The Spectrum of *GRIN2A*-Associated Disorders

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Summary

Alterations of the N-methyl-D-aspartate (NMDA) receptor subunit GluN2A, encoded by the gene *GRIN2A*, have been associated with a spectrum of neurodevelopmental and epilepsy disorders comprising epileptic encephalopathy such as Landau-Kleffner syndrome (LKS) and epilepsy with continuous spikes and waves during slow-wave sleep (CSWS) as well as less severe epilepsy disorders such as typical or atypical Rolandic epilepsy. These disorders do not only share a common underlying pathophysiology but also show phenotypic similarities, such as affection of speech development and centro-temporal EEG abnormalities.

In the present article, we aim to review what is known so far on *GRIN2A*-associated epilepsy disorders by focusing on genotype-phenotype correlations as well as on functional consequences of mutations.

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Keywords: Rolandic epilepsy, Landau-Kleffner syndrome, CSWS, epileptic encephalopathy

Das Spektrum *GRIN2A*-assoziierter Erkrankungen

Mutationen der N-Methyl-D-Aspartat (NMDA) Rezeptoruntereinheit GluN2A, welche durch das Gen *GRIN2A* kodiert wird, werden mit einem breiten Spektrum von neurologischen Entwicklungsstörungen und Epilepsieerkrankungen inklusive epileptischer Enzephalopathien wie etwa Landau-Kleffner-Syndrom (LKS) oder Epilepsie mit kontinuierlichen „Spikes and Waves“ während des „slow-wave“-Schlafs (CSWS) sowie milderen Epilepsieerkrankungen wie der Rolando-Epilepsie assoziiert. Diese Erkrankungen teilen nicht nur eine gemeinsam zugrunde liegende Pathophysiologie, sondern zeigen zudem phänotypische Ähnlichkeiten wie etwa Sprachentwicklungsstörungen und zentrotemporale EEG-Auffälligkeiten.

In der vorliegenden Arbeit möchten wir einen Überblick über den derzeitigen Kenntnisstand zu *GRIN2A*-assoziierten Epilepsien geben und dabei auf Genotyp-Phänotyp-Korrelationen und funktionelle Konsequenzen der Mutationen eingehen.

Schlüsselwörter: Rolando-Epilepsie, Landau-Kleffner-Syndrom, CSWS, epileptische Enzephalopathie

Le spectre des troubles associés à *GRIN2A*

Des altérations de la sous-unité du récepteur N-méthyl-D-aspartate (NMDA) GluN2A, codée par le gène *GRIN2A*, sont associées à un spectre clinique allant des troubles du développement neurologique à diverses épilepsies, celles-ci incluant de véritables encéphalopathies épileptiques (comme le syndrome de Landau-Kleffner, et l‘épilepsie avec POCS) ainsi que des formes moins graves (comme l’épilepsie rolandique). Ces maladies ne partagent pas seulement une physiopathologie similaire, mais également des phénotypes qui se recoupent, en particulier sur le plan de l‘affection du langage et de l’EEG avec pointes pouvant prédominer dans les régions centro-temporales.

Dans cet article, nous vous donnons un aperçu de l’état des connaissances sur les épilepsies associées au gène *GRIN2A*, en nous concentrant sur les corrélations génotype-phénotype et sur les conséquences fonctionnelles des mutations.

Mots clés : Epilepsie Rolandique, syndrome de Landau-Kleffner, CSWS, encéphalopathie épileptique

Structure of the NMDA receptor

NMDA receptors are ligand-gated ion channels permeable to Na^+, K^+ and Ca^{2+} composed of two glycine-binding GluN1 subunits and two glutamate-binding GluN2/3 subunits (GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, GluN3B) resulting in either di- or tri-heterotetrameric ion channels [1, 2].

