Molecular Pharmacology Fast Forward. Published on April 22, 2015 as DOI: 10.1124/mol.115.097998
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Title Page

Ionotropic GABA and Glutamate Receptor Mutations and Human Neurological Diseases

Hongjie Yuan, Chian-Ming Low, Olivia A. Moody, Andrew Jenkins, and Stephen F. Traynelis

Department of Pharmacology, Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta GA 30032 (H.Y., A.J., S.F.T.)

Department of Pharmacology and Department of Anaesthesia, Yong Loo Lin School of Medicine, NUS Graduate School for Integrative Sciences and Engineering, and Neurobiology/Ageing Programme, National University of Singapore, 28 Medical Drive, S117456 (C-M.L.)

Department of Anesthesiology, Emory University School of Medicine, Rollins Research Center, 1510 Clifton Road, Atlanta GA 30032 (O.A.M., A.J.)

Molecular Pharmacology Fast Forward. Published on April 22, 2015 as DOI: 10.1124/mol.115.097998
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Running Title Page

Running Title: GABA/glutamate receptor mutations in neurological diseases

Corresponding Author: Stephen F. Traynelis

1510 Clifton Road, Rollins Research Center, Atlanta, GA 30322.

Phone: (404) 727 0357

Fax: (404) 727 0365

E-mail: strayne@emory.edu

Molecular Pharmacology Fast Forward. Published on April 22, 2015 as DOI: 10.1124/mol.115.097998
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Abbreviations: (in alphabetical order)

ADHD: attention deficit hyperactivity disorder

AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ASD: autism spectrum disorder

ATD: amino terminal domain

BECTS: benign epilepsy with centrotemporal spikes

CACN: calcium channel gene, voltage-dependent

CAE: childhood absence epilepsy

CFTR: cystic fibrosis transmembrane conductance regulator

CHRN: cholinergic receptor gene, nicotinic

CNS: central nervous system

CTD: carboxy terminal domain

DD: developmental delay

DNM: *de novo* mutations

DS: Dravet syndrome

EIEE: early infantile epileptic encephalopathy

FS: febrile seizures

GABA: gamma-amino butyric acid

GABR: $GABA_A$ receptor gene

GWAS: genome-wide associational studies

ID: intellectual disability

GEFS+: generalized epilepsy with febrile seizures plus

KA: kainate acid

KCN: potassium channel gene

IGE: idiopathic generalized epilepsy

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JME: juvenile myoclonic epilepsy.

LBD: ligand binding domain

MR: mental retardation

NMDA: *N*-Methyl-D-Aspartate

SCN: sodium channel gene, voltage gated

SNP: single nucleotide polymorphism

UTR: untranslated region

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Abstract

The advent of whole exome/genome sequencing and the technology-driven reduction in the cost of next generation sequencing, as well as the introduction of diagnostic targeted sequencing chips, have resulted in an unprecedented volume of data directly linking patient genomic variability to disorders of the brain. This information has the potential to transform our understanding of neurological disorders by improving diagnoses, illuminating molecular heterogeneity underlying diseases, and identifying new targets for therapeutic treatment. There is a strong history of mutations in GABA receptor genes being involved in neurological diseases, particularly the epilepsies. In addition, a substantial number of variants and mutations have been found in GABA receptor genes in patients with autism, schizophrenia, and addiction, suggesting potential links between the GABA receptors and these conditions. A new and unexpected outcome from sequencing efforts has been the surprising number of mutations found in glutamate receptor subunits, with the *GRIN2A* gene encoding the GluN2A NMDA receptor subunit being most often affected. These mutations are associated with multiple neurological conditions, for which seizure disorders comprise the largest group. The GluN2A subunit appears to be a locus for epilepsy, which holds important therapeutic implications. Virtually all AMPA receptor mutations, most of which occur within *GRIA3*, are from patients with intellectual disabilities, suggesting a link to this condition. Similarly, the most common phenotype for kainate receptor variants is intellectual disability. Herein we summarize the current understanding of disease-associated mutations in GABA and glutamate receptor families, and discuss implications regarding the identification of human mutations and treatment of neurological diseases.

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Introduction

The control of ion flow across the lipid membrane is essential for many cellular functions, including hormone secretion, volume regulation, motility, muscle contraction and neuronal excitability. Inherited and *de novo* mutations in channels and transporters, the conduits that convey ions through lipid bilayers, are associated with many diseases, including diabetes, hypertension, cardiac arrhythmia, asthma, cystic fibrosis as well as multiple neurological diseases, some of which we will focus on here. The term "channelopathy" refers to a disease that arises due to the defect in a particular ion channel. Despite origins within a single molecular species (a channel), channelopathy etiology is often defined by a complex interaction between many processes, and thus similar symptoms can arise from mutations in different channels. In order to understand pathogenesis of channelopathies, we must disentangle these effects, especially if we are to design targeted therapeutics to mitigate the ramifications of the causative mutation. For example, long-QT syndrome, a delay in cardiac ventricular repolarization, can be caused by mutations in several different voltage-gated ion channel genes, including KCNQ1, KCNH2, KCNJ2, SCN5A, CACNA1C (Campuzano et al., 2010). Most instances of cystic fibrosis can be attributed to 1 of 5 CFTR classes of mutations, yet a myriad of disease-related mutations have been reported (De Boeck et al., 2014). Mutations in another ABC transporter (ABCC8) can cause 3 diseases: familial hyperinsulemic hypoglycemia and two forms of neonatal diabetes (Aittoniemi et al., 2009). The same 3 diseases can result from mutations in the Kir6.2 inwardly rectifying potassium channel gene KCNJ11 (Aittoniemi et al., 2009). In the CNS, mutations in both SCN1A and GABRG2 are associated with Dravet syndrome (Huang et al., 2012). Another epilepsy (EIEE) can be categorized into multiple subtypes, many being associated with a different channelopathy in a different gene (KCNQ2, SCN2A, SCN8A, KCNT1; Kim 2014). Finally, mutations in the CHRNA2, CHRNA4, CHRNB2, result in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) while mutations in CHRNA1, CHRNB1, CHRND and CHRNE result in congenital myasthenic syndrome (Steinlein and Bertrand, 2010; Kim 2014). The examples given above emphasize that these diseases are multifactorial, and that individual channel isoforms subserve many functions. This is especially true for the complex disorders of the central nervous system.

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The brain is comprised of networks of neurons interconnected by excitatory and inhibitory synapses, which generate patterns of activity that encode and convey information. Inhibitory synaptic signaling in the brain is mediated primarily by the vesicular release of the neurotransmitter GABA, which acts on a large family of postsynaptic ligand-gated ion channels that are pentameric assemblies of $GABA_A$ receptor subunits (Benarroch 2007; Brickley and Mody, 2012; Lee and Maguire, 2014; Kowalczyk and Kulig, 2014). The vast majority of excitatory synaptic transmission involves the vesicular release of the neurotransmitter glutamate, which activates a group of tetrameric receptors that can be divided into three subtypes (AMPA, kainate, NMDA) on the basis of pharmacology and structure (Traynelis et al., 2010; Paoletti et al., 2013). In addition to fast synaptic signaling, slower neurotransmission also occurs for both glutamate and GABA, and can involve both ionotropic and metabotropic receptors. The balance of excitation and inhibition in the CNS is controlled by multiple mechanisms, and aberrations in this balance can lead to abnormal neuronal firing, altered network activity, and neuropathology. When excitatory synaptic transmission proceeds unchecked due to attenuation of GABAergic transmission, it can lead to hypersynchronous neuronal firing, abnormal burst generation, and seizures (Hines et al., 2011; Macdonald et al., 2010). Overactivation of Ca^{2+}-permeable glutamate receptors can be neurotoxic, and exacerbates neuronal death in brain injury (Garthwaite and Garthwaite, 1991; Choi 1994). Excess inhibition can cause several different clinical conditions, including absence seizures (Crunelli et al., 2011; Yalçın 2012), pathological sleepiness and dysphoria (Rye et al., 2012).

