Overlaps, gaps, and complexities of mouse models of Developmental and Epileptic Encephalopathy

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Abstract

Mouse models have made innumerable contributions to understanding the genetic basis of neurological disease and pathogenic mechanisms and to therapy development. Here we consider the current state of mouse genetic models of Developmental and Epileptic Encephalopathy (DEE), representing a set of rare but devastating and largely intractable childhood epilepsies. By examining the range of mouse lines available in this rapidly moving field and by detailing both expected and unusual features in representative examples, we highlight lessons learned in an effort to maximize the full potential of this powerful resource for preclinical studies.

Keywords

Mouse models; Epileptic encephalopathy; Disease modeling; Seizure

1. Human DEE and mouse genetic models: a tally

The past three decades witnessed the unfolding realization that epilepsy has a significant genetic component, culminating with the striking discovery that a sizeable fraction of the most devastating form of disease – a mixed set of childhood epilepsies that are together termed Developmental and Epileptic Encephalopathy (DEE) – results from single gene *de novo* mutations (Epi4K et al., 2013). While currently over one-quarter of all DEE cases are genetically diagnosed with high confidence by whole exome sequencing, the underlying genetic heterogeneity, early age of disease onset, and severity present major challenges to effective treatment.

Through technological advances in genome editing, the past 5 years has produced a burst of new mouse models of epilepsy, primarily for DEE, which have been examined using transcriptomics, neuroimaging, neurophysiology and behavior testing. Despite a high degree of genetic, and to a lesser extent neurophysiologic similarity between human and mice, and while many construct-valid mouse models display some form of seizure susceptibility and abnormal neurodevelopment, navigating species differences proves to be no small matter. As DEE families and clinicians have begun to engage with researchers to seek diagnosis and

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therapy, there is a further need for clarity on mouse strains that model specific genetic DEEs and to reflect upon their potential for preclinical research.

Towards this end, we aligned human variant data with equivalent mouse mutations in the same genes. We began by surveying the NCBI ClinVar database (further guided by OMIM database annotation) for intragenic variants classified as pathogenic or likely pathogenic in the Molecular Consequence field, and containing terms in the Conditions field such as early infantile (or early onset) epileptic encephalopathy (EIEE, EOEE), severe myoclonic epilepsy in infancy (SMEI), mental retardation (intellectual disability) or similar conditions of neurodevelopmental origin where seizures are a prominent feature. After excluding genes that had only a single DEE variant, this effort resulted in 85 genes that collectively carried over 7200 DEE variants. These genes were then sub-grouped by *apparent* molecular mode of action (Table 1): A. Missense only (genes for which there were no predicted loss-of-function (LOF) DEE variants, *i.e.* no frameshifts, deletions, critical splice site or nonsense mutations), B. Loss-of-function only (genes for which no missense variants were listed), and C. DEE conditions that included both LOF and missense variants in the same gene.

These groupings are not intended as formal, but rather to guide comparative studies. Indeed, some genes have only a few pathogenic variants. Others may be exceptions, for example, while genes in Group C contain many loss-of-function variants and it is reasonable to assume that most missense variants in these genes for the same disease are also null or hypomorphic, some may have gain-of-function (GOF) properties. Nevertheless, any division between GOF (emergence of new functional properties) and LOF (deficiency in essential properties) begs the question of whether the genes align with different biological processes. To test this idea at a basic level, we performed *in silico* assessment using Gene Ontology (GO) functional annotation clustering (Fig. 1), from which two conclusions emerge: i) Across the most significant GO terms, Group A clusters were almost 9 orders of magnitude more significant than Groups B/C, despite having one-third fewer genes. This may suggest that genes with missense-only DEE variants are more likely to converge on common biological processes; ii) In fact, one o GO term ("Regulation of Membrane Potential") was 16.2 orders of magnitude more significant in the missense-only group. Although, ultimately specific genes and variants need to be tested in the lab, such clues that suggest higher-level convergence is good news for the potential for future treatments to span multiple DEEs.

We then cross-tabulated DEE genetic variant types by whether a mouse model exists for a human variant using the Mouse Genome Informatics database as a guide (http://www.informatics.jax.org), confirmed by review of the primary literature or supplemented with knowledge of recent studies or those pending publication. From this straightforward comparison we can make some general observations regarding the representation of mouse models for DEE genetic variants. First, it is very clear that only a small fraction (22%, or 8 of 36) from the missense-only type (Table 1, Row A) have been modeled in mice, whereas 100% of genes that have a LOF DEE is represented by mouse mutants, mostly knockouts (Table 1, Rows B and C). This contrast is not surprising given that knockout mice (whether studied as homozygous or heterozygous) have been mainstays in the mouse genetics community for many years, whereas facile generation of missense mutations in mice (*e.g.* by CRISPR/Cas9) has come about only recently.