All subunits share a similar structure containing analogous domains (**Figure 1**):

- an extracellular amino-terminal domain (NTD) containing binding sites for subtype specific allosteric modulators (e.g. Zn^{2+})
- an extracellular ligand-binding domain (LBD) binding agonists (e.g. glutamate, glycine)
- a channel pore-forming transmembrane domain

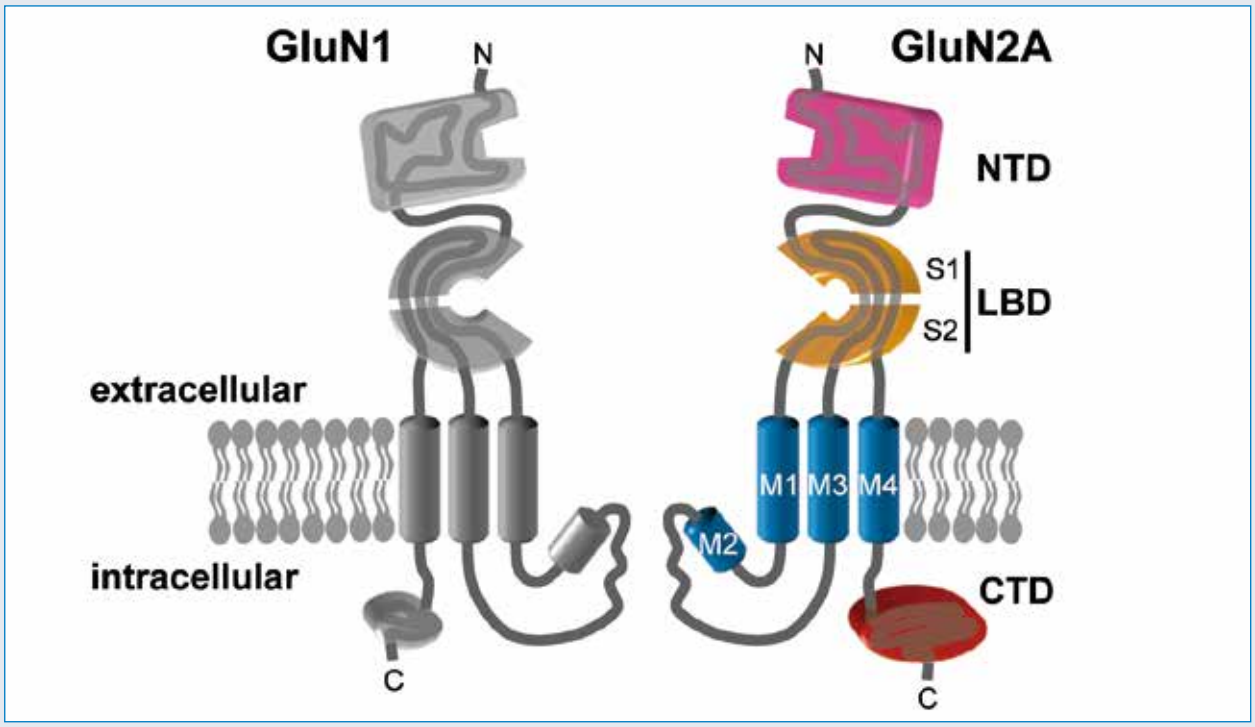

Figure 1: The structure of the NMDA receptor

(TMD) comprising four hydrophobic segments (M1-4), with M2 only partially entering the membrane
- an intracellular carboxyl-terminal domain (CTD) associating with postsynaptic density proteins mediating intracellular signalling

The receptor is characterised by relatively slow current kinetics, a voltage-dependent block by extracellular Mg^{2+} and high Ca^{2+} permeability [3]. It has a specific spatio-temporal expression pattern and is involved in brain development, plasticity as well as learning and other higher cognitive functions.

