Phenotypic Spectrum and Severity of Disease Depending on the Mutated Protein Domain of NMDA Receptor-Encoding Genes

Neurodevelopmental disorders such as intellectual disability, epilepsy, autism spectrum disorders, and impaired development of language and communication skills may result from mutation(s) affecting one or several genes governing brain development. In contrast to classical mendelian syndromes, a particular gene may be involved in several distinct clinical disorders [Poot, 2017]. Once the affected gene and mutation has been identified, the next task for medical genetics is to assess the phenotypic spectrum and the severity of the disorder, and to provide an informed guess regarding the prognosis of the patient. A particular challenging case represents the genes encoding components of the *N*-methyl-D-aspartate receptors (NMDARs), which are expressed throughout the brain and mediate excitatory neurotransmission in processes such as learning, memory, and other higher cognitive functions. The NMDARs are composed of di- or triheterotetrameric ligand-gated ion channels consisting of 2 glycine-binding GluN1 (encoded by *GRIN1*) and 2 glutamate-binding GluN2 subunits (encoded by the *GRIN2A–GRIN2D* genes) [Traynelis et al., 2010]. Of these genes, *GRIN1*, *GRIN2A*, *GRIN2B*, and *GRIN2D* have thus far been linked to human disease [Carvill et al., 2013; Lemke et al., 2013; Lesca et al., 2013; Hu et al., 2016; Fry et al., 2018]. The phenotypic spectrum associated with *GRIN1* mutations consists of profound developmental delay, severe intellectual disability with absent speech, muscular hypotonia, hyperkinetic movement disorder, oculogyric crises, cortical blindness, generalized cerebral atrophy, bilateral polymicrogyria, and epilepsy

[Lemke et al., 2016; Fry et al., 2018]. The *GRIN2A* gene has been associated with the broadest phenotypic spectrum, including disorders of the epilepsy-aphasia spectrum and developmental and epileptic encephalopathy, such as Landau-Kleffner syndrome and epileptic encephalopathy with continuous spike-and-wave during slow-wave sleep, respectively [Carvill et al., 2013; Lemke et al., 2013; Lesca et al., 2013]. Patients with *GRIN2B* mutations presented with a spectrum consisting of hypotonia, movement disorder, cortical visual impairment, cerebral volume loss, and epilepsy [Platzer et al., 2017]. Notably, 6 patients presented with a consistent malformation of cortical development between the tubulinopathies and polymicrogyria [Platzer et al., 2017]. While *GRIN1* mutations cluster within the transmembrane segments and result in loss of channel function with a dominant-negative effect, those in *GRIN2B* affected the transmembrane segments and the ligand-binding sites and likely operated via both gain-of-function and loss-of-function mechanisms [Lemke et al., 2016; Platzer et al., 2017].

Conceivably, similar phenotypes can result from single nucleotide variants (SNVs) that either enhance or reduce NMDAR function. Thus, the disease phenotype alone cannot predict how NMDARs have been dysregulated in each individual patient. To resolve this question, the functional impact of SNVs on NMDAR function was analyzed by a 2-step approach [Swanger et al., 2016]. First, SNVs were prioritized by bioinformatic procedures [Swanger et al., 2016]. Comparison of *GRIN2A* and *GRIN2B* SNVs in patients with non-neurologic disorders