We next examined whether seizure susceptibility was observed in a corresponding mouse mutant, whether or not that mutation was equivalent to the DEE variant. The results were very promising for genes with only missense human variants: 88% of mutant mouse strains with a DEE variant (7 of 8) were reported with obvious spontaneous seizures (Table 1, Row A; Table 2; *DNM1*, *GNB1*, etc). Interestingly, 14 DEE genes that have mouse strains with LOF variants or missense variants not orthologous to human DEE variants also showed seizure susceptibility (Table 1, Row A; *ALG13, ARV1, etc.*). This is a likely source of confusion to the uninitiated as these mouse lines might appear to be suitable construct-valid DEE models when they may model a different type of epilepsy entirely.

Similar examination of seizure susceptibility in models based on putative loss-of-function human DEE variants gave more mixed results. Seizure susceptibility was demonstrated in only 41% (20 of 49) mutant mouse strains (Table 1, Rows B, C). Certainly the lower rate in this group is affected by negative ascertainment bias, namely a) many knockout mice were not developed by epilepsy researchers who might not recognize subtle seizure behaviors, b) some knockouts are lethal prior to seizure onset or detection, and heterozygotes are often not examined to the same degree, c) as we will discuss later, non-convulsive seizures are common in mouse models but require dedicated *in vivo* electrophysiology for detection, d) focal seizures can easily be missed with conventional rodent EEG, and e) detection of seizures in pre-weaning age mice is not yet commonplace even among epilepsy researchers despite the fact that this developmental period corresponds best with children. In contrast, the relatively high seizure susceptibility yield of the missense-only group almost certainly reflects a degree of positive ascertainment bias – these models were likely developed for the explicit purpose of studying the respective human epilepsy.

Many further subtleties and important details influence comparison of DEE mouse models to the respective human genetic disease, including phenotypic heterogeneity, genetic background and various technical challenges to detecting and defining seizure types or predisposition in small rodents, not to mention other neurological or neurosensory abnormalities, present in one form or another in all mouse DEE models (Table 2). We will discuss some of these issues in examples below. However, even our straightforward assessment shows that while there are already over 80 mouse mutants in genes for human DEE, seemingly a large number available to study, there remain both significant gaps (*e.g.* only 22% of potential GOF models have been generated in mice), and sources of overlap and confusion (*e.g.* GOF and LOF mouse mutants in the same gene can cause seizures).

Gaps, overlaps and complexities aside, several studies from the mouse literature provide useful, state-of-the-art examples of studying genetic construct-valid models for DEE. The following examples illustrate both desirable features in mouse models, plus some quirks that need to be reckoned with as the field moves forward.

2.   *SCN8A* epileptic encephalopathy: strong models, paradox included

Genetic variants in genes encoding sodium channels were among the earlier channelopathies recognized in human epilepsy; first *SCN1B*, then *SCN1A* (the primary cause of Dravet Syndrome, the most common genetic DEE), *SCN2A* (Meisler et al., 2001), and more

recently *SCN8A* encoding the Nav1.6 sodium channel, first described for a DEE in 2012 (Veeramah et al., 2012). As Meisler described in a recent review (Meisler, 2019), heterozygosity for gain- or altered-function variants in *SCN8A* are associated with the most severe DEE whereas haploinsufficiency is associated with significant but less severe disease, notably, intellectual disability accompanied by mild epilepsies. Currently in ClinVar, there are 110 *SCN8A* missense variants, with 60% (66/110) being annotated with epileptic encephalopathy, while only 9 loss-of-function variants are documented.

Mice carrying *Scn8a* loss-of-function variants were first reported in the 1960's and 1970's as recessive spontaneous neurological mutants with motor phenotypes and premature lethality (Dickie, 1965; Lane, 1976). It was not until 2009 that loss-of-function *Scn8a* heterozygotes were shown to display spontaneous spike-wave discharges, considered to model absence or petit-mal seizures (Papale et al., 2009). Unusually, these same mice confer relative resistance to convulsive seizures whether induced chemically, electrically, or by genetic mutation (Hawkins et al., 2011; Martin et al., 2007; Sun et al., 2013) – a feature that was recently exploited to show the effectiveness of *Scn8a* antisense gene therapy in reducing or eliminating seizures in the *Scn1a* mouse model of Dravet Syndrome (Lenk et al., 2020). This apparent paradox of seizure sensitivity and resistance in the same genetic model provided a glimpse into the allelic heterogeneity and complex cellular etiology of epilepsy, complexities that are becoming increasingly appreciated for *SCN8A* and other genetic DEEs.