Spectrum of *GRIN2A*-associated phenotypes

Reutlinger et al. 2010 were the first to assign *GRIN2A* alterations to neurodevelopmental phenotypes [4]. They identified different microdeletions on 16p13 in three individuals with intellectual disability (ID), dysmorphic features and epilepsy. All three individuals had a centrotemporal EEG focus. The minimal region of overlap of the three microdeletions contained only one gene: *GRIN2A*. Shortly thereafter, Endele et al. 2010 identified the *de novo* mutation p.N615K in *GRIN2A* in a girl with early-onset epileptic encephalopathy [5]. The girl was described with epileptic spasms associated with massive myoclonus (onset at 3 months of age) as well as severe ID. The EEG showed generalised slowing and bilateral independent posterior spikes. In addition to this *de novo* case, Endele et al. 2010 described two familial cases. In one family, the mutation p.Q218* segregated with mild ID or learning disability and febrile and focal seizures in infancy [5]. The EEG showed centro-temporal spikes in two individuals, with one individual being diagnosed with CSWS. The second family carried a chromosomal translocation disrupting *GRIN2A* resulting in diffuse EEG abnormalities and tonic-clonic seizures starting in infancy and persisting to youth or early adulthood.

Lesca et al. 2012 investigated a cohort of patients with epileptic encephalopathies of the LKS and CSWS spectrum and revealed an LKS patient (without electrical status epilepticus during slow-wave sleep) carrying a microdeletion disrupting *GRIN2A* [6]. One year later, three groups reported simultaneously on their observation of *de novo* mutations in *GRIN2A* as well as rare inherited variants in patients of the epilepsy-aphasia spectrum [7 - 9]. Lesca et al. 2013 identified mutations in *GRIN2A* in about 20% of individuals presenting with a clinical phenotype of LKS and/or CSWS as well a atypical benign partial epilepsy (ABPE) [9]. Many cases derived from larger families where the mutation segregated with a broad range of phenotypes, comprising benign childhood epilepsy, absence epilepsy, centrotemporal spikes on EEG without seizures, dysphasia and verbal dyspraxia. Carvill et al. 2013 described four families where the respective *GRIN2A* mutation segregated with autosomal dominant rolandic epilepsy with speech dyspraxia, LKS, CSWS or intermediate epilepsy-aphasia disorder [7]. Lemke et al. 2013 showed that nearly 8% of all patients (n=395) with idiopathic focal epilepsy carried mutations in *GRIN2A*, encompassing 5% (13/284) in Rolandic epilepsy (benign epilepsy with centrotemporal spikes, BECTS), 14% (5/37) in ABPE, 13% (3/23) in LKS and 18% (9/51) in CSWS [8]. In this study,

several mutations also segregated in the respective families with varying phenotypes comprising learning disability, focal epilepsy (including Panayiotopoulos epilepsy) and febrile seizures with centrotemporal spikes on EEG. Some mutation carriers showed no clinical abnormalities at all. Thus, the complete phenotypic spectrum ranges from clinically normal to severe early-onset epileptic encephalopathy with profound global developmental delay [10]. However, *GRIN2A*-associated epilepsy phenotypes frequently shared a centrotemporal EEG focus. On the other hand, mutations or copy number variations of *GRIN2A* have been excluded in larger cohorts of either temporal lobe epilepsy or idiopathic generalized epilepsy [11].

Interestingly, a recent study on three *GRIN2A* families previously reported [7] revealed a life-long persistence of speech abnormalities in mutation carriers, independent of other developmental or epilepsy phenotypes [12]. The findings comprised imprecise articulation (11/11, 100%), impaired pitch (monopitch 10/11, 91%) and prosody (stress errors 7/11, 64%) as well as hypernasality (7/11, 64%). There was a decrease of vowel duration (8/11, 73%) and repetition of syllables (10/11, 91%).

Distribution of *GRIN2A* missense mutations

In the initial description of *GRIN2A* mutations in epilepsies with centrotemporal spikes, mutations were spread over nearly the entire gene [8]. There was no apparent correlation between position of mutation and severity of phenotype. As only a minority of mutations occurred *de novo* and most others appeared to be familial, it turned out to be challenging to evaluate whether a DNA sequence alteration was a true mutation, a disease-associated rare variant or a variant with no clinical relevance.

