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Can mutation-mediated effects occurring early in development cause long-term seizures susceptibility in genetic generalised epilepsies?

Running title: Early developmental changes in genetic generalised epilepsy

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1 Figure and 0 tables

Abstract (159)

Epilepsy has a strong genetic component, with an ever increasing number of disease-causing genes being discovered. Most epilepsy-causing mutations are germ line and thus present from conception. These mutations are therefore well positioned to have a deleterious impact during early development. Here we review studies that investigate the role of genetic lesions within the early developmental window specifically focusing on genetic generalised epilepsy (GGE). Literature on the potential pathogenic role of sub-mesoscopic structural changes in GGE is also reviewed. Evidence from rodent models of genetic epilepsy support the idea that functional and structural changes can occur in early development leading to altered seizure susceptibility into adulthood. Both animal and human studies suggest that sub-mesoscopic structural changes occur in GGE. The existence of sub-mesoscopic structural changes prior to seizure onset may act as biomarkers of excitability in genetic epilepsies. We also propose that pre-symptomatic treatment may be essential for limiting the long term consequences of disease-causing mutations in genetic epilepsies.

- Genetic contributions are increasingly being recognised in the pathogenesis of epilepsy with mutations mostly present from conception.
- Disease-causing mutations can impact in early development to set seizure susceptibility into adulthood in animal models of disease.
- Sub-mesoscopic structural changes occur in genetic generalized epilepsy and may be biomarkers of excitability.
- Anti-epileptogenic drugs used before overt symptoms are observed may alleviate both seizures and comorbidities.

Epilepsy affects up to 4% of the population at some time in their lives ¹. It is characterized by recurrent paroxysms resulting from hyper-synchronous discharges in the brain. There are major challenges in clinical epilepsy care with at least 30% of patients resistant to current therapies. Even amongst those patients whose seizures are controlled, major issues of drug side-effects and comorbidities often affect quality of life. Although it is difficult to establish an exact proportion, it is becoming increasingly clear that genetic factors play a significant pathogenic role in a majority

of the epilepsies with well over 50 epilepsy genes now identified ²⁻⁵. Knowledge of these causative mutations provides an unprecedented opportunity to investigate the underlying mechanisms of epileptogenesis in the genetic epilepsies. Much effort is placed on understanding the acute impact of genetic variation on neuronal excitability with the view that the protein products of this variation drive cellular dysfunction. However, because of the coordinated control of gene expression, these genetic mutations can exert their effects at different periods throughout life and are therefore positioned to divert the course of brain development and consequently long-term seizure susceptibility (Figure 1). Here we review both clinical and experimental evidence suggesting that genetic mutations can impact early development and discuss this paradigm with specific focus on genetic generalised epilepsy (GGE).

Genetic epilepsies

The new ILAE Classification stipulates that "The concept of a genetic epilepsy is that it directly results from a known or presumed genetic mutation in which seizures are a core symptom of the disorder" ⁶. The archetypical genetic epilepsy is GGE, also known as idiopathic generalized epilepsy (IGE). This form of epilepsy has the highest heritability of the common epilepsies, although the majority of genes that increase the risk for GGE are yet to be identified. GGE is a family of related syndromes that are associated with a characteristic generalized spike-and-wave discharge on electroencephalogram (EEG) and usually beginning in childhood or adolescence ⁷. Common GGE syndromes include childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy and generalized tonic-clonic seizures alone ⁶. There is also an increasingly recognized genetic component to focal epilepsies ⁸ and the rarer but devastating epileptic encephalopathies have emerged as a group of predominantly *de novo* dominant disorders ³. Although the latter two groups are not the focus of this review it is of interest that genes for the epileptic encephalopathies also raise risk for GGE ² suggesting that biological observations made in GGE may be generally applied.

Functional developmental changes altering long-term seizure susceptibility in models of genetic epilepsy

Rodent models of generalised epilepsy have given insight into the potential role genetic lesions play in early development. Investigations using the Wistar albino

Glaxo rats of Rijswijk (WAG/Rij) model of polygenic GGE revealed that treatment with the antiepileptic drug ethosuximide during the early epileptogenic period has a sustained impact on disease progression⁹. A persistent suppression of seizures was observed even after the therapy was discontinued⁹. This early treatment also blocked changes in the expression of Na_v1.1, Na_v1.6, and HCN1 ion channels that are markers of disease in this rat model⁹. A long term protective effect of early antiepileptic drug treatment on seizure susceptibility was also observed in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), another inbred model of polygenic GGE¹⁰. Similarly, treatment in early development of a transgenic mouse model, based on a dysfunctional Kv7 channel, with the sodium-potassium-chloride cotransporter 1 (NKCC1) antagonist bumetanide normalised hippocampal structural changes, electrophysiological properties and some behavioural markers of hyperexcitability¹¹.