Two separate *SCN8A* DEE gain-of-function (GOF) mutations have now been introduced into mice – N1768D (constitutive) and R1872W (conditional) – respectively based on the heterozygous variant N1768D first identified in a patient with sudden unexpected death in epilepsy (SUDEP) (Veeramah et al., 2012), and R1872W first identified in a patient with prolonged tonic-clonic seizures from 3 months of age (Ohba et al., 2014). Although just two models, the severity of the *in vivo* phenotype in mice and the corresponding channel kinetics *in vitro* track with the respective disease severity (Meisler, 2019). Both heterozygous models exhibit spontaneous seizures resulting in lethality, although the seizure phenotype of R1872W of heterozygotes is fully penetrant and mice die at a much earlier age (2–3 weeks) than in N1768D (9 weeks). Electroencephalographic evidence of epileptiform activity was observed in both models including typical high amplitude polyspike activity followed by post-ictal depression, and at least one of the two exhibited myoclonic jerks prior to development of generalized tonic-clonic seizures (GTCS). N1768D heterozygous mice were tested for various neurological and behavioral deficiencies but found to be relatively normal compared with control, except for modest deficits motor coordination and social discrimination (Wagnon et al., 2015). The R1872W conditional model was activated using cre-recombinase expression in either inhibitory or excitatory neurons, which led to the discovery that expression of the variant in excitatory neurons is necessary for seizure activity (Bunton-Stasyshyn et al., 2019).

Overall *Scn8a* knockin mice appear to be a satisfying model for genetic DEE, displaying features representative of some other mouse models of DEE including lethal spontaneous seizures, allelic heterogeneity, and mild-to-moderate behavioral deficits that together make them suitable models for study. The R1872W conditional mutation adds further insight into the cellular etiology of disease. *Scn8a* heterozygous loss-of-function mutants also

show the value of studying a range of milder forms of disease with seizures over a range of severities (Inglis et al., 2020). Although the coexistence of spontaneous absence-like seizures and resistance to convulsive seizures in the same mice appears at first a paradox, it provides an object lesson about the biological complexity of developmental epilepsies, while underscoring how crucial correct Nav1.6 ion channel expression levels are for normal neuronal function. Using *Scn8a* conditional knockout mice, Makinson et al. (2017) reported that absence seizures are due to thalamic deficit while convulsive seizure resistance results from loss of *Scn8a* from cortical excitatory cells, providing an example of how seemingly paradoxical findings in *Scn8a* LOF mutants could be understood by considering how mutations influence distinct regions and networks differently.

3. *KCNT1* DEE: multiple seizure types, brain state-dependence

Inherited variants in potassium channels were among the first well-defined albeit relatively mild monogenetic epilepsies, now termed Benign Neonatal Familial Epilepsy (Wang et al., 1998). It was not for another ten years when variants in *KCNT1*, the gene encoding the Na^+ activated K^+ channel (Slack, K_{NA} 1.1) were associated with a range of more severe epilepsies, including EIEE, epilepsy of infancy with migrating focal seizures (EIMFS), and autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).

Two *KCNT1* GOF variants, Y796H (Y777H in mouse) and R474H (R455H in mouse), were described in mice recently, and both confer significant spontaneous seizure activity (Quraishi et al., 2020; Shore et al., 2020). The Y796H variant (c.2386 T>C) was identified as a heterozygous mutation causing both inherited and *de novo* cases of a severe, early-onset form of ADNFLE (Heron et al., 2012). The R474H variant (c.1421G>A) was reported in individuals with EIMFS or EOEE (Ohba et al., 2015). Both Y796H and R474H patients had intractable seizures with high frequency.

R455H homozygotes are embryonically lethal, and heterozygotes all have persistent interictal epileptiform discharges in the EEG (IED; average 16/h) and susceptibility to PTZ-induced convulsive seizures. Despite the fully penetrant IED, only half (3/7) showed spontaneous seizures in the EEG. Consistent with the respective human disease, Y777H mice have milder phenotypes and no selective lethality was observed in homozygotes. While seizures in heterozygotes are too rare to quantify, 93% of homozygotes (26/28 total) experienced seizures at an average of about one every 3.2 ± 0.4 h. In agreement with the epilepsy phenotype reported in Y796H patients [17], seizure episodes consisted of at least two types: GTCS typically lasting 30–60 s, and tonic seizures (TS) lasting 1–2 s (also observed in R455H but termed as myoclonic seizures). Frequent postical and interictal epileptiform discharges (IED) were also observed. Interestingly, 90% of seizure episodes occurred during NREM sleep, which provides meaningful parallel with the sleep-associated seizure pattern of ADFNLE patients. Both GTCS and TS were examined further using calcium imaging, revealing large amplitude calcium events localized primarily to the secondary motor cortex region. Epileptiform activity was also detected in pre-adolescent mouse pups by performing acute unanaesthetized *in vivo* electrocorticography with a novel neural interface device called NeuroGrid, which makes Y777H mice especially valuable for studying childhood genetic disease. Further examination of biophysical properties and

neuronal connectivity in primary neuron culture and acute slices also provide a first glimpse into the cellular basis of *KCNT1* DEE, with non-fast spiking GABAergic interneurons displaying a strong impairment in action potential generation due to increased K^+ current at subthreshold membrane voltages.