Whereas the vast majority of known channelopathies involve voltage-gated channels, many missense GABA receptor mutations have been reported over the past decade in patients with various neurological diseases, including epilepsy (Table 1). Even more recently, a surprising number of disease-associated glutamate receptor mutations have been identified, with >80% found within the NMDA receptor subfamily (Table 2, reviewed by Soto et al., 2014; Burnashev and Szepetowski, 2015). In both GABA and glutamate receptor families, mutations have been described that disrupt protein structure,

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conformation, abundance, or localization. Missense mutations encode a different amino acid at a specific position, splice junction mutations alter alternative splicing or lead to protein truncation, and insertions or deletions can lead to a frame shift and protein truncation by a premature stop codon in the new reading frame. In addition, some mutations alter protein trafficking or RNA stability, leading to changes in the level and location of receptors that reach the neuronal plasma membrane (e.g. Macdonald and Kang, 2012; see below). By changing critical channel properties, surface expression, or localization, mutations can alter neuronal signaling, which leads to changes in brain function that can underlie patient symptoms (e.g. Macdonald et al., 2010; Pierson et al., 2014; Yuan et al., 2014). Adaptive neurobiological consequences of perturbations in neuronal function also occur secondary to the effects of the mutations and thus functionally relevant mutations can alter circuitry to create neuropathological situations that subsequently persist and drive symptoms independent of the initial insult (e.g. Jefferys and Whittington, 1996). Moreover, molecularly distinct mutations can interact with adaptive changes to produce at times common physiological effects.

Recent advances in next generation sequencing technologies have led to a dramatic increase in the amount of exome sequencing data available, which has accelerated our understanding of human mutations in neurological disease, including mutations in the channels that mediate inhibitory and excitatory synaptic transmission. In this review, we summarize the current state of knowledge for human disease-associated mutations in ionotropic GABA and glutamate receptor families, with a discussion of the implications for advancing the understanding of these conditions. We focus here on *de novo* mutations (newly acquired mutation in the patient that is absent in the healthy parents) as well as inherited rare variants that occur with a frequency less than 1% in the general population, and thus are potential disease-associated mutations.

$GABA_A$ Receptors

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$GABA_A$ receptors are cys-loop ligand-gated chloride/anion channels that broadly dampen neuronal electrical excitability, as well as regulate spike timing, thereby controlling circuit function. Functional pentameric $GABA_A$ receptors can be homomeric, or heteromeric assemblies of up to 3 of 19 *GABR* gene products (*GABRA1-6, GABRB1-3, GABRG1-3, GABRR1-3, GABRD, GABRE, GABRP, GABRQ*, Olsen & Sieghart, 2009, Fig. 1). Receptors exhibit different spatial and temporal expression in the mammalian CNS (Pirker et al., 2000) and the structural differences in each subunit account for differences in receptor pharmacology, subcellular localization, and intrinsic channel kinetics (Lavoie et al., 1997). Receptors concentrated in the synapse provide brief but strong inhibition, whereas those located more diffusely in perisynaptic or extrasynaptic locations can cause a long-lived inhibitory shunt in response to ambient GABA, often amplified by neurosteroids or the presence of alcohol (Jia et al., 2007; Belelli et al., 2009).

It is not surprising that the disorders associated with the $GABA_A$ receptor are behaviorally complex and multifactorial, since $GABA_A$ receptors and GABAergic neurons are very heterogeneous and widespread throughout the CNS. The GABA receptor genes show a range of tolerances to mutations that are predicted to damage the encoded protein in the general population (Table 1), with genes that harbor fewer than expected protein-disrupting mutations (*GABRG1, GABRB2*) considered less tolerant to mutations (see Petrovski et al., 2013). The roles of most *de novo* or inherited mutations in disease progression are not well understood, even though the majority of these mutations have been associated with autism, epilepsy, schizophrenia or addiction disorders (see Table 1). Whereas most of the rare variants (<1% of the general population) are *de novo* mutations verified in trios, early infantile epileptic encephalopathy, generalized epilepsy with febrile seizures plus, idiopathic generalized epilepsy and febrile seizures have been linked to heritable mutations in *GABR* genes (Singh et al., 1999; Baulac et al., 2001; Wallace et al., 2001; Kananura et al., 2002; Dibbens et al., 2004; Lenzen et al., 2005; Audenart et al., 2006; Carvill et al., 2013a, 2014). In addition, susceptibility to alcohol dependence, childhood absence epilepsy, and juvenile myoclonic epilepsy are all strongly associated with *GABR* mutations (Cossette et al., 2002; Radel et al., 2005; Maljevic et al., 2006; Lachance-Touchette et al., 2011). These heritable

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diseases are restricted to 5 of the 19 genes at 4 vulnerable chromosomal locations: 5q34, 4p12, 15q12 and 1p36 (www.ncbi.nlm.nih.gov/omim).

$GABA_A$ receptors had long been considered to play a central role in epilepsy (Jasper, 1984), a view eventually confirmed at a molecular level (Baulac et al., 2001; Wallace et al., 2001, Cossette et al., 2002). In these human studies, an inherited mutation in the $GABA_A$ receptor γ2 subunit was associated with the manifestation of the disease. Since these important discoveries, many additional *GABR* mutations have been described in the literature (Supplemental Table S1). Of these, relatively few *GABR* missense mutations have been studied functionally, limiting insight into how the mutations might alter neuronal and circuit function, and ultimately impact neurological disease. One exception to the lack of a functional understanding has come from a body of work from Macdonald and coworkers, who have documented the functional effects of 15 mutations linked to inherited epilepsies in *GABRA1, GABRB3, GABRG1* and *GABRD* genes, advancing our understanding of this complex family of diseases (reviewed by Kang and Macdonald, 2009; Macdonald et al., 2010). Interestingly, 11 of these 15 mutations occur in the mature peptide, allowing the use of *in vitro* techniques to assess changes in functional and molecular properties. The remaining 4 mutations are either intronic, or reside in regions encoding the promoter or signal peptide.

The results of these studies are fascinating yet complex, and show that receptor dysfunction can occur via a wide array of deficits. For example, the mutations γ2(R82Q) and γ2(Q390X) (also referred to as R43Q and Q351X) are both retained in the ER, resulting in childhood absence epilepsy/febrile seizures (CAE/FS) and generalized epilepsy with febrile seizures plus/Dravet syndrome (GEFS+/DS), respectively (Wallace et al. 2001; Harkin et al., 2002; Kang and Macdonald 2004; Kang et al., 2009). Although αβ heteromers lacking the γ subunit are functional and exhibit higher potency (e.g. lower EC_{50}) for GABA than αβγ assemblies, the loss of the γ subunit impairs the targeting of αβ receptors to the synapse via the loss of interactions with the GABARAP-gephyrin trafficking-scaffolding machinery. In addition, β3(P11S), β3(S15F) and β3(G32R) all result in N-linked glycosylation errors, impairing normal GABA

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receptor mediated inhibition, resulting in GEFS+ and/or DS (Tanaka et al., 2008; Gurba et al., 2012). In addition to those noted above, ten more mutations in the most abundant alpha subunit, α1, have been linked with idiopathic epilepsies, Dravet syndrome and epileptic encephalopathies. The mutations include 2 gene deletions, 5 point mutations, an intronic insertion and a nonsense mutation. As with other epilepsy-associated GABR mutations, functional deficits include reduced cell surface expression and impaired receptor activation (see Suppl. Table S1; Fisher, 2004; Krampfl et al., 2005; Klassen et al., 2011; Lachance-Touchette et al., 2011; Mefford et al., 2011; Epi4K, 2013; Carvill et al., 2014; Olson et al., 2014). Premature termination codons can result in the production of truncated proteins, or truncated mRNAs that are degraded prior to translation. These effects appear to occur with α1(S326fs), γ2(Q40X), γ2(Q429X), as well as a splice site mutation in γ2 at the boundary of intron6/exon6 (Kananura et al., 2002; Hirose 2006; Maljevic et al., 2006; Sun et al., 2008; Huang et al., 2012; Tian and Macdonald, 2012), resulting in seizure disorders including DS, GEFS+, FS and CAE. Promoter mutations (*GABRB3* haplotype) result in CAE following impairment of transcription, which might reduce surface expression of this subunit (Urak et al., 2006). Finally, missense mutations can result in unincorporated, mis-folded subunits (e.g. α1(A322D)) or intact pentameric receptors that harbor mutant subunits, which alter channel kinetics (e.g. γ2(K328M), γ2(R177G), δ(E177A), δ(R220H)). In all 4 cases, impaired gating results in reduced inhibition and is proposed to cause GEFS+ or FS (Baulac et al., 2001; Dibbens et al., 2004; Audenaert et al., 2006). Thus, there are examples within the GABA receptor family of mutations that alter receptor function, cell surface density, and transcription and RNA processing. This could change the optimal balance of synaptic excitation and inhibition, perturb subcellular signaling in neurons, influence disease progression outright, or constitute a risk factor working in concert with other processes to contribute to neurological disease. In addition, compartmental changes in GABA receptor function associated with the various mutations could have large scale effects on circuit and brain function, as mutations that impact where and when GABA receptor operate could be as important as changes in

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biophysical properties. Thus, despite functional evaluation of biophysical and biochemical properties, additional work still remains to fully understand the circuit level pathology and potential drug targets.

