Review article

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Update on the genetics of the epilepsy-aphasia spectrum and role of *GRIN2A* mutations

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ABSTRACT – Formerly idiopathic, focal epilepsies (IFE) are self-limiting, "age-related" diseases that mainly occur during critical developmental periods. Childhood epilepsy with centrotemporal spikes, or Rolandic epilepsy (RE), is the most frequent form of IFE. Together with the Landau-Kleffner syndrome and the epileptic Encephalopathy related to Status Epilepticus during slow Sleep syndrome (ESES), RE is part of a single and continuous spectrum of childhood epilepsies and epileptic encephalopathies with acquired cognitive, behavioral and speech and/or language impairment, known as the epilepsy-aphasia spectrum (EAS). The pathophysiology has long been attributed to an elusive and complex interplay between brain development and maturation processes on the one hand, and susceptibility genes on the other hand. Studies based on the variable combination of molecular cytogenetics, Sanger and next-generation sequencing tools, and functional assays have led to the identification and validation of genetic mutations in the *GRIN2A* gene that can directly cause various types of EAS disorders. The recent identification of *GRIN2A* defects in EAS represents a first and major break-through in our understanding of the underlying pathophysiological mechanisms. In this review, we describe the current knowledge on the genetic architecture of IFE.

Key words: childhood focal epilepsies, Rolandic epilepsy, encephalopathy related to status epilepticus during slow sleep, epileptic-aphasia, *GRIN2A*, genetics

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The so-called "idiopathic" childhood focal epilepsies (IFE) correspond to a broad spectrum of childhood epilepsy syndromes with specific age-dependent onset and typical EEG features such as multifocal spikes or spike-waves of various topographies. Childhood epilepsy with centro-temporal spikes (ECTS), also known as Rolandic epilepsy (RE), is the most frequent IFE. The relationships between RE and various comorbid manifestations and conditions, such as migraine, cognitive and behavioral issues, or reading impairment, have increasingly been recognized. Furthermore, the association with transient or permanent speech and/or language impairment has long been reported; hence the identification of the genetic syndrome of RE with verbal dyspraxia (Scheffer *et al*., 1995). Epileptic Encephalopathy related to Status Epilepticus during slow Sleep (ESES) and Landau-Kleffner syndrome (LKS) -also known as 'acquired' epileptic aphasia - are two closely related epileptic encephalopathies (EEs) that represent more severe and less frequent forms of the IFE continuum. Indeed, all those syndromes are now considered different clinical expressions of the same pathological spectrum (Rudolf *et al*., 2009). They all share the association of usually infrequent seizures with paroxysmal EEG discharges activated during drowsiness and sleep, sometimes fulfilling, in a subset of the patients, the criteria of status epilepticus during slow-wave sleep (SES), with more or less severe acquired neuropsychological and behavioral deficits.

General considerations about the genetic architecture of childhood focal epilepsies

A more modern view, that takes into account the recent advances in the genetic origin of various types of epilepsies, has recently challenged the classical distinction between idiopathic and symptomatic epilepsies (Berg *et al*., 2010). Indeed, based on some of the many possible examples, it has somehow unexpectedly demonstrated that various types of EEs, such as the Dravet syndrome or the Ohtahara syndrome, can have simple, monogenic causes (Depienne *et al*., 2012; Allen *et al*., 2013). Conversely, epilepsies of genetic origin (formerly considered as 'idiopathic') can be associated with comorbid neurological conditions (*e.g.* migraine, behavioral or cognitive issues) or with structural lesions (*e.g.* cortical dysplasia). In the IFE, the possible existence of behavioral and cognitive issues, for instance, inherently challenged the use of the 'benign' term. It had long been assumed that in contrast to generalized epilepsies, most focal epilepsies are caused by lesions, infections, tumors, etc. and are hardly under genetic influence. Twin studies and familial concurrences then indicated that focal epilepsies could also be sustained by genetic factors (Ryan, 1995). As an example, the mapping and the subsequent identification of the first 'monogenic epilepsy' gene (*CHRNA4*) encoding a nicotinic acetylcholine receptor subunit was obtained for autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein *et al*., 1995). Since then, several genes responsible for rare types of monogenic focal epilepsies have been identified (Berkovic *et al*., 2006).