Taken together, the *KCNT1* DEE models are satisfying as they cover different disease severities and forms consistent with the range of human patients. Although epileptic phenotypes were only tested in R455H adult mice, the model may phenocopy many key aspects of human EIMFS. For the Y777H model, while it is odd and a perhaps species-specific quirk that homozygosity is required to confer frequent seizures in mice, the relationship between seizures and activity state and the topographical mapping is surprisingly consistent with that of the ADNFLE patient.

4.   *GABRB3*: Lennox-Gastaut Syndrome in a mouse model?

Type A GABA receptors (GABA$_A$Rs), formed by pentameric assemblies of different subunit subtypes, are ligand-gated chloride channels that mediate fast inhibitory synaptic transmission. Because of the crucial inhibitory role of GABA$_A$Rs, variants in many GABA$_A$R subunit genes (*e. g. GABRA1, GABRA2, GABRA5, GABRB1, GABRB2, GABRB3, GABRG2*) are associated with DEE. *GABRB3* variants specifically are implicated in several types of epileptic encephalopathies including infantile spasms and Lennox-Gastaut Syndrome (LGS; (Epi4K et al., 2013)). In ClinVar, 12 out of 29 missense variants are annotated with epileptic encephalopathy, and all others with annotated conditions, including 4 putative loss-of-function, are annotated with other epilepsy types. Children with missense mutations in *GABRB3* have common symptoms including infantile seizure onset of multiple seizure types, and associated comorbidities such as neurodevelopmental delay, autistic behavior, and intellectual disability (Epi4K et al., 2013; Papandreou et al., 2016).

Gabrb3 knockout mice were first reported in 1998 (DeLorey et al., 1998) as a model for Angelman Syndrome since *GABRB3* is within the Angelman Syndrome locus (15q11–13) together with *UBE3A*, but it was not until 2020 that a *Gabrb3* D120N knock-in mouse was reported as the first genetic model for Lennox-Gastaut Syndrome (Qu et al., 2020). Although other missense mutations in mouse *Gabrb3* (N265M and S408/9A) were described previously (Vien et al., 2015; Zeller et al., 2007), only D120N is based on a human DEE. The *de novo* missense mutation in *GABRB3* - D120N (c.358G>A) – has been identified in two individuals with different symptoms (Epi4K, 2016) one diagnosed with LGS and another one with milder febrile and tonic-clonic seizures. The LGS patient had infantile spasms (IS) at 10 months of age (Epi4K, 2016) and later developed additional seizure types including generalized tonic-clonic, atypical absence, myoclonic and atonic seizures. EEG showed bursts of 2 Hz sharp spike-wave complexes. EEG from the other patient showed 2.5 Hz–3 Hz generalized spike-waves and diffuse slowing. It is notable that a phenotypic range arises in patients with the exact same *GABRB3* variant.

While homozygous *Gabrb3* D120N/D120N mouse mutants are neonatal lethal, heterozygotes do have frequent spontaneous spike-wave discharges that appear to model

atypical absence-like seizures (3.0 Hz – 5.5 Hz), and less frequent tonic, myoclonic, atonic and GTCS. Spontaneous epileptic spasms were observed in young heterozygotes, which aligns with the observation that IS precedes LGS in one D120N patient. Other behavioral comorbidities seen in LGS patients such as impaired learning and memory, hyperactivity, and impaired social interactions appear to be mimicked in *Gabrb3* D120N/+ heterozygotes as well. The authors assert that the heterozygotes are a convincing model of LGS because they recapitulate both the occurrence and semiology of LGS seizures.

Gabrb3 D120N/+ mice, like *Kcnt1* Y777H/Y777H mice, are early examples of how specific mouse model seizure phenotypes may line-up with associated features of the human disease, while many of the accompanying behavioral features of these mice are quite mild. Moving forward, it will be very helpful to develop milder *GABRB3* missense models to begin exploring the phenotypic heterogeneity and range and thus how precisely the mouse model phenotype aligns with a specific human DEE subtype – LGS.

5. *CDKL5* Deficiency Disorder: nominal seizure susceptibility in an epilepsy model

Loss-of-function variants in the Chromosome X-linked gene *CDKL5*, encoding a serine-threonine kinase, were first shown to cause a DEE in the early 2000's (Evans et al., 2005). Now termed *CDKL5* Deficiency Disorder (CDD), disease associated mostly with loss-of-function *CDKL5* variants provides a good example of early-onset intractable neurodevelopmental epilepsies with a wide range of overlapping clinical diagnoses such as autism, intellectual disability and atypical Rett's Syndrome, usually accompanied by seizures.