To test this idea more directly, a conditional mouse model based on the GABA_A $\gamma 2_{R43Q}$ GGE mutation was engineered allowing the investigation of the impact of a single known epilepsy gene in early development^{12; 13}. Although rare, patients harbouring the GABA_A $\gamma 2_{R43Q}$ mutation have epilepsy phenotypes that are common, including childhood absence epilepsy and febrile seizures¹³. Understanding the principal pathogenic mechanisms caused by the GABA_A $\gamma 2_{R43Q}$ are expected to have broader implications, although their relevance for understanding GGE due to other genetic mechanisms is not currently known. Mice in which the mutation had been silenced during early development were less susceptible to a proconvulsant challenge than mice that had expressed the epilepsy-causing mutation from conception¹⁴. This suggests that the GABA_A $\gamma 2_{R43Q}$ mutation is most likely altering neuronal network architecture in the early developmental window to set seizure susceptibility later in adulthood.

Similar findings have been observed in other mouse models of epilepsy. *SLC2A1* encodes for GLUT1 that is responsible for glucose transport across the blood brain barrier with mutations causing deficiency in this protein accounting for up to 1% of GGE¹⁵. GLUT1 is also a cause of early onset CAE¹⁶. A *Slc2a1* haploinsufficient mouse recapitulates many of the features of GLUT1-deficiency including a spike and wave discharge phenotype¹⁷. Recent efforts have demonstrated that viral-mediated gene replacement therapy was able to reverse many of the disease phenotypes in the

Glut1-deficient mouse including epileptiform activity¹⁸. Interestingly, Tang and colleagues show that the effectiveness of gene therapy is largely limited to treatment in a narrow pre-symptomatic postnatal window¹⁸. Although not GGE specific, another example includes a transgenic mouse model of familial nocturnal frontal lobe epilepsy based on a *CHRNA2* mutation that causes a gain-of-function in the encoded $\beta 2$ nicotinic receptor¹⁹. When expressed throughout life mice carrying this mutation display a spontaneous epileptic phenotype with high frequency interictal spikes and seizures¹⁹. In a conditional model of this disease, the silencing of the transgene expression during early development was sufficient to prevent the occurrence of seizures in adulthood¹⁹. Further, seizures in a mouse model of Angelman syndrome (an epileptic encephalopathy) based on the loss of function of the maternal ubiquitin protein ligase E3A (UBE3A) are only suppressed when the protein is reinstated in an early developmental period²⁰. Together, these studies strongly suggest that there are pre-symptomatic changes occurring in neuronal networks that are caused, at least in part, by underlying genetic factors. Altered neuronal network structure during early development is one mechanism which may cause long-term increases in seizure susceptibility and is discussed next.

Structural brain abnormalities in generalized epilepsy

Human studies

There is strong evidence linking structural changes in the brain and seizure genesis in acquired focal epilepsy. Temporal lobe epilepsy, the archetypal acquired epilepsy syndrome, is sometimes associated with structural changes, particularly hippocampal sclerosis²¹. Similarly, for genetic epilepsies such as tuberous sclerosis and familial multiple cavernous malformation syndrome, brain malformations are evident and clearly contribute to hyperexcitability. Surgical removal of the brain malformation in both these genetic diseases can be effective in reducing seizure burden^{22; 23}.

In contrast, GGE mesoscopic scale structural lesions cannot be detected by conventional diagnostic methods such as gross anatomical analysis or magnetic resonance imaging (MRI). Historically this was part of the basis for the designation of these epilepsies as 'idiopathic'. However, studies based on more advanced imaging technologies have begun to resolve subtle differences in brain structure in GGE²⁴⁻²⁷. For example, MRI volumetry and voxel-based morphometry studies have reported

regional changes, particularly in the thalamus, of GGE patients ^{24; 26}. Changes in cortical gray matter volumes have also been reported ^{24; 28}. Whether these more subtle structural changes are a contributing factor to, or a consequence of, seizures is not known. It is also not clear if these structural changes occur in pre-symptomatic patients when they could act as ideal biomarkers of disease progression. Animal models that enable investigation of neuronal morphology changes in the pre-seizure brain will help us to resolve the relationships between these structural changes and disease progression.