As commonly agreed, the most convincing factors influencing the evaluation of a sequence variant being likely pathogenic are: i) *de novo* origin, ii) position in a highly conserved and functionally important domain of the protein, and iii) a phenotype in line with other mutation carriers. Regarding only published *de novo* mutations in *GRIN2A*, the distribution of mutations along the gene differs from what was expected initially [5, 7 - 10, 13, 14]. The most likely pathogenic mutations now appear to cluster in or around ligand-binding sites and transmembrane domains (**Figure 2**). Surprisingly, this region was spared from mutations in the description of the first few carriers [8]. Individuals carrying *de novo* mutations within this region appear to have a more severe and encephalopathic phenotype. However, this observation is based on only a few patients and may be influenced by some degree of selection bias as patients with less severe phenotypes are probably less likely to undergo similar extensive genetic testing as patients with severe epileptic encephalopathy.

Spectrum of *GRIN2A* mutations

All sorts of mutations have been reported in *GRIN2A*, comprising missense mutations, truncating mutations, splice variants and copy number variations. In the first small subset of patients (n=27) [8], there was a surprising correlation between the type of mutation and the severity of the associated phenotype. Approximately 80% of individuals with BECTS had been described to carry missense mutations. With increasing severity of the phenotype, the proportion of missense mutation carriers decreased significantly and had been <20% in the most severe CSWS encephalopathies (**Figure 3**). However, considering all *GRIN2A* mutations published up to date (n=50), this striking initial correlation almost disappears completely (**Figure 4**). All four analysed categories (BECTS, ABPE, LKS, CSWS) now appear to have an almost identical ratio of approximately ¾ missense mutation carriers versus approximately ¼ carriers of truncating or splice site variants.

Functional consequences of *GRIN2A* mutations

Only a minority of five mutations have so far been investigated for their functional consequences (**Table 1**). All five missense mutations result in a gain of NMDA receptor function, partly mediated through different

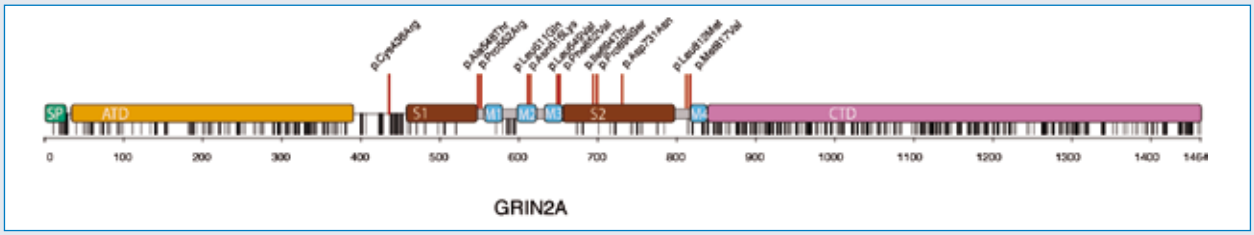

Figure 2: Distribution of published *GRIN2A* de novo mutations
Published *de novo* mutations cluster in and around ligand-binding sites and transmembrane domains. According to the ExAC database, this region shows significantly less rare and recurrent (grey and black bars) single nucleotide variants (SNV). So far, no *de novo* mutations have been observed in the amino-terminal and C-terminal domains, whereas these areas appear more tolerable to genetic variation and show an obvious enrichment of likely benign SNV.

