ mutant in some cases(Barburic et al., 2008; Walz et al., 2015)
NRXN1	Yes	No	(HOMO) Normal	(HOMO) Normal	Impaired PPI, grooming, impaired nest building, enhanced motor learning(Etherton et al., 2009), altered social behavior(Grayton et al., 2013)	Increased novelty response in males(Laarakker et al., 2012)	Enhanced motor learning(Etherton et al., 2009)	Only effects on excitatory synapses are known(Etherton et al., 2009)	NA	Reduced glutamatergic synapse number(Etherton et al., 2009)	NA	NA	NA	Rat model is also present(Esclassan et al., 2015)
DYRK1A	Yes	Yes	(HET) Reduced body size(Fotaki et al., 2002)	(HET) Reduced brain size, normal cytoarchitecture(Fotaki et al., 2002)	NA	Hypoactivity(Fotaki et al., 2004), reduced response to dopaminergic agents(Martinez de Lagran et al., 2007), impaired spatial memory(Arque et al., 2009; Arque et al., 2008)	Impaired spatial memory (Arque et al., 2009; Arque et al., 2008)	Effect on multiple neuronal types(Souchet et al., 2014)	Reduced threshold for PTZ seizures(Souchet et al., 2014)	NA	Reduced neuronal complexity (Benavides-Piccone et al., 2005), reduced striatal dopamine,	NA	Very diverse roles in neurogenesis and neuronal differentiation(Tejedor and Hammerle, 2011)	Gene dosage of DYRK1A gene is critical, and overexpression model is studied(Ahne et al., 2006; Altafaj et al., 2001; Altafaj et al., 2013; Garcia-Cerro et al., 2014; Souchet et al., 2014; Thomaizeau et al., 2014)
MYH9	Yes	Yes	(HOMO)Embryonic Lethality (Conti et al., 2004)	NA	NA	NA	NA	Low expression in glia relative to neurons(Ozkan et al., 2015)	NA	None observed (Ozkan et al., 2015)	NA	NA	Defects in cell adhesion and tissue organization in embryos (Conti et al., 2004)	
SETD5	Yes	Yes	(HET)Reduced lean mass (IMPC)	NA	NA	Reduced grip strength (IMPC)	NA	NA	NA	NA	NA	NA	NA	

Gene Symbol	KO	HOMO KO KO Lethal	Mouse Growth/ Survival	Gross Brain Morphology	Behavioral Endophenotyping KO	Behavioral Endophenotyping g Het	Learning and Memory	Cell Type/ Brain Region Specificity	Seizures	Synaptic Phenotypes KO	Synaptic Phenotype s Het	Synaptic Plasticity	Developmental Phenotypes	Notes
TRIO	Yes	Yes(O'Brien et al., 2000)	NA	Disrupted cerebellar formation, hippocampus, inferior olivary, and facial motor nuclei(Backer et al., 2007; O'Brien et al., 2000; Peng et al., 2010)	Ataxia(Peng et al., 2010)	NA	NA	(HOMO) Cerebellum malformed(Peng et al., 2010)	NA	NA	NA	NA	NA	Ogden et al.
ARID1B	Yes	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
RELN	Yes	No	(HET) Normal body weight (Tueting et al., 1999)	(HOMO) Reduced brain size (Badea et al., 2007)	Well characterized see (Tueting et al., 2006)	Well characterized see(Tueting et al., 2006)	Deficit in contextual fear conditioning (Qui et al., 2006)	Effect both glutamatergic and GABAergic neurons(Katsuyama and Terashima, 2009)	Yes(Patrylo et al., 2006)	Up regulation of NMDA receptors(Isozaka et al., 2006)	NMDA receptor up regulation (Isozaka et al., 2006), Decrease spine density(Liu et al., 2001)	Yes, impaired short and long-term plasticity (Qiu et al., 2006)	Well characterized roles in cellular positioning (Katsuyama and Terashima, 2009)	A rat model is also available (Yokoi et al., 2003)
HUWE1	Yes	Yes(Zhao et al., 2009)	NA	(Brain-specific HOMO KO) disorganized laminar pattern in cortex(Zhao et al., 2009)	NA	NA		NA	NA	NA	NA	NA	NA	
PTCHD1	Yes	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA	

Abbreviations: NA, no data is available; IMPC, International Mouse Phenotyping Consortium

The IMPC states that PTCHD1 KO mice are available and some metabolism data are available for the homozygous KO.

HOMO KO - homozygous knockout; HET - heterozygous knockout; HI - haploinsufficiency; LTP - long-term potentiation; ctx - cortex; hip - hippocampus; cb1m - cerebellum; brstm - brain stem; pyr - pyramidal; IPSC - inhibitory postsynaptic current; sIPSC - spontaneous IPSC; PAM - positive allosteric modulator; EPSC - excitatory postsynaptic current; LTD - long-term depression; WT - wild-type; IMPC - International Mouse Phenotyping Consortium; PPI - prepulse inhibition; PTZ - pentylenetetrazol; GABA - gamma-aminobutyric acid; NMDA - N-methyl-D-aspartate; NA - no data is available