Rodent Models

Preclinical MRI studies in rodent models support the emerging view that structural brain changes can occur in GGE. Structural MRI has detected differences in GAERS including increases in amygdala, cortical and ventricular volumes ²⁹. Axonal connectivity changes measured using diffusion tensor imaging suggest that chronic seizures in the WAG/Rij and GAERS rat models of GGE causes reduction in myelin and/or decreased axon fibre density changes in the corpus callosum ³⁰. Functional connectivity studies using MRI have also demonstrated abnormally high degree of cortical-cortical correlations in the WAG/Rij model supporting the idea that neuronal connectivity is altered in these mice ³¹. However, studies reporting structural changes all investigate rodents that are already epileptic making it difficult to assess if these morphological changes are a result of seizure activity or are themselves the cause of seizures. In support of the former, axon fibre density changes are not seen in pre-seizure WAG/Rij rats suggesting that at least some changes occur as a result of the seizures themselves ³⁰. Unfortunately, evidence for structural changes in the absence of overt seizures is limited. In one study, high field MRI in fixed brains of the GABA_A $\gamma 2_{R43Q}$ mouse model of GGE revealed a small increase in dentate granule cell layer volume of the hippocampi of non-seizing mice ³². This marks one of the first indications that morphological changes can occur in GGE due to the direct impact of an epilepsy mutation and not as a consequence of seizure activity. Although these morphological changes are well placed to contribute to enhanced excitability, experiments have not yet been performed which can elucidate their role in pathogenesis. Ongoing imaging studies in other genetic rodent models of GGE are required to assess if similar pre-symptomatic morphological changes are a common hallmark of the disease.

Current imaging techniques are not sensitive to various microscopic changes in neuronal morphology such as changes in neuron shape; and position, arborisation and density of dendritic spines and axonal boutons. Such changes can provide structural substrates for pathological changes in excitability. In the GABA_A $\gamma 2_{R43Q}$ mouse model stereological analysis of seizure free brains showed a 33% increase in dentate granule cell density in the hippocampus³². Further quantitative morphological analysis of the GABA_A $\gamma 2_{R43Q}$ mouse using three-dimensional reconstruction of cortical columns showed that changes in inhibitory neuron numbers and positions presaged the occurrence of seizures³³. Specifically, the densities of GABA-, calretinin-, parvalbumin-, and calbindin-containing inhibitory neurons were increased in the cortex of GABA_A $\gamma 2_{R43Q}$ mice³³. Morphological analysis has also revealed changes in the microarchitecture of GAERS brains. Similar to that observed in the GABA_A $\gamma 2_{R43Q}$ mouse, GABAergic neuron numbers are increased in the lateral geniculate, ventral posteromedial, and ventral posterolateral thalamic nuclei of GAERS model when compared to Wistar controls³⁴. Although this seems paradoxical, others have proposed circuit level increases in GABA_A receptor-mediated transmission can be epileptogenic³⁵. Finally, in the WAG/Rij model, Golgi staining reveals differences in dendritic arborisation in the cortex³⁶. Collectively, this data strongly supports that microarchitecture changes occur in the genetic epilepsies prior to and after the appearance of seizures and that subtle change in brain structure may be part of an underlying epileptogenic and post seizure pathogenic processes. More advanced non-invasive imaging modalities that are capable of detecting these microscopic changes are needed to test these ideas in pre-clinical animal models and humans.

Exploring the impact of a GABA_A epilepsy mutation in early development

To develop new therapeutic approaches targeted within early developmental windows we will need to resolve underlying pathogenic mechanisms. Dysfunctional proteins may exert their impact through a raft of different mechanisms (Figure 1), during different developmental time periods and each mutation may do so in a unique way. However, in the genetic epilepsies it is likely that common mechanistic pathways will emerge that could be used as drug targets to treat patients before seizures occur.

One broad pathogenic mechanism may be the disruption of early developmental oscillatory behaviour that is critical for the refinement of neuronal networks. Discontinuous structure of EEG activity, alternating between periods of oscillatory discharges and network silence, in premature infants are thought to reflect such activity³⁷. Rodents at birth provide ideal models for investigating these early events given they correspond to the second gestational trimester in humans³⁸. Neonatal spindle bursting is an example of an early developmental oscillatory network behaviour in rodents that is similar to that seen in humans³⁷. Giant depolarising potentials (GDPs) are localised synchronised events that can be measured in brain slices cut from rodents early in development allowing more detailed molecular mechanisms to be resolved³⁹. The coordinated activation of a range of ion channels is critical to the generation of GDPs, with excitatory GABA_A receptor transmission a particularly important driver of this activity³⁹. We next discuss structural and functional evidence obtained from the GABA_A $\gamma 2_{R43Q}$ mouse model of GGE to highlight how disruptions in early oscillatory activity could lead to long-term changes in network excitability.

