Rodent genetic models of neurodevelopmental disorders and epilepsy

Title: Rodent Genetic Models of Neurodevelopmental Disorders and Epilepsy

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Abstract: Neurodevelopmental disorders (NDDs) are characterized by cognitive, social and motor deficits and are highly comorbid with intractable epilepsies. Through advances in genetic sequencing technologies a vast number of genes have been implicated in NDDs. State-of-the-art gene-editing techniques have led to the generation of hundreds of mouse models of NDDs. As an example, rodent models of Rett and Dravet syndromes as well as the syndromes caused by mutations in *CDKL5* and *Syngap1* display cognitive deficits in conjunction with seizure phenotypes. These models allow researchers to understand the underlying mechanisms as well as develop novel treatment strategies that can potentially be translated to the clinic. Furthermore, it may be possible to gain insights into the contribution of epilepsy to the progression of cognitive, social and motor phenotypes in NDDs.

Keywords (6): Neurodevelopmental disorders, epilepsy, MECP2, SCN1A, CDKL5, SYNGAP1.

1.0 Introduction

Neurodevelopmental disorders (NDDs) including autism spectrum disorders (ASDs) and intellectual disability (ID) can affect cognitive, social and motor abilities[1]. NDDs are commonly associated with severe and intractable epilepsies with approximately 26% of patients having seizure comorbidities[1]. Thus far, even if anti-epileptic drugs (AEDs) are effective in treating seizures, it is unclear whether neurodevelopmental symptoms are improved upon seizure control.

Recent advances in genetic sequencing have identified over 100 genes[2–4] implicated in syndromic ASD and a further 700 x-linked, autosomal-dominant and autosomal-recessive genes that can be utilized to diagnose NDDs[5–7]. Coinciding with an increase in clinical data on the association of these genes with NDDs and epilepsy, gene-editing technologies such as CRISPR-Cas9 have made it increasingly faster, accurate and inexpensive to generate rodent models of these disorders with over 250 mouse models generated to study ASD alone[8,9],

Animal models allow researchers to investigate the underlying mechanisms of these disorders, identify biomarkers for the clinic and develop potential novel treatment strategies. Furthermore, as many of the genes identified converge across biological pathways, it is important to define whether this results in symptomatic convergence and therefore possible common treatments.

For animal models to be effective it is necessary that they display face validity that resembles symptoms in patients. Although genes in brain disorders are frequently conserved in rodents, their function in other species may not be as critical to the complex behavioural disabilities associated with NDDs. Furthermore, it is possible that compensatory mechanisms come into effect upon gene-editing which are not present or have more of an impact than compensation in patients. Nonetheless, mouse models have shown the highest level of construct validity across species so far tested[10–12]

As recently reviewed by Silverman and Ellegood[12], deficits in many behavioural assays in rodent models of NDDs pertinent to ASD and ID include social communication experiments such as the three-chambered approach, reciprocal dyad interactions, social recognition, social place preference, and ultrasonic vocalizations. Cognitive inflexibility and insistence on sameness has also been modelled in NDD models[13,14]. Other relevant behavioural deficits found in mouse models include quantification of spontaneous behaviours such as self-grooming, circling, jumping, back flipping and overall hyperactivity, which may be linked to abnormal or epileptic brain network activity.

Spontaneous epileptic activity has been recorded both through behavioural monitoring and electrophysiology in multiple genetically modified mouse models of NDDs[15–18]. A range of spontaneously emerging seizure types have been reported including tonic-clonic, atypical and typical absence, myoclonic spasms and interictal spikes. Increases in susceptibility to audiogenic seizures, hyperthermia and chemically induced seizures is also prevalent across genetic rodent models of NDDs[18–23]. These models offer the opportunity to test the effect of anti-epileptic drugs (AEDs) and to disentangle the contribution of seizures to the progression of NDD's cognitive, social and motor phenotypes. Below, we summarize findings about epileptic activity in four mouse models of NDDs.

2.1 methyl-CpG-binding protein 2 (*MECP2*)

In Rett syndrome, both ID and ASD develop due to gain and loss of function of the X-linked MECP2 gene. Patients develop progressive dementia, infantile hypotonia, gait abnormalities and seizures following normal early development for the first 2 years of life[24]. Approximately half of patients with MECP2 gene duplication suffer from epilepsy during their lifetime with a high level of refractoriness of approximately 32 to 56%[25,26]. Lennox-Gastaut syndrome, in which patients suffer from a variety of seizure types is the most common form of epilepsy in patients with MECP2 duplication. Whereas patients with late truncating deletions have a lower prevalence of epilepsy, several mutations exist with varying risks for epilepsy.

Mouse and rat models of MECP2 overexpression and duplication reliably replicate the seizure phenotype[15,16], with animals developing tonic-clonic seizures and increasing the sensitivity to pentylenetetrazole (PTZ)-induced and kainic acid-induced seizures. Loss of function models display cortical discharges consistent with absence epilepsy[19].

Seizure and ND phenotypes are reversable in Rett syndrome rodent models utilizing gene modification strategies[27,28] and implementation of gene therapy in the clinic may be soon attainable. Pharmacological strategies that target downstream targets of MECP2 are also possible but made difficult to implement as the exact function of the gene is still unknown. Nonetheless, the effectiveness of AEDs in treating seizures in Rett syndrome have not been extensively tested in rodent models and could inform clinicians on potential therapeutics for Rett syndrome.