A conventional *Cdkl5* knockout mouse model was first reported in 2012 (Wang et al., 2012), and more recently an independent nonsense mutation, R59X, was described (Tang et al., 2019; Yennawar et al., 2019). While hemizygous males in these studies were described as having autistic-like behaviors and. Hyperactivity and reduced pain sensitivity (Gao et al., 2020b; Montanara et al., 2020), no seizure susceptibility was observed. Lately, evidence for excessive NMDAR-mediated neurotransmission was described in these models (Okuda et al., 2017; Tang et al., 2019). Although *Cdkl5* knockout mice are not sensitive to the standard pro-convulsant kainic acid, one study observed increased seizure susceptibility induced by NMDA (Okuda et al., 2017). Interestingly, following anecdotal observations of behavioral seizures, it is now known that aged *Cdkl5* heterozygous females (> 300 days) of both the R59X and the original knockout line actually do exhibit brief epileptic spasms and interictal spiking (Mulcahey et al., 2020). As in CDD, spasms in mice tended to occur in clusters along with increased power in the gamma and theta frequency band during quiet wake and non-REM sleep. The presence of epileptiform features in aged hemizygous males was not examined.

From a disease modeling perspective, the lack of readily-apparent seizure activity in genetic construct-valid mice might reasonably be argued as less than ideal. And, as we highlighted above, mouse knockouts published for over half of the genes representing loss-of-function DEE do not have any demonstrated seizure susceptibility (Table 1, Rows B, C). While

most of those studies apparently did not include systematic efforts to detect seizures beyond passive observation, the *Cdkl5* example shows that even dedicated epilepsy research labs sometimes need to dig deep to detect seizures in their mouse models. It also reinforces the reality that while some mouse models, such as *Scn8a*, *Kcnt1* and *Gabrb3* above have readily observed seizures - some with features that are strikingly similar to human - it is unrealistic to expect precise phenotypic matching in an animal model where brain maturation occurs earlier and more accelerated in real time compared with human. Notwithstanding our desire for better alignment, the *Cdkl5* story and others emerging like it nevertheless show that much may be learned mechanistically at the basic cellular and network levels from studying construct-valid models, and the excitability features that may reflect key aspects of seizure predisposition.

6. Prominence of spike-wave discharges and the "absence" of characteristic DEE electrographic features in mouse models

Spike-wave discharges (SWD), the electrographic correlate of seizures in rodents characterized by behavioral arrest, are thought to be analogous to the generalized spike and wave discharges observed in human absence seizures. SWD are promiscuous in laboratory rodents having been demonstrated in many mutant mouse strains (Maheshwari and Noebels, 2014; Noebels, 1995), and they are also observed at lower incidence in several so-called normal inbred strains, although they are not typically observed in the three most commonly used strains for genetic engineering, C57BL/6J, FVB/NJ, 129/Sv (Letts et al., 2014). The reason for this predisposition is unknown – perhaps it is endemic to all mice, or alternatively it may have evolved in the ancestors of laboratory mice as a quirk due to relaxation of selective pressure over the centuries of commensality culminating with their use in biomedical research. Regardless, like *Gabrb3* and *Scn8a* models noted above, several DEE mouse mutants exhibit spontaneous SWD (including *Cacna1a* (Fletcher et al., 1996; Jun et al., 1999; Noebels and Sidman, 1979)*, Cacna2d2* (Barclay et al., 2001), *Gabra1* (Arain et al., 2012), *Gabrg2* (Tan et al., 2007), *Gnb1* (Colombo et al., 2019), *Pcdh19* (Pederick et al., 2018), *Scn2a* (Ogiwara et al., 2018) and *Stxbp1* (Chen et al., 2020)) well above the genetic background rate, indicating that the DEE variant is the primary determinant. Among DEE mouse models, *Stxbp1* is a notable because SWD is the most prominent seizure phenotype in the otherwise fairly mildly impaired *Stxbp1* haploinsufficiency mouse model (Chen et al., 2020; Miyamoto et al., 2017). *STXBP1* is one of the most prevalent DEEs (Hamdan et al., 2011; Saitsu et al., 2008) but absence seizures are only one of several types of seizure observed in patients (Carvill et al., 2014).

The study of SWD in mouse models of DEE is a kind of double-edged sword. If absence seizures are not prominent in human DEE, and if laboratory mice are particularly SWD-sensitive, the corresponding mouse model might not be viewed by purists as suitable if SWD is the predominant or only seizure phenotype. On the other hand, if the defective gene is indeed the initiating cause of excitability phenotypes, then study of SWD in these models nevertheless provides a convenient means of uncovering pathogenic mechanisms at the cellular and network level which, in turn, could be generalized to other neurological systems.