Table 1: Published mutations in *GRIN2A* and their functional consequences

DNA	Protein	Domain	Phenotypes	Effect	Consequence	Reference
c.728C>T	p.Ala243Val	NTD	BECTS	Altered Zn2+ binding, loss Zn2+ inhibition	Gain of function	Lemke 2013 [8]
c.1553G>A	p.Arg518His	LBD	CSWS	Altered glutamate binding, increase of glutamate sensitivity	Gain of function	Lesca 2013 [9]
c.1845C>A	p.Asn615Lys	M2	EE	Elimination of Mg2+ block	Gain of function	Endele 2010 [5]
c.1954T>G	p.Phe652Val	M3	CSWS	Prolonged channel opening time	Gain of function	Lesca 2013 [9]
c.2434C>A	p.Leu812Met	Pre-M4	EE	Incomplete reduction of Mg2+ block	Gain of function	Yuan 2014 [10]

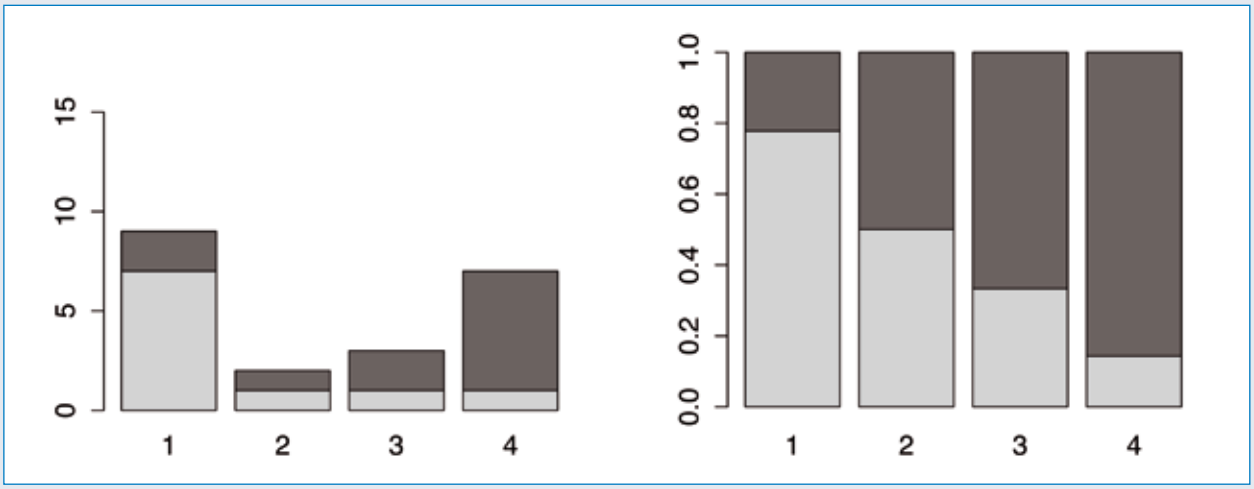

Figure 3: Absolute and relative distribution of types of *GRIN2A* mutations according to the initial publication of Lemke et al., 2013
Compared to the number of truncating mutations (dark grey), Lemke et al., 2013 observed significantly more missense mutations (light grey) in benign phenotypes such as BECTS (1) and ABPE (2). This relation changed towards an excess of truncating mutations in more severe phenotypes, such as LKS (3) and CSWS (4). The absolute patient number is shown in the left diagram, whereas the relative proportions are illustrated on the right.