A key difference in the GABAergic system in early development compared to adulthood is how neurons regulate intracellular chloride ions (Cl⁻). At these early developmental time-points neurons primarily express NKCC1. This transporter pumps Cl⁻ into the cell while the K-Cl cotransporter 2 (KCC2) that is expressed in adult neurons efficiently pumps Cl⁻ out⁴⁰. As a result of this expression profile immature neurons have higher internal Cl⁻ concentrations. This high internal concentration shifts the reversal potential of Cl⁻ so that at typical holding potentials Cl⁻ leaves the neuron and the resulting depolarisation takes it closer to the threshold for action potential firing⁴⁰. Synaptic release of GABA acting on GABA_A receptors under these conditions is therefore excitatory. Recurrent spontaneous GDPs are an early developmental neuronal network phenomenon that relies on this excitatory GABA_A receptor-mediated transmission^{41; 42}. These events cause neuronal synchronized firing and set up Ca²⁺ oscillations that help the establishment and refinement of network circuitry⁴³.

Neonatal hippocampal slices from the GABA_A $\gamma 2_{R43Q}$ mouse model of GGE have a lower frequency of GDPs when compared to their wildtype littermates⁴⁴. A similar

reduction in GDP frequency can be achieved pharmacologically using bumetanide which blocks NKCC1 and consequently shifts the reversal potential of Cl^- so that it is no longer excitatory^{45; 46}. Interestingly, wild-type mice treated with bumetanide in the antenatal period have a long-lasting increase in thermogenic seizure susceptibility that is comparable with that observed in the $\text{GABA}_A \gamma 2_{\text{R43Q}}$ mouse⁴⁴. It should be noted that bumetanide given early in development has a protective effect in a genetic mouse model based on dysfunctional Kv7 channels, normalising several cellular and behavioural markers of hyperexcitability¹¹. Related work has also shown that manipulations of GABAergic transmission, using other pharmacological tools, in this early developmental window have a long-lasting impact on neuron circuitry, potentially influencing a range of behaviours including altered seizure susceptibility in adulthood⁴⁷⁻⁴⁹.

Perturbation of the GABAergic system in early development has previously been postulated to influence the migratory behaviour and differentiation of cortical neurons⁵⁰. For instance, GABA-signalling stimulates the migration of embryonic cortical cells with concentration-dependent effects^{47; 51; 52}. A causative link has not yet been established between reductions in GDP frequency and changes in neuron positioning in the $\text{GABA}_A \gamma 2_{\text{R43Q}}$ mouse³³. Further, how changes in neuronal migration, proliferation, differentiation may alter long-term network excitability remains an open question. One speculation is that subtle changes due to the mis-positioning or change in numbers of certain GABAergic neurons may alter the excitatory-inhibitory balance of local circuits leading to a susceptibility to increased excitability. Long-term structural modifications to neuronal morphology may also occur. As an example, reduced dendritic branching has been implicated as part of the pathogenic mechanism of increased neuronal excitability in both acquired and genetic epilepsy^{53; 54}. Although the precise mechanism may remain unclear, the early developmental GABAergic system is well positioned to contribute to morphological changes in the young brain and consequently long-term neuronal hyper-excitability. This system is also likely to be amenable to therapeutic intervention, for example, increasing GDP frequency in $\text{GABA}_A \gamma 2_{\text{R43Q}}$ patients using drugs that increase GABA_A receptor activity may reduce future seizure risk.

Implications for comorbid pathologies in epilepsy

There is a vast literature implicating dysfunction in early development as a predisposing factor for a range of neurological conditions. Interestingly, studies in humans indicate that major depression, bipolar disorders, psychiatric history and cognitive impairment are strongly associated with an increase in the risk of developing epilepsy⁵⁵⁻⁵⁹. These clinical relationships suggest that common network pathologies may underlie both changes in seizure susceptibility and increased risk of psychiatric and cognitive disorders. There is also evidence for this at the neuronal structure level. For example, dynamic changes in dendritic spines are associated with epilepsy; they are also seen in a number of neuropsychiatric and cognitive disorders, including autism spectrum disorders, schizophrenia and Alzheimer's disease^{60; 61}. As a consequence, early therapeutic intervention that normalises network dysfunction may also alleviate comorbid states. One experimental example of this is that the anxiety-like behaviour, that is a comorbid state in GAERS, is reduced in adult rats that had only been treated during the pre-symptomatic period¹⁰.