Conversely, several electrographic seizure types that are characteristic of DEE have not yet been described in mouse models. The most notable include: hypsarrhythmia –EEG is characterized by chaotic background with nearly continuous random asynchronous high-voltage slow wave and spikes arising from multiple foci; paroxysmal fast activity, *i.e.* as seen in LGS patients; and abnormal sleep architecture. Although it is easy to conclude that the absence of these features so far, along with the dominance of SWD may be inevitable limitations of mouse models, the jury is still out because the vast majority of EEG studies in freely-moving mice is done in adults, and with a nominal number of electrodes. The novel recording device NeuroGrid noted in the *KCNT1* section above, designed specifically to cover the dorsal cortical surface of a pup brain, allows recording from multiple areas simultaneously without damaging the brain in the same way that implanted electrodes would. Further development and implementation of advanced *in vivo* array recording technologies will be valuable for determining the full spectrum of normal and pathogenic electroencephalographic patterns in mouse pups.

7. Genetic background: expected and unexpected players

Genetic background is one of many potential sources of diverse clinical presentation for neurodevelopmental disorders and epilepsies. Although background is well known to impact seizure threshold in mouse strains and some mutant lines, including models that exhibit SWD (Frankel et al., 2001; Loscher et al., 2017), there are not yet enough data to know the degree to which it plays a role in spontaneous seizure incidence or form in some of the newer DEE models. Certainly, background genetics is known to have a very significant impact in *Scn1a* heterozygous knockout mice, the most widely studied model for Dravet Syndrome (Hawkins et al., 2016; Kang et al., 2019). While homozygotes are early lethal, heterozygotes have spontaneous tonic-clonic seizures with an onset, incidence and severity that varies considerably with mouse strain background. Heterozygotes on the 129/Sv mouse strain background do not have any spontaneous seizures, whereas those on either of the major C57BL/6 substrains (or F_1 hybrid background between 129/Sv and C57BL/6), have lethal seizures as young adults with varying degrees of penetrance. Efforts to use *Scn1a* haploinsufficiency as a driver for identifying modifier genes have begun, and candidates have begun to emerge, such as that encoding a hypomorphic variant of *Gabra2* in C57BL/6 J mice (Hawkins et al., 2016). However, nomination of genetic modifiers based on apparent, sensical candidate genes, such as *Gabra2*, may be self-limiting and come with ascertainment bias. For example, very recently it was determined that all C57BL/6 J mice – historically the most widely-used C57BL/6 substrain - have a natural LOF mutation in a neuronal tRNA gene, initially discovered as a genetic modifier of neurodegeneration (Ishimura et al., 2014). This mutation also significantly increases electroconvulsive and chemoconvulsive threshold compared with C57BL/6N substrains and even mitigates the incidence of SWD due to the *Gabrg2* R43Q DEE variant (Kapur et al., 2020). Thus, while it is appealing to nominate existing candidate genes that account for some genetic background effects, this latest study suggests that fresh, unbiased approaches to identify unexpected players may also have significant value in shedding new light on genes and pathways that modify disease severity.

8. Genetic-based therapies and future expectations

So far at least three mouse DEE models ($Cdkl5^{-/Y}$, $Scn1a^{-/+}$, $Dnm1^{Ftfl/Ftfl}$) have been deployed to illustrate the power of gene therapy for both loss-of-function and missense childhood epilepsies (Aimiuwu et al., 2020; Colasante et al., 2020; Gao et al., 2020a; Lenk et al., 2020; Yamagata et al., 2020), an approach that could supplement if not eventually replace conventional drug therapies to mitigate symptoms and improve quality of life. Although there are significant differences in timing of onset and progression of disease in rodents compared to human, conventional mouse genetic tools, such as conditional mutations, and somatic tools, such as antisense oligonucleotides and virally-delivered RNAi vectors, can be deployed to model both the genetic specificity as well as effective therapeutic windows for mouse DEE models. A recent review has discussed in detail the uses and limitations of utilizing mouse models to screen for genetic-based therapy strategies (Turner et al., 2020). It is potentially feasible to direct genetic therapies at the defective gene or RNA at or before the onset of symptoms to mitigate most, if not all, clinical features of some DEEs.