2.2 *SCN1A*

Dravet syndrome is a rare form of epilepsy accompanied by neurodevelopmental deficits and is mediated by the SCN1A gene, which encodes for the alpha subunit of the voltage-gated sodium channel[29]. The syndrome is characterised by multiple seizure types including febrile and febrile generalised tonic-clonic and focal-clonic, tonic, status epilepticus, myoclonic and atypical absences. The epileptic symptoms are highly refractory to AEDs and the onset of the disease occurs within the first year of life[30].

As recently reviewed by Griffen at al.[31], multiple mouse models of Dravet with *SCN1A* loss of function exist. Rodent models display ictal and interictal activity, as well as myoclonic jerks and flexions[17]. Seizures in *SCN1A* knock-out mice are also inducible via hyperthermia[20].

First line AEDs are ineffective in treating seizures and sodium channel modulators such as carbamazepine are known to exacerbate seizures[32]. However, recent clinical trials show positive seizure reduction utilizing stiripentol, cannabidiol, and fenfluramine with 50 to 70% reduction in seizures and nearly a quarter of patients attaining seizure freedom[33]. In mouse models, stiripentol iw effective in reducing hyperthermia-induced seizures[34], while

cannabidiol similarly reduced spontaneous seizures[35]. Fenfluramine was found to be effective in a zebrafish model of Dravet[36].

Successful face validity of Dravet animal models suggests that novel treatments to achieve a higher level of seizure control and reversal of neurodevelopmental deficits may be possible. Studies to modify gene expression in rodent models of Dravet Syndrome have shown to effectively reduce seizures[37]. The utility of animal models in Dravet is therefore likely to yield fruitful new treatment paths to test in the clinic.

2.3 cyclin-dependent kinase-like-5 (CDKL5)

CDKL5 is likely involved in correct dendritic spine structure and synapse activity in excitatory neurons by binding and phosphorylating the NGL-1 cell adhesion molecule which allows it to form a stable association with PSD95[38]. *CDKL5* is amongst the most common single-gene predictors of epilepsy diagnosis[39]. Mutations in *CDKL5* lead to a NDD with severe developmental delay and early-onset epilepsy. Other features include impaired hand function with stereotypies, impaired social interaction with autistic features and severe motor impairment with only exceptional patients who are independently ambulant[40]. 98% of patients present with a history of epilepsy including infantile spasms, myoclonus and prolonged generalized tonic-clonic seizures[41]. Most of these types of epilepsy appear to be refractory to AEDs.

CDKL5 models have been generated and display certain phenotypes including hyperexcitability[21], visual impairments[42], social interaction deficits, motor control decreases and loss of fear memory[43]. Nonetheless, despite the prevalence of seizures in patients, mouse models have not been reported to display spontaneous ictal or interictal activity. It is likely that either compensatory mechanisms exist to compensate *CDKL5* loss of function or the gene does not play as critical a role in rodents. CDKL5 mice however are susceptible to NMDA-induced seizures[21]. More research is necessary to elucidate whether animal models of this disorder have translational value.

2.4 *SYNGAP1*

SYNGAP1 is associated with NMDAR-activated Ras signalling dynamics in dendritic spines as well as AMPA receptor membrane insertion[23,44]. Although mutations are relatively rare, heterozygous loss-of-function in *SYNGAP1* results in a genetically defined NDD with ID, which also predisposes patients to ASD. Symptoms of this syndrome include cognitive impairment, loss of language abilities and seizures[45,46]. In a recent clinical report, 56 of 57 patients had epilepsy with a median age of onset of 2 years[47]. Most seizures are generalized, with absence seizures reported in 53 of the patients, although focal seizures also occur. 65% of patients had intractable epilepsy with valproate and lamotrigine commonly prescribed as well as cannabidiol.

As found in patients[48], mouse models display increased risk-taking behaviours as assessed by the elevated plus maze as well as cognitive and learning impairments[45]. *SYNGAP1* haploinsufficient mice have spontaneous interictal activity and have a reduced fluorothyl-induced seizure threshold as well as being prone to audiogenic seizures[18,22,23]. Restoration of the gene in adult mice is able to improve behavioural and electrophysiological measures of memory and seizures[18]. More work is needed to test potential treatment strategies in these animals and determine whether these are translatable to the clinic.

3.0 Conclusion

Rodent models display phenotypes reminiscent of symptoms in genetically defined NDDs such as those affecting cognition, motor abilities and sociability. Genetically defined NDDs very frequently are comorbid with severe intractable epilepsy. Brain network dysfunction resulting in seizure activity is common in rodent models. Furthermore, if spontaneous epilepsy is absent, such as in the case of *CDKL5* models, the mutant animals can still be challenged to determine whether they have compromised seizure thresholds.

Pharmacology treatments as well as other strategies such as gene therapy can be tested on rodent models and findings can be translated to the clinic as with the positive results of

stiripentol[34], cannabidiol[35], and fenfluramine[36] being effective in seizure control in animal models. Rodents give researchers the opportunity to develop new treatments by probing deeper into the mechanisms resulting in NDDs. An enticing avenue of research with rodent genetic models of NDDs is to test the hypothesis that if seizures are controlled early enough in development, they are able to arrest the neurodevelopmental deficits.

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