listed in the Exome Aggregation Consortium (ExAC) dataset (release 0.3.1) with those in patients with epilepsy showed that the transmembrane and the cytoplasmic domains of the encoded proteins were highly overrepresented among the latter patients [Lek et al., 2016; Swanger et al., 2016]. Strikingly, the frequency of SNVs in the agonist-binding domain (ABD) of *GRIN2A* was not elevated in epilepsy versus ExAC patients, while those in *GRIN2B* were significantly overrepresented. This indicates that these variants are likely to be deleterious, which warrants further scrutiny. However, evaluation of a single NMDAR property in isolation may lead to incomplete or even misleading conclusions. Therefore, the authors cloned *GRIN2A* and *GRIN2B* genes containing these SNVs into vectors and subsequently transfected those into human embryonic kidney-293 cells. SNVs in ABD of either *GRIN2A* or *GRIN2B* were associated with neurological disease such as intellectual disability and intractable epilepsy, and found to alter the glutamate-binding affinities of the affected proteins. Thus, these SNVs in the ABD may affect the strength and time course of synaptic transmission by the neurons harboring them. In addition, the cell surface-to-total NMDAR protein ratios and/or the total NMDAR protein levels were found to be reduced for all SNVs, except for the GluN2A-p.Val734Leu variant. This suggests that SNVs affecting the ABD may reduce NMDAR levels at their functional sites in the neuronal dendrites. In contrast, the SNVs near the transmembrane domain linkers affect NMDAR channel gating, leading to a gain-of-function and ultimately to neurological symptoms. This property of these specific SNVs opens the possibility of pharmacologic modulation of the mutant NMDARs in order to alleviate the clinical symptoms in the patients. The authors note that *GRIN2A* and *GRIN2B* are expressed in both excitatory and inhibitory neurons in the brain. Therefore, the impact NMDAR affecting SNVs may have on the balance of excitation and inhibition in brain circuits will be influenced by their function (i.e., post-, non-, or presynaptic) and expression levels of these genes in different types of neurons. Conceivably, SNVs in other genes and compensatory mechanisms could affect brain development and lead to paradoxical effects on neuronal circuit function. By localizing the amino acid residue affected by each specific SNV within the tertiary structure of the NMDAR protein and clarifying the biochemical effects of the mutations, this study identified distinct underlying disease mechanisms and indicated treatment options for individual patients.

A recent study of a cohort of 248 patients with *GRIN2A* SNVs extends this approach further and sheds

new light on the involvement of NMDAR components on epilepsy and other neurodevelopmental disorders [Strehlow et al., 2019]. In this cohort, the phenotypic spectrum ranged from normal or near-normal development with mild epilepsy and speech delay/apraxia to severe developmental and epileptic encephalopathy up to the epilepsy-aphasia spectrum. The authors found that missense SNVs in transmembrane and linker domains (misTMD+Linker) were associated with severe developmental phenotypes, whereas missense SNVs within amino terminal or ligand-binding domains (misATD+LBD) and null variants provoked less severe phenotypes. Further phenotypes such as MRI abnormalities and some types of epilepsy were also significantly different between the 2 groups. Notably, this was reflected by electrophysiological results, such that misTMD+Linker SNVs predominantly led to gain of NMDAR function, while misATD+LBD SNVs exclusively caused loss of NMDAR function. Both misTMD+Linker and misATD+LBD SNVs are correlated with opposing electrophysiological consequences of the NMDAR. With respect to null variants, the authors found that *Grin2a*^{+/-} cortical rat neurons also showed reduced NMDAR function. Thus, in cases with *GRIN2A* SNVs, there is no evidence supporting a compensatory mechanism involving overexpression of other NMDAR components as previously postulated [Swanger et al., 2016]. Remarkably, *GRIN2A* null cases are associated with comparable clinical consequences as misATD+LBD (i.e., resulting in loss of function) and with clearly less severe clinical consequences than misTMD+Linker (resulting mostly in gain of function). The authors predict that individuals with developmental and epileptic encephalopathy due to misTMD+Linker SNVs are prone to having an underlying gain of NMDAR function. These patients represent promising candidates for treatment with NMDAR blockers, such as memantine [Pierson et al., 2014]. Conversely, patients with SNVs leading to complete or partial loss of channel function (misATD+LBD or null variants) may potentially respond to positive allosteric modulators of the NMDAR [Zhu and Paoletti, 2015; Addis et al., 2017]. These studies illustrate that systematic investigation of clinical phenotypes in large cohorts of individuals with a monogenic disease not only reveals novel genotype-phenotype correlations, but may also contribute to a better understanding of the underlying functional mechanisms. The latter opens the option of developing precision medicine tailored to the genetic and functional subtype of each individual patient.

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