Schizophrenia is a disease of multifactorial origin that affects up to 1% of the population, with some cases showing a heritable component (Tsuang et al., 2001). Perturbation in GABAergic interneuron biology and function have been observed in schizophrenic patients, in addition to changes in glutamatergic, serotonergic and dopaminergic neurotransmission (Benes and Berretta, 2001; Coyle, 2012). Whereas *GABR* mutations have not been widely reported in schizophrenic patients, the importance of $GABA_A$ receptor perturbations was emphasized when five SNPs in the $GABA_A$ receptor β2 gene were shown to be associated with schizophrenia (Lo et al., 2004). In common with the majority of the epilepsy mutations, many recently described SNPs lead to protein changes that appear to impair the delivery of functional receptors to the cell surface, compromising normal inhibition. For example, hypermethylation occurs in the vicinity of one schizophrenia-associated SNP, and 2 other SNPs introduce a CpG methylation site (Pun et al., 2011). In both cases, studies of trios indicated that these SNPs, and hence aberrant methylation, may play a role in *GABRB2* imprinting and the risk of developing schizophrenia. Also in common with epilepsy-associated mutations (Baulac et al., 2001; Dibbens et al., 2004; Audenaert et al., 2006), some mutations result in an alteration of the channels delivered to the neuronal surface. *GABRB2* can be expressed as a long or short alternative RNA splice variant. Notably, the short isoform lacks exon 10, which encodes residues that form a consensus phosphorylation site for calmodulin protein kinase II (Thr365) that might play a role in receptor retention in the membrane (Pun et al., 2011). Two schizophrenia-linked SNPs reduce the amount of the longer isoform in favor of the shorter, less stable isoform. This generates a population of surface $GABA_A$ receptors that are more prone to receptor desensitization and rundown and ultimately, which results in a long-term dis-inhibition (Zhao et al., 2006, 2009).

Autism, a developmental disorder that is characterized by deficits in reciprocal social interactions, impaired communication and repetitive behaviors, is estimated to occur in 1 in 1000 children. However,

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the risk to siblings of autistic children is as high as 3% (Bolton et al., 1994; Bailey et al., 1995) with a male:female risk ratio of 4:1 (McLennan et al., 1993). Genetic abnormalities have been described in the Angelman critical region 15q11-13 in several individuals with autism (Tager-Flusberg et al., 2001). This region of chromosome 15 contains the $GABA_A$ receptor genes encoding the α5, β3 and γ3 subunits (Bass, 2000). Whereas rare mutations have not been identified, two SNPs in the $GABA_A$ receptor γ3 gene are significantly associated with autism (Menold et al., 2001), raising the idea that this gene, or one proximal to it contributes a significant risk in autistic disease (e.g. β3, Buxbaum et al., 2002). In addition, partial tetrasomy of this region can result in autistic-like behavior, coupled with seizures, and intellectual disability (Battaglia et al, 2010). More recently, significant risk for autism has been hypothesized via complex gene-gene interactions involving *GABRA4* and *GABRB1* with other *GABRs* potentially playing an important role as well (Ma et al., 2005), although how the changes in gene expression affect GABAergic neurotransmission is not well understood.

Finally, many neuropsychiatric disorders, including addiction, do not manifest symptoms until patients become adults. More than half of the phenotypically-linked SNPs and mutations are associated with drug abuse (predominantly alcohol http://www.ncbi.nlm.nih.gov/SNP/), which supports a role for $GABA_A$ receptors in addiction risk (Anstee et al., 2013; Li et al., 2014). In particular, *GABRA6* has been identified as an inheritable locus for developing alcohol dependence (Radel et al., 2005). Little of the available data point toward coding region mutations in critical alcohol targets. Instead, UTR and intronic mutations are commonly reported to lead to a reduction in human brain *GABR* gene transcription and RNA processing (Haughey et al., 2008). In a separate study, while no link between alcohol dependence and *GABRA2* SNPs was found, SNPs in this gene were associated with an abnormal EEG phenotype that might be rectified by self-administration of ethanol (Lydall et al. 2011). However, as with the majority of *GABR* SNPs, more data is needed to understand specifically how each SNP leads to a change in GABAergic function. If the same approach taken in the evaluation of epilepsy-linked mutations can be

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applied here (e.g. Gallagher et al., 2007, Gurba et al., 2012), our understanding of addiction could be substantially enhanced.

NMDA-selective glutamate receptors:

NMDA-selective glutamate receptors are tetrameric complexes comprised of two glycine-binding GluN1 and two glutamate-binding GluN2 subunits. GluN3 subunits are thought to co-assemble in some NMDA receptors, but there is incomplete understanding of the nature of GluN3 stoichiometry. Receptor activation requires binding of both glutamate and glycine, which are often referred to as co-agonists (Fig. 2). The eight possible alternative mRNA splice variants of the single GluN1 gene (*GRIN1*), four genes encoding the GluN2 subunits (*GRIN2A-D*), and two genes encoding GluN3 subunits (*GRIN3A-B*) endow the receptor with divergent single channel, pharmacological, and temporal signaling properties (Traynelis et al., 2010; Paoletti et al., 2013). While the GluN1 subunit is broadly expressed throughout the CNS, GluN2 subunits show different spatial and temporal expression patterns (Monyer et al., 1994; Dunah et al., 1998; Dunah and Standaert, 2003; Lopez de Armentia and Sah, 2003; Salter and Fern, 2005). GluN2B and GluN2D subunits are highly expressed prenatally and decline after birth in most brain regions, whereas GluN2A and GluN2C subunits are mainly expressed after birth (Akazawa et al., 1994). NMDA receptors mediate a slow, Ca^{2+}-permeable synaptic current that is voltage-dependent due to channel block by extracellular Mg^{2+} (Traynelis et al., 2010). The requirement for depolarization and synaptic release of glutamate renders NMDA receptors a trigger for synaptic plasticity, a cellular correlate of learning and memory (Huganir and Nicoll, 2013; Lisman 2003). NMDA receptors participate in development of the CNS (Colonnese et al., 2005; Colonnese and Constantine-Paton, 2006; Kelsch et al., 2012). In addition, over-activation of NMDA receptors can promote seizures and cell death (Choi, 1994; Rothman and Olney, 1995; Obrenovitch et al., 1997; Dirnagl et al., 1999; Yurkewicz et al., 2005), and NMDA receptor hypofunction is a leading hypothesis for schizophrenia (Coyle, 2012; Menniti et al., 2013). Thus, there is considerable interest in factors that control NMDA receptor expression and function.

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Early GWA studies suggested that *GRIN2A* but not *GRIN2B* was a modifier gene for Parkinson's disease (Hamza et al., 2011; Lee et al., 2011; Yamada-Fowler et al., 2014). Whereas a large GWA study did not correlate epilepsy to any of the NMDA receptor genes (ILAE 2014), the first potential disease-causing mutations in NMDA receptors were described by Endele et al. (2010) in *GRIN2A*, the gene encoding the GluN2A subunit of the NMDA receptor. One of the mutations, N615K, resided at the tip of a re-entrant pore loop at a position known to control voltage-dependent Mg^{2+} block (Wollmuth et al., 1998). The mutation removed voltage-dependent Mg^{2+} block, thereby increasing the amount of current flowing when NMDA receptors were activated at normal resting membrane potentials. The profound increase in current produced by this mutation seems likely to drive aberrant excitation and potentially contribute to neuronal loss, and consequently the patients' clinical symptoms. In the subsequent years, a large number of missense mutations and deletions/truncations (>100) have been identified through whole exome and genome sequencing (reviewed by Soto et al 2014; Burnashev and Szepetowski, 2015), and are scattered across all domains in NMDA receptor subunits (Table 1, Supplemental Table S2; Hamdan et al., 2011; Myers et al., 2011; Tarabeux et al., 2011; de Ligt et al., 2012; O'Roak et al., 2012; Carvill et al., 2013b, DeVries and Patel, 2013; Epi4K and EPGP Investigators, 2013; Freunscht et al., 2013; Kenny et al., 2014; Lemke et al., 2013a,b; Lesca et al., 2013; Adams et al., 2014; Andreoli et al., 2014; Fromer et al., 2014; Pierson et al., 2014; Redin et al., 2014; Venkateswaran et al., 2014; Yuan et al., 2014; Turner et al., 2015; see Bunrashez & Szepetowski, 2015). More recently, several case-control studies have isolated *de novo* and inherited mutations in the *GRIN2A* gene in patients diagnosed with different forms of epilepsy, including continuous spike-and-waves during slow-wave sleep syndrome, epileptic encephalopathy, Landau-Kleffner syndrome, and Rolandic epilepsy (Endele et al., 2010; Carvill et al., 2013b; Lemke et al., 2013a; Lesca et al., 2013, reviewed by Burnashev and Szepetowski, 2015). These studies suggest that *GRIN2A* constitutes a locus for mutations in a subset of patients with early-onset seizures (Table 2, Fig. 3). The exceptional number of mutations in *GRIN2A* could reflect the post-partum expression of GluN2A, precluding catastrophic pre-term neurological complications and enabling patients

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to survive full-term but with neurological complications that later appear as *GRIN2A* expression ramps up.