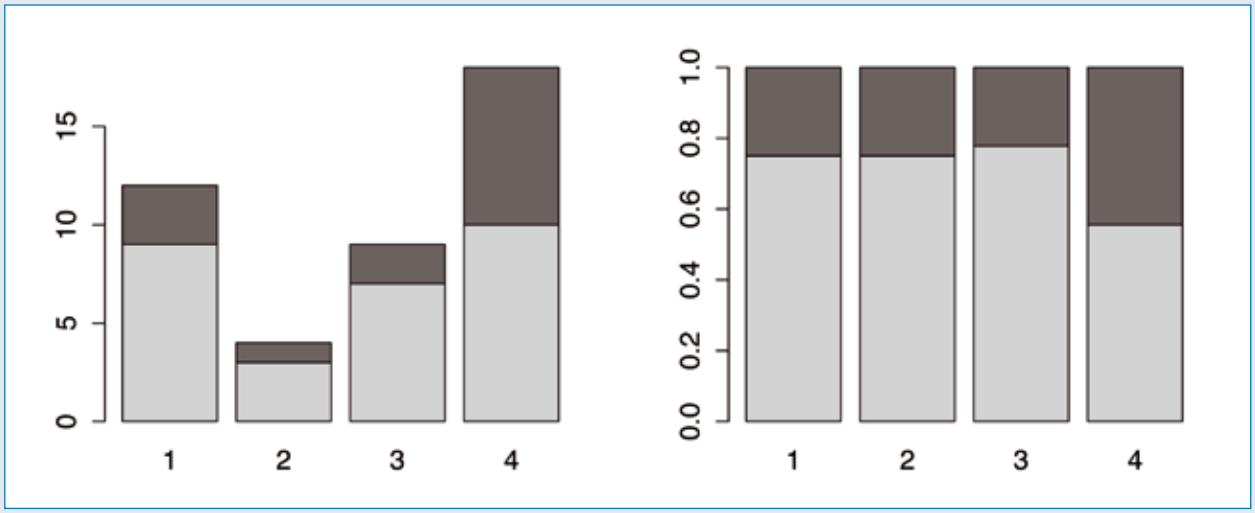

Figure 4: Absolute and relative distribution of types of *GRIN2A* mutations according to all published cases since 2013
In contradiction to Figure 3, the increase of published patients since 2013 lead to a harmonization of proportions of missense versus truncation mutations. For all phenotypes, this relation appears to be approximately 3:1, irrespective of the clinical severity.

mechanisms, such as decrease of antagonist binding, increase of agonist binding or reduction of pore blocking.

Surprisingly, the phenotypic spectrum of the missense mutations listed above is identical to individuals carrying truncating mutations. Truncations are considered to result in haploinsufficency and nonsense-mediated mRNA decay putatively leading to a loss of expression of the affected allele. This appears to be in contrast to a consecutive gain of NMDA receptor function. However, a possible mechanism might be the compensation of a decreased GluN2A expression by an increase of GluN2B expression. This would lead to an altered composition of the NMDA receptor. As GluN2B is associated with a longer channel opening time, a replacement of a GluN2A subunit by GluN2B within the NMDA receptor can be equated with a gain of function. Therefore, secondary changes on constitution of subunits, assembly of the receptor protein or other aspects are likely to influence the phenotype of a *GRIN2A* mutation carrier as well.

The finding of a gain of channel function mediated by *GRIN2A* mutations is of particular interest as hyperfunction of NMDA receptors have been successfully restored by memantine and other NMDA-blocking compounds. Pierson et al. 2014 recently reported a patient with *GRIN2A* mutation and refractory epilepsy, who responded well to memantine [15]. This finding gives hope for the development of individualised therapeutic approaches targeting the molecular defects of patients suffering from NMDA receptor-dependent epilepsy.

Conclusions

Mutations in *GRIN2A* play an important role in idiopathic focal epilepsies of childhood. They are particularly associated with phenotypes sharing a centrotemporal EEG focus, such as BECTS, ABPE, LKS and CSWS. Speech and intonation may be affected life-long – independent from epilepsy and other neurodevelopmental issues.

As some mutation carriers present with significantly milder or even subclinical phenotypes, *GRIN2A* mutations should be considered as high risk factors predisposing to the phenotypic spectrum described above, especially in the settings of an inherited familial mutation.

Gain of NMDA receptor function appears to be a common pathomechanism resulting from *GRIN2A* mutations. Thus, patients with *GRIN2A*-related epilepsies may benefit from application of memantine and similar drugs.

With increasing knowledge and rising numbers of identified patients, the *GRIN2A*-associated mutational distribution and spectrum can be revised and does not necessarily comply with initially observed or predicted findings.

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