Each of the 85 DEE genes recognized in this simple survey have corroborative evidence for a causative role in DEE, ranging from nominal to overwhelming. With the considerable genetic heterogeneity that underlies DEE, the number should rise steadily with increasing genetic diagnosis. While it is very unlikely that new genes will overtake the major contributors *SCN1A*, *CDKL5*, *STXBP1*, *SCN8A* in terms of population incidence, we expect that the continued identification and laboratory modeling of rare players will contribute, perhaps significantly, to understanding and convergence of pathogenic mechanisms, in turn providing new opportunities for effective therapy that may cut across multiple genetic DEEs. Despite the complexities of modeling a neurodevelopmental human disease in a rodent, in parallel with alternatives such as the currently popular human iPSC-derived neuron culture approach (Ardhanareeswaran et al., 2017; Di Lullo and Kriegstein, 2017) and continued use of other animal models ranging from flies, to nematodes, to fish (Bessa et al., 2013; Gatto and Broadie, 2011; Sakai et al., 2018), laboratory mouse models are likely to remain prominent tools for *in vivo* research on DEE.

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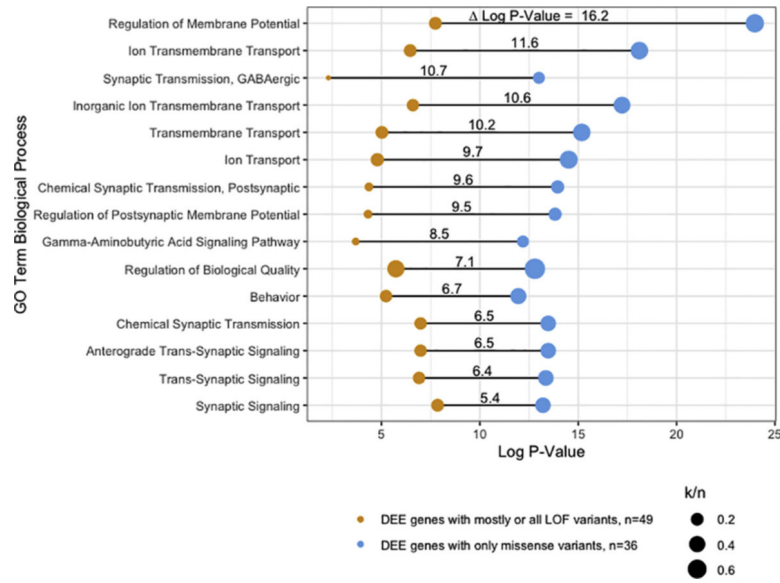
Fig. 1.
Functional annotation clustering of DEE genes by variant type. Visualization of enriched biological process Gene Ontology (GO) terms in two categories of genes – DEE genes with mostly or all LOF variants and DEE genes with only missense variants. Only GO terms with at least 5 genes in either category were included. Δ Log P-Value: difference in log p-value for clustering between the two gene categories; n: total number of genes in each category; k: number of genes in the total gene set annotated to the GO term.

Table 1

Seizure susceptibility in mouse models of human DEE.

Human DEE variant type	Mutant mouse strain with DEE variant		Other mutant mouse strain (no DEE variant)		No mutant mouse reported	Total (unique)
	Seizure susceptibility		Seizure susceptibility			
	Yes	None reported[a]	Yes	None reported[a]		
A. Missense only	DNM1[b], GNB1[c], GRIN2A, KCNT1, GABRB3[c,f], SCN1B, SCN8A	ALG13[d]	ALG13, ARV1, GABBR2, GABRB3, GABRG2[f], KCNQ2, KCNQ3, NEUROD2, SCN1B, SCN2A[c], SCN8A[c], SLC1A2, SLC25A12, SYNGAP1[e]	CACNA1E, CUX2, CYFIP2, DNM1, EEF1A2, FGF12, GABRA2, GABRA5, GABRB1, GABRB2, GRIN2B, GRIN2D, KCNT1, KCNT2, MEF2C, RNF13, SETD1A, ST3GAL3, USP19	RHOBTB2	36
B. Loss-of-function only	AP3B2, CPLX1, PLCB1, SZT2[f] LOF mouse	DMXL2, DOCK7, FRRS1L, PIGQ, TRAK1 In LOF and missense				9
C. Both LOF & missense	ARX[c], CACNA2D2[c], CDKL5, GABRA1[c,e], GNAO1, IQSEC2, MECP2, SCN1A, SLC12A5 CACNA1A[c], HNRNPU, KCNA2[e], PCDH19[f], STXBP1[c], SYNJ1, TBC1D24	AARS[e], ACTL6B, ADAM22[f], ARHGEF9, CAD, CHD2, DENND5A, GLS, GOT2[f], HCN1[e], ITPA, KCNB1, MDH2[e], PIGP[e], PNKP, PPP3CA, SCN3A, SIK1, SLC13A5, SMC1A, SPTAN1, UGDH, WWOX, YWHAG				40

[a] Excluding seizures described without documentation in manuscript text.

[b] The missense mutation in *Dnm1*[Ftfl] mice is not based on a human DEE variant but phenocopies multiple variants in the same domain.