Familial aggregation has long been recognized in RE (Neubauer *et al*., 1998). Relatives of RE patients display higher risk of epilepsy (notably RE, LKS or ESES) than control individuals (De Tiege *et al*., 2006; Vears *et al*., 2012; Dimassi *et al*., 2014). Most RE, however, do not show a simple inheritance. In contrast to RE, the genetic influence in LKS and ESES has long remained controversial (Landau and Kleffner, 1957; Rudolf *et al*., 2009) and a role for autoimmunity has even been hypothesized (Connolly *et al*., 1999; Nieuwenhuis *et al*., 2006). Recent advances in molecular cytogenetics and in next-generation DNA sequencing have dramatically helped in solving this issue. Consistent with the existence of genomic defects (copy number variations) that may have possible pathophysiological influence in numerous human disorders including the epilepsies (Helbig *et al*., 2009; Mefford *et al*., 2010), the screening of a series of 61 patients with LKS or ESES led to an overall picture with highly heterogeneous genomic architecture (Lesca *et al*., 2012). A large number of potentially pathogenic alterations corresponded to genomic regions or genes (*e.g.* encoding cell adhesion proteins) that were either associated with the spectrum of autism disorders, or involved in speech or language impairment - which was of interest given the well-known association of LKS and ESES with autism-like manifestations (*e.g.* regression, disturbance of social interactions, perseveration) and with language disorders.

The role of *GRIN2A* mutations in RE/ESES/LKS

The study by Lesca and colleagues led to detection of several *de novo* genomic alterations including deletions of the NMDA glutamate receptor (NMDAR) subunit gene *GRIN2A* (Lesca *et al*., 2012). Besides the obvious crucial function of NMDARs in the brain (Burnashev & Szepetowski, 2015), a few *GRIN2A* defects had previously been reported in patients with severe neurodevelopmental disorders (Reutlinger *et al*., 2010; Endele *et al*., 2010). Since then, the crucial and direct causal role of *de novo* or inherited *GRIN2A* mutations (microdeletions, splice-site, nonsense and missense mutations) in LKS, in ESES, and in RE with

verbal dyspraxia has been demonstrated in three parallel studies (Carvill *et al*., 2013; Lemke *et al*., 2013; Lesca *et al*., 2013). The mutations were found in all the different domains of the corresponding GluN2A (formerly known as NR2A) subunit, making it difficult to draw any clear genotype-phenotype correlation (Burnashev and Szepetowski, 2015; von Stülpnagel et al., 2017). However, *de novo* mutations of *GRIN2A* might cluster in and around the ligand-binding sites and the transmembrane domains (Strehlow *et al*., 2015).

The existence of a simple, unifying pathophysiological mechanism remains elusive: whereas the existence of microdeletions and nonsense mutations indicated loss-of-function (LOF) effects, some missense mutations seemed to lead to gain-of-function (GOF), at least *in vitro*, and some may even have multiple effects (Yuan *et al*., 2014; Swanger *et al*., 2016; Sibarov *et al*., 2017). Interestingly, a recent study has indicated that some *GRIN2A*-associated EEs caused by gain-of-function mutations might be treatable by the NMDA receptor blocker, memantine (Pierson *et al*., 2014). This study confers hope for more targeted therapeutic strategies in *GRIN2A*-related seizure disorders caused by GOF mutations. Nevertheless, an alteration in the Glun2B-to-GluN2A developmental switch, as suggested by altered kinetics of the mutant NMDARs (Carvill *et al*., 2013; Lesca *et al*., 2013), and a dysfunction of the thalamocortical network are likely. As a matter of fact, *Grin2a* knockout mice one month old, exhibit a series of transient brain microstructural alterations that involve the thalamus and the neocortex, and also display rare spontaneous epileptiform discharges in the third postnatal week (Salmi *et al*., 2018). A case report suggested that the epileptic discharges could have a triggering role in the speech deterioration observed in children carrying a deleterious variant of *GRIN2A* (Sculier *et al*., 2017).