Surprisingly, the incidence of *de novo* and inherited NMDA receptor mutations in all subunits in patients with early onset neurological problems was ~6%, with 202 patients with *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, or *GRIN2D* mutations identified in 3549 patients subjected to exome/genome sequencing. In addition, variants in two genes (GRIN3A and GRIN3B) encoding the poorly understood GluN3 NMDA receptor subunits have been reported in patients with intellectual disability, schizophrenia, autism, and amyotrophic lateral sclerosis, although their relation to these diseases is uncertain (Niemann et al., 2008; Hamdan et al., 2011; Tarabeux et al., 2011; Matsuno et al., 2015).The frequency of NMDA receptor mutations found in epilepsy patients with slow wave sleep syndrome and benign epilepsy with centrotemporal spikes (BECTS) (Carvill et al., 2013b; Lemke et al., 2013a), together with the rates of incidence of these conditions (from the Centers for Disease Control, http://www.cdc.gov/epilepsy/basics/fast_facts.html; Pavlou et al., 2012; Singhal and Sullivan, 2014), suggests that 1000's of North American pediatric epilepsy patients have undiagnosed NMDA receptor mutations. In addition to seizure disorders, NMDA receptor mutations have been identified in patients with Alzheimer's disease, attention deficit hyperactivity disorder, autism spectrum disorder, developmental delay, schizophrenia, and intellectual disability (Table 2). The number of mutations in NMDA receptor subunits is an important new development in pediatric neurology, and presents an opportunity to better understand a subset of previously undiagnosed developmental diseases in children (Burnashev & Szepetowski, 2015). Moreover, the identification of mutations in all domains (ATD, LBD, transmembrane domain, C-terminal) and all subunits (Table 3) provides an opportunity to gain new insight into the structural basis underlying NMDA receptor function.

Despite the increasing identification of new NMDA receptor mutations, there remains only minimal functional analysis of missense mutations and minimal evaluation of the effects of mutations on complex processes that govern receptor trafficking (Horak et al., 2014). For example, of more than a 100 published mutations in NMDA receptor subunits, functional data is reported for only 12 (Endele et al.,

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2010; Hamdan et al., 2011; Carvill et al., 2013b; Lemke et al., 2013a,b; Lesca et al., 2013; Adams et al., 2014; Pierson et al., 2014; Yuan et al., 2014). The lack of functional information for *de novo* mutations in genes with a strong genetic link to disease underscores a pressing need. Within GluN2A, functional data exists for the previously mentioned mutation that alters Mg^{2+} sensitivity (Endele et al., 2010). In addition, a mutation in the Zn^{2+} binding amino terminal domain, GluN2A(A243V), impaired the negative allosteric modulation by nanomolar concentrations of Zn^{2+} (Lemke et al., 2013a). Two more GluN2A mutations in ligand binding domain (T531M, R518H) and a mutation in the transmembrane domain (F652V) are proposed to increase mean channel open time (see Burnashev and Szepetowski, 2015). Another mutation, GluN2A(L812M), lies adjacent to known gating elements at a conserved position in the linker preceding the M4 transmembrane helix/domain (Fig 4A). This mutation was identified in a pediatric patient suffering from intractable seizures, early-onset epileptic encephalopathy, cortical parenchymal cell loss, thinning of the corpus callosum, retinal degeneration, and other neurological problems including developmental delay. Functional studies in NMDA receptors for which stoichiometry was controlled so that receptors contained a single copy of the GluN2A(L812M) (Yuan et al., 2014) were hyper-active as a result of increased agonist potency (Fig. 4B), decreased sensitivity to negative modulators (Mg^{2+}, Zn^{2+}, protons), prolonged deactivation time course (Fig. 4C), and increased single channel open time and open probability (Fig. 4D). This profound increase in receptor function likely contributes to seizure activity, and has the potential to trigger excitotoxicity, which may have contributed to parenchymal cell loss.

Since this patient's seizures were resistant to all antiepileptic regimens tested, several FDA-approved drugs known to inhibit NMDA receptors (even weakly) were screened in an effort to identify those with the potential to inhibit the overactive mutant NMDA receptors in this patient with similar potency and efficacy compared with the wild type receptors. Among them, memantine (Namenda® , Fig. 4E), previously well-tolerated in a pediatric population, was selected for off-label use as a potentially effective antagonist of the mutant NMDA receptors (Owley et al., 2006; Chez et al., 2007; Erickson et al., 2007). While only a single patient was examined, addition of memantine to valproate treatment (see also

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Urbanska et al., 1992) nevertheless decreased the frequency of seizures from >11 per week to ~3 per week with associated improvement of EEG and abnormal motor function, suggesting that the seizures involve excessive NMDA receptor excitatory drive (Fig. 4F; Pierson et al., 2014). This example illustrates the potential utility of comprehensive functional and pharmacological data in the context of understanding and treating disease-associated highly penetrant or *de novo* mutations, and suggests further studies and carefully controlled clinical trials should be informative. These results emphasize the need to fill the large gap in our functional understanding of the rapidly expanding list of ion channel mutations revealed by gene sequencing programs for patients with refractory epilepsy, developmental delay, autism spectrum disorders, and other neurological conditions. Gain-of-function mutations that increase NMDA receptor function raise the possibility that each individual mutation could be tested for sensitivity to a host of FDA-approved low affinity channel blockers that can inhibit NMDA receptor function, some of which appear to be safe in a pediatric population. These drugs may allow an attenuation of NMDA receptor overactivation, which could slow excitotoxic damage to preserve grey matter in pediatric patients, and perhaps partially rectify circuit imbalances that develop from NMDA receptor dysfunction.

In addition to *GRIN2A*, *de novo* mutations have been described in all other NMDA receptor subunits (Table 2). Functional analysis has been performed on two *GRIN1* mutations (Ser560dup reduces current amplitude and Glu662Lys has no effect on glycine potency and Mg^{2+}sensitivity; Hamdan et al., 2011, see Supplemental Table S2) and three *GRIN2B* mutations that reduce Mg^{2+} sensitivity (GluN2B(R540H), GluN2B(N615L), GluN2B(V618G); reviewed by Burnashev and Szepetowski, 2015, see Table 2, Supplemental Table S2). A fourth mutation (GluN2B(E413G)) that caused a 50-fold reduction in glutamate potency was recently described, which should diminish current responses to GluN2B-containing NMDA receptors (Adams et al., 2014). No functional information is available yet for missense mutations in other NMDA receptor subunits (see Supplemental Table S2).

AMPA-selective glutamate receptors:

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AMPA-selective glutamate receptors are tetrameric assemblies of GluA1-4 subunits encoded by *GRIA1-4* genes. AMPA receptors interact with multiple accessory proteins (e.g. TARP, cornichon; Straub and Tomita, 2012) and are localized to the postsynaptic density where they interact with scaffolding and other proteins (Specht and Triller, 2008; Huganir and Nicoll, 2013). AMPA receptors bind to and are activated by synaptically-released glutamate, which triggers the rapid opening of a cation conductance. The *GRIA2* mRNA is often edited in a region encoding the apex of a reentrant pore-loop, which confers Ca^{2+} impermeability to mature receptors containing the edited GluA2 subunit (Traynelis et al., 2010). Modification of adenosine deaminase and RNA editing of GluA2 subunits could be relevant in epileptic foci (Grigorenko et al 1998). The AMPA receptor-mediated conductance underlies the majority of excitatory synaptic signaling in the central nervous system, and is typically brief (on the order of a few milliseconds) because glutamate rapidly unbinds from AMPA receptors and is removed from the synaptic cleft by diffusion and active transport. The AMPA receptor-mediated current during synaptic transmission leads to a brief depolarization, which is critical for virtually all circuits, and thus is an indispensable aspect of normal brain function. Therefore, it is not surprising that this gene family is predicted to be largely intolerant to mutagenesis that disrupts protein function (Table 4).