[c] Mouse mutants that exhibit frequent spike-wave discharges (SWD).

[d] C57BL/6NJ-*Alg13*[N107S] mice exhibit no spontaneous seizures behaviorally or by conventional EEG and have normal electroconvulsive and PTZ seizure threshold (W. Frankel, unpublished results).

[e] Only one human loss of function DEE variant described.

[f] *SZT2* individuals are compound heterozygous, whereby one of the two allelic variants is always loss-of-function.

Table 2

Mouse models carrying orthologous variants (or equivalent) to human DEE and exhibiting seizure or comorbidity phenotypes.

Gene	DEE variant(s) Missense	LOF[a]	Seizure phenotype Spontaneous[e]	Susceptibility to induction[e,f]	Other neurological abnormalities Growth[g]	Cognitive	Social	Locomotor[h]	Reference
Ap3b2		✓	✓					✓	(Seong et al., 2005)
Arx	P355R, P355L	✓		✓	✓	✓			(Collombat et al., 2003; Kitamura et al., 2009)
Cacna1a		✓	✓	✓	✓			✓	(Fletcher et al., 1996; Mark et al., 2011; Miki et al., 2008; Rose et al., 2014)
Cacna2d2		✓	✓[d]	✓	✓			✓	(Ivanov et al., 2004)
Cdkl5		✓		✓		✓	✓	✓	(Amendola et al., 2014; Wang et al., 2012)
Cplx1		✓	✓		✓			✓	(Reim et al., 2001)
Dnm1	A408T (Ftfl)		✓	✓	✓			✓	(Aimiuwu et al., 2020; Asinof et al., 2015)
Gabra1		✓	✓		✓			✓	(Vicini et al., 2001)
Gabrb3	D120N		✓		✓	✓		✓	(Qu et al., 2020)
Gnao1	G203R	✓		✓	✓	✓	✓	✓	(Feng et al., 2019; Jiang et al., 1998)
Gnb1	K78R		✓	✓	✓	✓			(Colombo et al., 2019)
Grin2a	S644G		✓[b]	✓	✓	✓		✓	(Amador et al., 2020)
Hnrnpu		✓		✓	✓				(Dugger et al., 2020; Ye et al., 2015)
Iqsec2	A350V	✓	✓[c]		✓	✓	✓	✓	(Rogers et al., 2019; Sah et al., 2020)
Kcna2		✓	✓	✓	✓				(Brew et al., 2007)
Kcnt1	Y796H, R474H, P924L		✓	✓		✓		✓	(Quraishi et al., 2020; Shore et al., 2020)
Mecp2	R106Q, T158A, Y120D, A140V, P225R, R306C, T158M, R106W, P225R, P322L	✓	✓[b,c]	✓	✓	✓	✓	✓	(Dong et al., 2020; Gandaglia et al., 2019; Shahbazian et al., 2002; Zhang et al., 2014)
Pcdh19		✓	✓[d]		✓				(Pederick et al., 2018; Uemura et al., 2007)
Plcb1		✓	✓	✓	✓	✓		✓	(Kim et al., 1997)
Scn1a	R1648H, A1783V	✓	✓[b,c]	✓	✓	✓		✓	(Kuo et al., 2019; Martin et al., 2010; Tsai et al., 2015)
Scn1b	C121W	✓	✓[b,c]	✓	✓				(Kruger et al., 2016; Wimmer et al., 2010)

Gene	DEE variant(s)		Seizure phenotype			Other neurological abnormalities				Reference
	Missense	LOF[a]	Spontaneous[e]		Susceptibility to induction[e,f]	Growth[g]	Cognitive	Social	Locomotor[h]	
Scn8a	R 1872W, N1768D	✓	✓[b,c]			✓			✓	(Bunton-Stasyshyn et al., 2019; Jones and Meisler, 2014; Wagnon et al., 2015)
Slc12a5		✓	✓		✓	✓				(Silayeva et al., 2015; Woo et al., 2002)
Stxbp1		✓	✓				✓	✓	✓	(Chen et al., 2020)
Synj1		✓	✓			✓			✓	(Cremona et al., 1999; Wimmer et al., 2010)
Szt2		✓	✓		✓					(Frankel et al., 2009)
Tbcld24		✓	✓							(Tona et al., 2019)

[a] One or more knockout or hypomorphic mouse mutation has been described.

[b] Observed in one or more missense mutation.

[c] Observed in one or more LOF or hypomorphic mutation.

[d] Spike-wave discharges (SWD) only.

[e] Blank cells mean either not tested or not susceptible.

[f] May include pentylenetetrazole, kainic acid, electroconvulsive, audiogenic, fluorothyl or febrile stimuli.

[g] May include growth delay, reduced body weight, small stature.

[h] May include ataxia, dystonia, hyperactivity, hypoactivity.