Based on the initial studies, it has been estimated that up to 20% of patients with RE, LKS or ESES have a mutation in *GRIN2A*. A recent exome-wide study of the mutational burden in patients with typical and atypical RE showed that *GRIN2A* was the only gene with a significantly enriched burden associated with deleterious and LOF mutations (Bobbili *et al*., 2018). However, interestingly, the statistical significance of this burden disappeared after excluding atypical RE patients, indicating that *GRIN2A* is more likely to cause phenotypes within the severe end of the disease spectrum (Bobbili *et al*., 2018).

Other rare monogenic causes of ESES

Besides *GRIN2A*, other rare monogenic causes, particularly for ESES, have been reported (*table 1*). ESES has been reported in patients with LOF mutations in *KCNA2* encoding the potassium channel subunit $K_v1.2$. The patients usually present with febrile and multiple afebrile, often focal seizure types, multifocal epileptiform discharges strongly activated by sleep, mild to moderate intellectual disability, delayed speech development, and sometimes ataxia (Syrbe *et al*., 2015; Masnada *et al*., 2017). Interestingly, mutations in another potassium channel gene, *KCNB1*, have also been detected in patients with developmental and epileptic encephalopathies and an ESES-like pattern on the EEG (*figure 1*) (de Kovel *et al*., 2017; Marini *et al*., 2017).

Furthermore, several genetic and genomic defects of the *CNKSR2* gene, encoding an adaptor protein of the postsynaptic density, have been identified in patients with clinical features reminiscent of the epilepsy-aphasia spectrum (Lesca *et al*., 2012; Vaags *et al*., 2014; Damiano *et al*., 2017). Last but not least, recent publications also suggest that ESES may be a frequent and underestimated feature in patients with Christianson syndrome, a severe neurodevelopmental disorder due to mutations in *SLC9A6*, encoding the endosomal solute carrier (Na K/H) exchanger 6 (NHE6) (Zanni *et al*., 2014; Mathieu *et al*., 2018).

Genetic susceptibility factors

16p11.2 microduplications encompassing *PRRT2* have not been identified in patients with RE or atypical RE (Dimassi *et al*., 2014; Reinthaler *et al*., 2014). *PRRT2* has been associated with a wide range of paroxysmal neurological disorders including infantile convulsions, paroxysmal dyskinesia (mostly kinesigenic), or hemiplegic migraine (Cloarec *et al*., 2012; Lee *et al*., 2012), thus duplication of this gene may also confer risk for the development of RE.

Other potential genetic risk factors for RE have been suggested. For example, a dominant-negative rare variant previously identified in the *SRPX2* (Sushi-Repeat Protein, X-linked 2) protein (Roll *et al*., 2006) co-segregated with a p.Ala716Thr *GRIN2A* missense mutation in most affected members of a family with RE, verbal dyspraxia, and intellectual disability (Lesca *et al*., 2013). The *GRIN2A* mutation likely plays a key role in this family, although it had no obvious effect on a subset of NMDAR properties *in vitro* (Sibarov *et al*., 2017). On the other hand, *SRPX2* causes transcriptional down-regulation of *FOXP2* (Roll *et al*., 2010), which is associated with verbal dyspraxia (Lai *et al*., 2001). Furthermore, knockdown of *Srpx2 in utero* had dramatic consequences on neuronal migration in the developing rat cerebral cortex and led to postnatal epileptiform activity that was prevented by maternal administration of a tubulin deacetylase inhibitor (Salmi *et al*., 2013). Moreover, it was shown in mice that *Srpx2*

Table 1. Monogenic causes of RE/LKS/ESES.

	GRIN2A	***KCNA2***	***KCNB1***	***SLC9A6***	***CNKSR2***
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant	X-linked recessive	X-linked recessive
Functional effect	LOF, GOF	LOF	LOF	LOF	LOF
Seizure type	Focal motor seizures, GTCS, myoclonia, dyscognitive seizures	Febrile and multiple afebrile, often focal seizure types	Tonic, focal-clonic, myoclonia, spasms atypical absences, eyelid myoclonia	Absence, myoclonia, generalized seizures	Generalized or focal seizures
Clinical regression	+/-	No	+/-	Yes	Yes
Pharmacoresistant	No	No	Yes	Yes	No
Possible on sleep EEG	Centrotemporal spikes or ESES	Multifocal epileptiform discharges strongly activated by sleep/ESES	Multifocal or GS/PS strongly activated by sleep/ ESES	ESES	ESES
ID	Possible	Severe	Severe	Severe	Severe
Autistic features	Possible	Possible	Possible	Yes	NK