Intellectual disability is a neurodevelopmental disorder affecting 2-3% of general population (Chelly and Mandel, 2001), and has been explored for potential links to gene families involved in synaptic transmission. It has traditionally been characterized by lower intelligence test scores and deficits in at least two behaviors related to adaptive functioning. For the *GRIA* gene family, there have been reports of a fusion transcript in *GRIA2*, a *de novo* interstitial deletion of chromosome 4q32 that contains the *GRIA2* loci, missense mutations in the ligand binding and transmembrane domains of the GluA3 subunit (*GRIA3*), partial tandem duplication that reduced *GRIA3* transcript levels, as well as frameshift in *GRIA3* gene (Fig. 5; Supplemental Table S3; Chiyonobu et al., 2007; Wu et al., 2007; Poot et al., 2010; Bonnet et al., 2009,2012; Tzschach et al., 2010; Hackmann et al., 2013; Philips et al., 2014). These data suggest that mutations within the *GRIA* gene family participate in a small subset of patients

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with intellectual disability; however, few cellular or mechanistic studies of these modifications have been reported.

Kainate-selective glutamate receptors

Kainate receptors are tetrameric assemblies of GluK1-5 subunits encoded by *GRIK1-5*. Kainate receptors mediate a rapidly activating inward current, which can persist longer than AMPA receptor signaling following removal of glutamate from the synaptic cleft (Traynelis et al., 2010). RNA editing of certain kainate receptor subunits can render kainate receptors Ca^{2+} impermeable in a similar fashion to editing of the AMPA receptor subunit GluA2, and editing of GluK2 may be regulated at epileptic foci (Grigorenko et al 1998). A number of publications report that genetic variants in *GRIK* genes influence kainate receptor ion channel function in humans. An early genome scan highlighted chromosome 6q21, which contains *GRIK2*, as a candidate region for autism (Jamain et al., 2002). One missense mutation, GluK2(M867I), in a highly conserved domain of the C-terminal region, was identified in the *GRIK2* gene (Fig. 5; Table 4). Characterization of the M867I missense mutation revealed no detectable effect on GluK2 receptor gating (Han et al., 2010), but revealed a modest ~1.6-fold slowing of the desensitization time course. Additional SNP association studies on various populations support a role of *GRIK2* in autism (Shuang et al., 2004; Dutta et al., 2007; Kim et al., 2007; Holt et al., 2010; Casey et al., 2012; Griswold et al., 2012).

An insertion/deletion variant located at the 3'UTR just downstream of the *GRIK4* stop codon (Pickard et al., 2008; Supplemental Table S4) was suggested to result in a higher cellular transcript level of *GRIK4*, which may confer a genetic protective effect against bipolar disorder. Furthermore, there was an overrepresentation of the deletion-carrying transcript in the hippocampus and cerebral cortical regions of diagnostically unaffected heterozygous individuals from a brain tissue repository. Further follow-up studies showed positive correlation between this deletion variant with increased hippocampal activities in humans via magnetic resonance imaging as well as significantly higher GluK4 protein distribution in frontal cortex and hippocampus of the post-mortem human brain tissue in the deletion group (Whalley et

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al., 2009; Knight et al., 2012). SNPs in *GRIK5* have also shown association with bipolar disorder (Gratacòs et al., 2009).

A mutation in *GRIK2* causing a partial deletion of the ATD and transmembrane domain resulting in loss-of-function was reported in patients with intellectual disability (Motazacker et al., 2007). Similarly, a reported micro-deletion involving the *GRIK3* gene was detected in a patient diagnosed with severe developmental delay (Takenouchi et al., 2014). As *GRIK3* $^{-/-}$ mice exhibit impaired synaptic transmission, the functional deletion of *GRIK3* gene may lead to development delay (Pinheiro et al., 2007).

The glutamatergic dysfunction hypothesis suggests genes involved in glutamatergic transmission are candidates for schizophrenia susceptibility genes. The *GRIK3* variant that encodes GluK3(S310A) showed a significant association with schizophrenia (Begni et al., 2002; Ahmad et al., 2009; Djurovic et al., 2009; Minelli et al., 2009; Dai et al., 2014; see also Lai et al., 2005). *GRIK1* and *GRIK4* gene variants have also been studied as schizophrenia susceptibility genes, however, no consistent association has been identified (Shibata et al., 2001; Pickard et al., 2006; Li et al., 2008).

Delta receptors

Two poorly understood subunits in the glutamate receptor family, delta-1 (δ1, GluD1) and delta-2 (δ2, GluD2), bear distant resemblance to ionotropic glutamate receptors through sequence homology (Araki et al., 1993; Lomeli et al., 1993). When expressed alone or with other glutamate receptors, δ2 does not form functional glutamate-gated ion channels, although δ2 does bind the glycine-site ligand D-serine (Naur et al., 2007). While the physiological function of both gene products in humans has not been well-defined, a number of case-control association studies on SNPs and CNVs in *GRID1* encoding δ1 focused on schizophrenia, cognition deficits and depression (Fallin et al., 2005; Guo et al., 2007, Griswold et al., 2012; see also Treutlein et al., 2009; Nenadic et al., 2012). In addition, mice expressing the δ2 Lurcher mutation altered cerebellum development, which resulted in the animals displaying ataxia

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and jerky movement of the hindlimbs (Kashiwabuchi et al., 1995; Zuo et al., 1997; Lalouette et al., 1998). Recently, exon deletions in *GRID2* gene encoding δ2 were reported in patients with cerebellar ataxia and autism spectrum disorder (Hills et al., 2013; Utine et al., 2013; see also Huang et al., 2014; Fig.5). Immunohistochemical evidence suggests that the exclusive expression of *GRID2* at parallel fiber-Purkinje cell synapses observed in mice is preserved in the human cerebellum (Hills et al., 2013). The phenotypic resemblance and similarity in protein expression pattern between humans and mice suggest deletions of *GRID2* could contribute to cerebellar ataxia.

Functional genomics and future directions

Tremendous advances in our understanding of the genetic basis of neurological disease have occurred over the last 20 years. In addition, there has been a virtual tsunami of genetic data from accelerating sequencing studies designed to help with diagnosis of complex neurological diseases (Heinzen et al., 2015). For the ionotropic glutamate and GABA receptor families, many rare *de novo* and inherited mutations appear to be associated with multiple diseases, and in some cases clearly define a phenotype via an identifiable pathway or mechanism. While individual mutations are rare, some genes appear to harbor many mutations (e.g. *GABRA1*, *GABRA2*, *GABRB2*, *GABRB3*, *GRIA3*, *GRIN2A*), suggesting these genes may be a locus for disease-associated mutations. These advances herald the entry into a new era of personalized medicine, in which specific genes or mutations will allow precision diagnostics of neurological disease in an ever growing number of patients. The advent of this information will improve clinical care by reducing unnecessary and costly tests, thereby allowing physicians to focus on treatment rather than identification of the underlying condition. Identification of growing numbers of mutations will allow new therapeutic strategies to be considered that take advantage of genetic and functional knowledge of variants, and rule out ineffective therapies. In addition, the increasing understanding of how modified genes contribute to disease will advance our understanding of the disease, and catalyze development and testing of new preclinical disease models and therapeutic strategies. For complex receptors, we expect mutations in different regions to have a myriad of effects on circuits. For

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example, within the NMDA receptor family, one would predict distinct changes in circuit function to result from mutation-linked alterations in Zn^{2+} inhibition, glutamate potency, glycine potency, receptor surface expression, or channel opening frequency. Each of these actions could alter synaptic and non-synaptic response time course and consequent signaling as well as spike timing in subtle and potentially different ways.