Precision medicine implications

The understanding of the genetic basis of many of the epilepsies provides an opportunity to generate precision medicine strategies. Part of this strategy will be to understand the early developmental consequences of variants that raise risk for epilepsies and its comorbidities. Here we have highlighted how animal models are beginning to shed light into how they exert their effect on the developing brain. Another particularly promising approach is the use of human stem cell-based neural models. The fundamental mechanisms of neurogenesis can be modelled by the *in vitro* differentiation of neurons from pluripotent cells, such as embryonic stem or patient derived induced pluripotent (iPS) cells. For instance, *in vitro* protocols for neural differentiation enable the faithfully reproduction of the temporal appearance, spatial organisation and gene expression profiles of developing neurons *in utero*⁶²⁻⁶⁵. These methods have been used to model neural microarchitecture changes that accompany epilepsy disease progression, such as impaired neurite outgrowth in iPS-derived neurons from individuals with Ohtahara syndrome, a severe epileptic encephalopathy⁶⁶. Further, iPS cell-derived GABA positive inhibitory neurons carrying a mutation in *SCN1A* have been shown to replicate disease pathology previously identified in animal models including a reduction in voltage dependent Na⁺ currents in inhibitory neurons⁶⁷. Recently, neurodevelopmental and synaptic changes

in a human stem cell-based model of tuberous sclerosis were rescued using pharmacological intervention⁶⁸. This growing body of research indicates that stem cell-based models will be integral to helping our understanding of early developmental pathogenic changes caused by epilepsy mutations, many of which may be difficult to study in whole organisms, and for which novel therapeutic strategies can be developed.

Taking these ideas to the clinic

The major conclusion of this review is that disease-targeted intervention may need to occur in pre-symptomatic GGE patients to be effective. This will require the identification of patients that will benefit from such early intervention. As our understanding of the genetic architecture improves and pre- and post-natal genetic screening becomes more widely employed it will be possible to identify individuals who have an increased risk of developing epilepsy. However, as incomplete penetrance and variable expressivity is commonly observed it may be difficult to predict accurately the clinical outcome simply from DNA sequence analysis alone⁶⁹. Additional biomarkers are therefore going to be essential to make informed decisions regarding the suitability of individuals for early therapeutic intervention. Improving human imaging technologies are enabling us to better map the brain from both a structural and functional perspective allowing us to identify morphological and/or functional markers of excitability in GGE and other genetic epilepsies. Although limited in scope, evidence from animal models suggests that subtle changes in pre-seizure brains can indeed be detected. Finally, it is important to note that we must develop new therapeutic strategies, moving away from the development of classical anti-epileptic drugs that treat the symptoms of epilepsy ('anti-seizure') to those that are anti-epileptogenic and target the underlying disease mechanism to prevent the development of epilepsy and its comorbidities.

Figure 1

Cartoon illustrating the potential that an epilepsy mutation can alter neuronal network structure and function in early development to set seizure susceptibility in adulthood.

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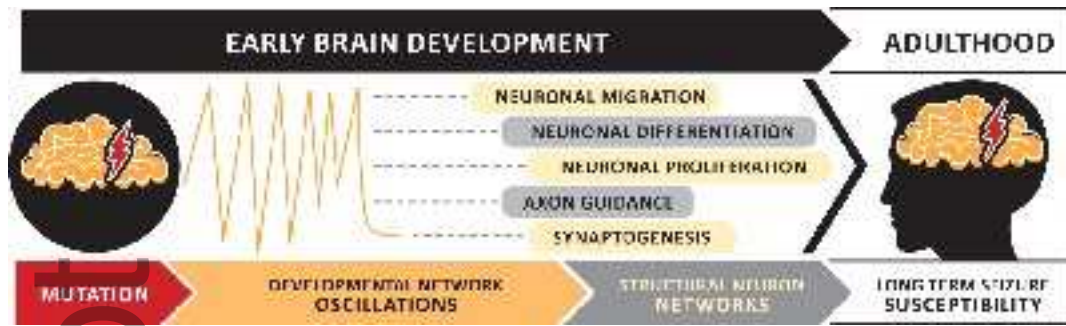
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