Abbreviations: LOF: loss of function, GOF: gain of function, ESES: Encephalopathy related to Status Epilepticus during slow Sleep, GS/PS: generalized spikes/polyspikes, NK: not known, GTCS: generalized tonic-clonic seizures

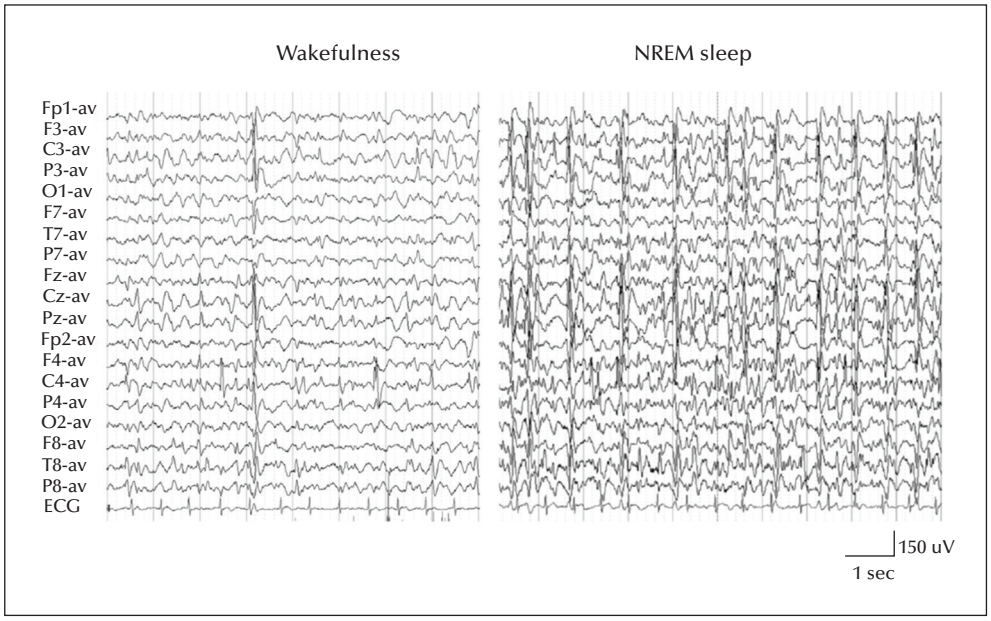

Figure 1. Extreme activation of epileptic activity (spike-wave index during NREM sleep up to 98%) consistent with an ESES EEG pattern in a three-year-old boy with KCNB1 encephalopathy.

also influences synaptogenesis and ultrasonic vocalization (Sia *et al*., 2013). Overall, rare *SRPX2* variants might correspond to genetic risk factors for various neurodevelopmental disorders; as a matter of fact, one splice-site *SRPX2* mutation was reported in a patient with autism (Lim *et al*., 2013), and the p.N327S rare variant was detected in a novel patient with LKS (Reinthaler *et al*., 2014).

Furthermore, *ELP4* has been proposed as a susceptibility gene for RE (Strug *et al*., 2009), however, this has never been confirmed. Mutations that might influence RE were also identified in the paralogous *RBFOX1* and *RBFOX3* neuronal splicing regulator genes (Lal *et al*., 2013), in the mammalian target of rapamycin regulator gene *DEPDC5* (Lal *et al*., 2014), and recently also in the *GABRG2* gene (Reinthaler *et al*., 2015).

Conclusion

In conclusion, several genes have been associated with LKS/ESES/RE, however, mutations in *GRIN2A* represent by far the most common genetic cause identified so far. As *GRIN2A* mutations unambiguously participate in the epilepsy-aphasia spectrum, whether and how genetic modifiers and environmental factors together with *GRIN2A* mutations contribute to the huge phenotypic variability, seen in the affected patients and families, emerges as an important question that surely deserves future investigation. Identification of other genetic and non-genetic factors and the ongoing studies with corresponding animal models, such as the *Grin2a* KO murine model, will help our understanding of the pathophysiology of this fascinating group of disorders situated at the crossroads between epileptic, cognitive, behavioral, and speech and language disorders. □

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