While there has been tremendous increase in information relating neurological disease to specific genes, variants, or mutations, the ability to generate genomic data has not been matched by complementary advances in an understanding of the functional effects of mutations. Indeed, data on disease-linked mutations appear to be orders of magnitude more plentiful than functional data on these mutations, and this ratio is increasing. For ion channels, one reason for this mismatch is that functional studies have not seen the cost reduction or increases in efficiency witnessed by DNA sequencing technology. Thus, there currently exists a large chasm between the volume of information known about mutations in the coding region of specific proteins and our understanding of how individual mutations impact protein function. This chasm is widening with the accelerating pace of sequencing, and is poised to eclipse the scientific community's ability to functionally investigate the enormous volumes of newly generated sequence data. While computational methods have provided some guidance as to which mutations might be harmful, these algorithms are a poor substitute for functional evaluation. For example, an algorithm suggesting a mutation is deleterious cannot predict whether the mutation enhances or reduces protein function, making these predictions of dubious value in terms of guiding the development of treatment options and understanding of the underlying disease mechanisms. Thus, there is a strong need for resources by which clinically-oriented laboratories can obtain functional insight into the effects of mutations uncovered in candidate genes.

The promise of precision medicine and the lack of functional data highlight the need for future development of technical means to efficiently explore the functional effect of mutations identified in patients. For ion channels, this is relatively straightforward in concept, yet slow and tedious in practice, with cell-by-cell patch clamp studies still the gold standard for determining how a mutation alters receptor

function. Improving this throughput is essential in order to provide an efficient means for clinical investigators to obtain high quality functional data on mutant receptors. This will enable the community to capitalize on the opportunity for deeper understanding of neurological disease brought about by technical revolution that has accompanied DNA sequencing. It is also critical to enhance the ability to broadly screen the library of FDA-approved medications against *in vitro* assays of altered receptor function, looking for safe, approved compounds that might rectify functional problems associated with specific mutations. Development of a means to obtain these data quickly and at low cost could enable clinical investigators to understand the mechanisms underlying disease caused by mutant receptors. This will assist in advancing understanding of the disease and new therapeutic strategies, which in some cases involve re-purposing of a drug. For glutamate and GABA receptors, there appears to be a clear path forward to understanding functional effects given that ion channels are amenable to functional studies.

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Acknowledgements

We thank Dr. John DiRaddo for his help with figure making and Drs. John DiRaddo, Erin Heinzen, Johannes Lemke, Robert Macdonald, Steve Petrou, and Sharon Swanger for critical comments on the manuscript.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Yuan, H., Low, C-M., Moody O.A, Jenkins, A., and Traynelis, S.F.

Footnotes

This work was supported by National Institute of Neurological Disorders and Stroke [Grant S036654, NS065371] (to S.F.T.), by the Eunice Kennedy Shriver National Institute Of Child Health & Human Development of the National Institutes of Health under Award Number R01HD082373 (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health) (to H.Y.), by National Institutes of Health [HHSN268201400162P and HHSN268201400169P] (to H.Y.), by Emory+Children's Pediatric Center Seed Grant Program (to H.Y.), by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000454 (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health) (to H.Y.), and by National University of Singapore [Grants C171000216411, R184000233112] (to C-M.L.).

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Figure Legends

Figure 1. Architecture and domain organization of ionotropic gamma-amino butyric acid A receptor family.

(A) Top-down and (B) side view of a homomeric β3 $GABA_A$ receptor (pdb 4COF). (C) Linear representation of modular domains LBD (blue) and TMD (yellow) within a subunit polypeptide chain. (D) Schematic illustration of a $GABA_A$ receptor subunit topology with the extracellular domain (blue) and membrane associated elements (yellow) color-coded to match the linear polypeptide chain in (C). Short peptide linkers between domains are shown in black lines.

Figure 2. Architecture and domain organization of ionotropic glutamate receptor family.

(A) Top-down and (B) side view of an NMDA receptor (pdb 4PE5; Karakas and Furukawa, 2014). (C) Linear representation of modular domains ATD (green), LBD (blue), TMD (yellow) and the C-terminal domain (grey) within a subunit polypeptide chain. (D) Schematic illustration of a glutamate receptor subunit topology with the extracellular domain (blue, green) and membrane associated elements (yellow) color-coded to match the linear polypeptide chain in (C). Short peptide linkers between domains are shown in black lines.

Figure 3. Published human *GRIN2A* mutations in the coding sequence identified in neurological disorders. S1, S2 comprise the LBD and M1, M2, M3, M4 comprise the transmembrane domains; see Figure 2 for domain organization. fs* denotes a mutation leading to a frame shift.

Figure 4. Functional analysis of GluN2A mutation (L812M) and personalize therapy. (A) Location of mutant L812M (green spacefill) and possible van der Waals interaction with the adjacent GluN1 subunit pre-M1 helix (purple) and SYTANLAAF (yellow) of the NMDA receptor gate as predicted from the homomeric GluA2 structure. (B – D) The GluN2A(L812M) mutation changes the pharmacology and

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channel properties of NMDA receptors, and shows increased glutamate potency (B), prolonged deactivation time course (C), increased open probability (D) with tri-heteromeric NMDA receptors with 0, 1 or 2 copies of the L812M mutation in each complex (B-D reproduced from Yuan et al., 2014). (E) GluN2A(L812M) modestly reduces the sensitivity to the FDA-approved drug memantine. (F) Adjunct-antiepileptic drug treatment with memantine reduced seizure frequency after progressive weaning off of lacosamide and rufinamide between weeks 40 – 60 while valproate dosing remained unchanged (E and F reproduced from Pierson et al., 2014).

Figure. 5. Published human *GRIA, GRIK,* and *GRID* mutations in the coding sequence identified in neurological disorders. Refer to Fig.2 for domain organization.

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Table 1: Human $GABA_A$ receptor mutations in neurological disorders*

Gene, Subunit	Total	RVIS (%)	AD	ASD	DD/MR	Epi	SZ	ADD
***GABRA1*, α1**	13	24	0	0	0	12	1	0
***GABRA2*, α2**	11	34	0	1	1	0	0	9
***GABRA6*, α6**	3	68	0	0	0	0	2	1
***GABRB2*, β2**	7	15	0	2	0	0	5	0
***GABRB3*, β3**	7	22	0	1	0	5	0	1
***GABRG1*, γ1**	4	12	0	0	0	0	0	4
***GABRG2*, γ2**	9	25	0	0	0	8	1	0
***GABRG3*, γ3**	2	46	1	1	0	0	0	0
***GABRR2*, ρ2**	6	59	0	1	0	0	0	5
***GABRD*, δ**	2	59	0	0	0	2	0	0
Total	64		1	6	1	27	9	20

All missense mutations with a frequency of <1%, as well as stop codons and splice junction mutations are included. Total indicates number of published *de novo* or inherited mutations in each subunit. AD: Alzheimer's disease; ASD: Autism Spectrum Disorder; DD: Developmental Delay; MR: Mental Retardation; Epi: Epilepsy; SZ: Schizophrenia; ADD: addiction. *many mutations have more than one phenotype. RVIS is the Residual Variation Intolerance Score in percentile, for which lower numbers reflect genes less tolerant to mutation (see Dataset S2 in Petrovski et al., 2013; www.plosgenetics.org/article/fetchSingleRepresentation.action?uri=info:doi/10.1371/journal.pgen.1003709.s002). See Supplemental Table S1 for references.

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Table 2: Human NMDA receptor mutations in neurological disorders*

Gene, Subunit	Total	RVIS (%)	AD	ADHD	ASD	DD/MR	Epi	ID	SZ
***GRIN1*, GluN1**	7	7	0	0	1	0	2	4	2
***GRIN2A*, GluN2A**	67	4	0	8	5	32	54	16	8
***GRIN2B*, GluN2B**	34	1	1	2	9	2	5	16	8
***GRIN2C*, GluN2C**	24	--	0	0	6	0	0	12	9
***GRIN2D*, GluN2D**	11	--	0	0	3	0	0	0	9
Total	143		1	10	24	34	61	48	36

All missense mutations with a frequency of <1%, as well as stop codons and splice junction mutations are included. Total indicates number of published *de novo* or inherited mutations in each subunit. AD: Alzheimer's disease; ADHD: Attention Deficit Hyperactivity Disorder; ASD: Autism Spectrum Disorder; DD: Developmental Delay; Epi: Epilepsy; ID: intellectual disability; MR: Mental Retardation; SZ: Schizophrenia. *many mutations have more than one phenotype. RVIS is the Residual Variation Intolerance Score in percentile, for which lower numbers reflect genes less tolerant to functional mutation in the population (see Dataset S2 in Petrovski et al., 2013; www.plosgenetics.org/article/fetchSingleRepresentation.action?uri=info:doi/10.1371/journal.pgen.1003709.s002). See Supplemental Table S2 for references.

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Table 3: Locations of human NMDA receptor mutations in various domains of GluN1 and GluN2 subunits (see Fig 2 for domains)

Domain	**GluN1**	**GluN2A**	**GluN2B**	**GluN2C**	**GluN2D**	**Total**
ATD	2	19	7	6	1	35
LBD (S1/S2)	1	20	10	2	2	35
TMs + linker	4	11	8	2	1	26
CTD	0	17	9	14	7	47
Total	7	67	34	24	11	**143**

ATD is the amino terminal domain, LBD is the ligand binding domain, S1 and S2 are portions of the polypeptide chain comprising the LBD, TMs are the membrane associated elements, linkers are short regions of the polypeptide chain between the various domains, and CTD is the C-terminal domain.

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Table 4: Human AMPA receptor, Kainate receptor and Delta receptor mutations in neurological disorders*

Gene, Subunit	Total *	RVIS (%)	AD	ADHD	ASD	DD/MR	Epi	ID	SZ	ATX
***GRIA1*, GluA1**	0	6	0	0	0	0	0	0	0	0
***GRIA2*, GluA2**	1	12	0	0	0	0	0	1	0	0
***GRIA3*, GluA3**	10	45	0	0	0	8	0	2	0	0
***GRIA4*, GluA4**	0	7	0	0	0	0	0	0	0	0
***GRIK1*, GluK1**	0	3	0	0	0	0	0	0	0	0
***GRIK2*, GluK2**	2	6	0	0	1	1	0	0	0	0
***GRIK3*, GluK3**	1	3	0	0	0	1	0	0	0	0
***GRIK4*, GluK4**	0	20	0	0	0	0	0	0	0	0
***GRIK5*, GluK5**	0	7	0	0	0	0	0	0	0	0
***GRID1*, GluD1**	0	2	0	0	0	0	0	0	0	0
***GRID2*, GluD2**	2	11	0	0	0	0	0	0	0	2
Total	16		0	0	1	10	0	3	0	2

All missense mutations with a frequency of <1%, as well as stop codons and splice junction mutations are included. Total indicates number of published *de novo* or inherited mutations in each subunit. AD: Alzheimer's disease; ADHD: Attention Deficit Hyperactivity Disorder; ASD: Autism Spectrum Disorder; ATX: Ataxia; DD: Developmental Delay; Epi: Epilepsy; ID: intellectual disability; MR: Mental Retardation; SZ: Schizophrenia. *many mutations have more than one phenotype. RVIS is the Residual Variation Intolerance Score in percentile, for which lower numbers reflect reduced tolerance to functional mutation in the population (see Dataset S2 in Petrovski et al., 2013; www.plosgenetics.org/article/fetchSingleRepresentation.action?uri=info:doi/10.1371/journal.pgen.1003709.s002). See Supplemental Tables S3, S4 and S5 for references.

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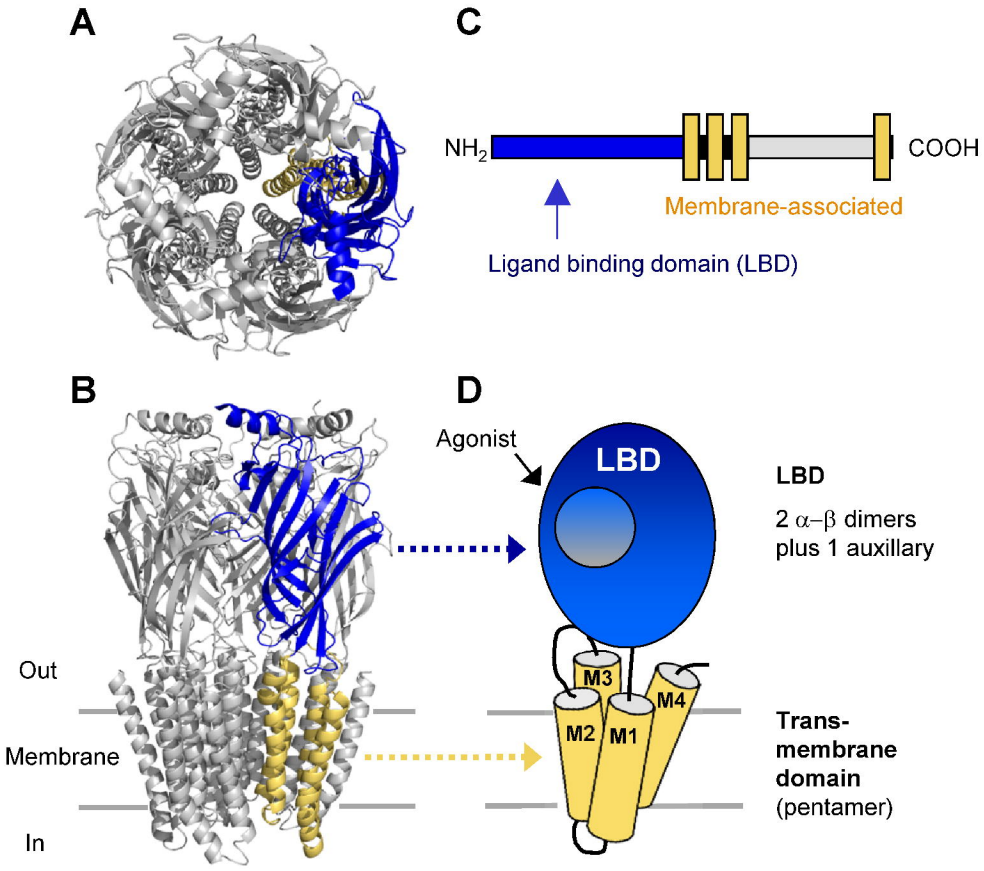

Figure 1. Architecture of GABA receptor family

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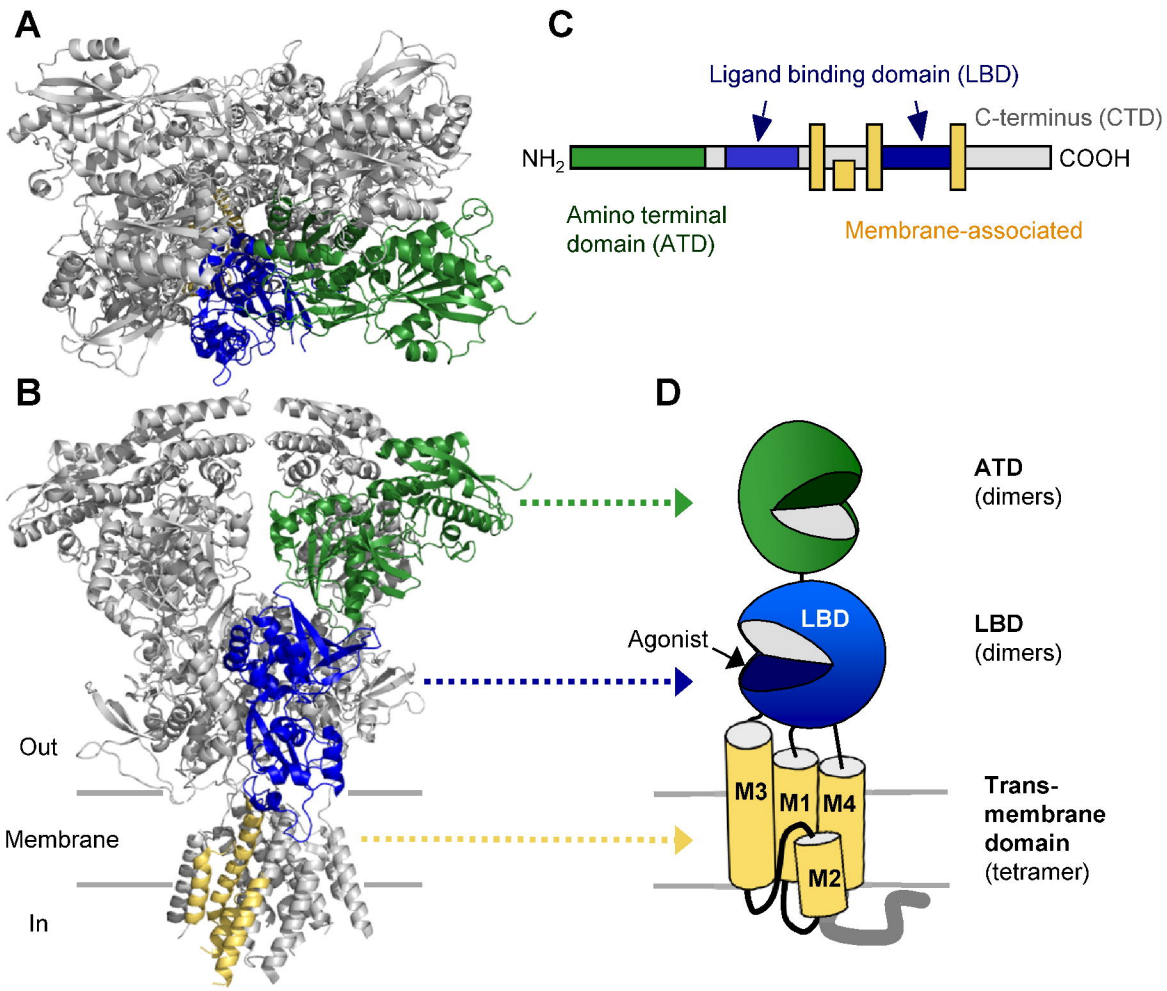

Figure 2. Architecture of glutamate receptor family

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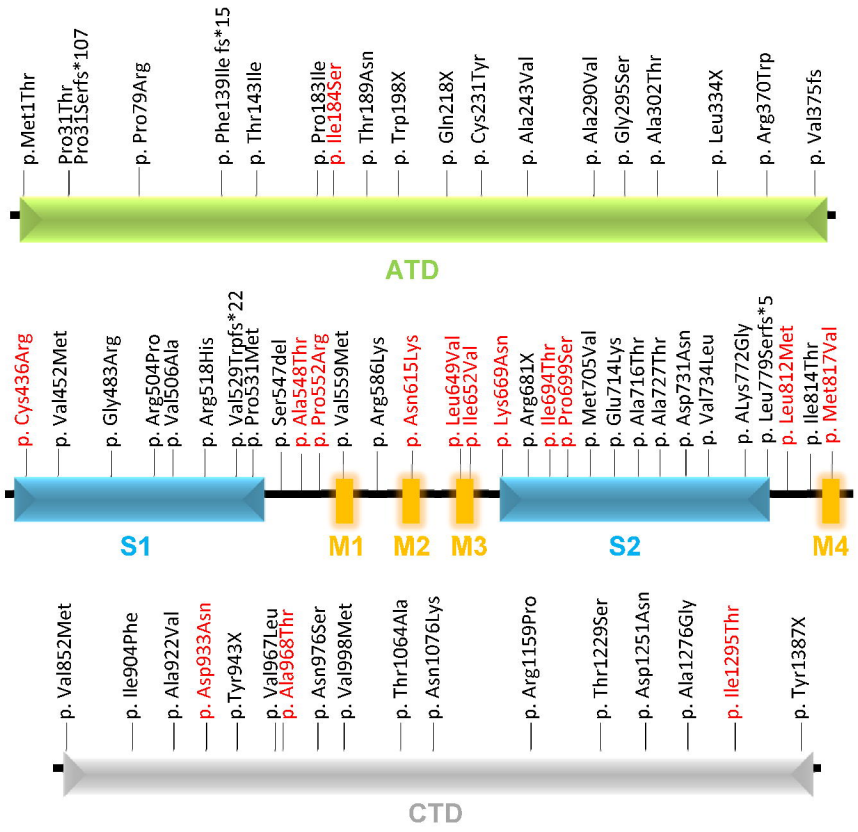

Figure 3. Locations of GluN2A mutations

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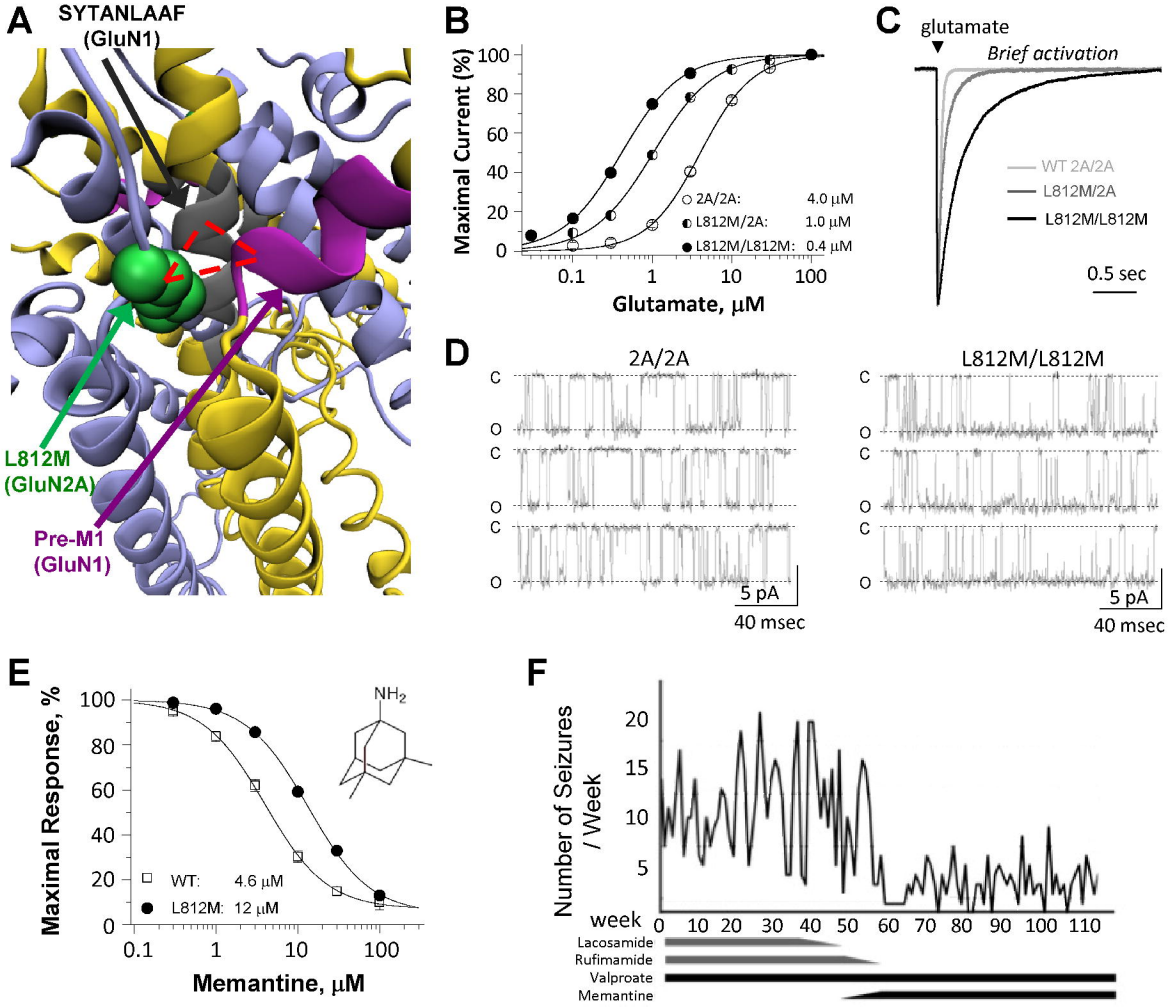

Figure 4. Functional analysis of a GluN2A Mutation (L812M) and personalized therapy

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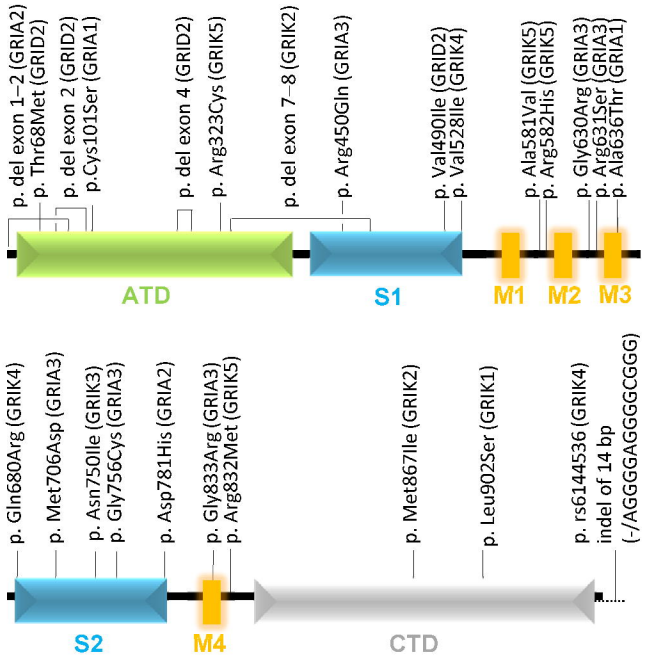

Figure 5. Locations of GluA, GluK and GluD mutations