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GRIN2A mutations cause epilepsy-aphasia spectrum disorders

Gemma L Carvill[1], **Brigid M Regan**[2], **Simone C Yendle**[2], **Brian J O'Roak**[3], **Natalia Lozovaya**[4,5,6,7], **Nadine Bruneau**[4,5,6,8], **Nail Burnashev**[4,5,6,8], **Adiba Khan**[1], **Joseph Cook**[1], **Eileen Geraghty**[1], **Lynette G Sadleir**[9], **Samantha J Turner**[2,10], **Meng-Han Tsai**[2], **Richard Webster**[11], **Robert Ouvrier**[11], **John A Damiano**[2], **Samuel F Berkovic**[2], **Jay Shendure**[3], **Michael S Hildebrand**[2], **Pierre Szepetowski**[4,5,6,8], **Ingrid E Scheffer**[2,10,12,*], and **Heather C Mefford**[1,*]

[1]Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, Washington, 98195, USA [2]Epilepsy Research Centre, Department of Medicine, University of Melbourne, Austin Health, Australia [3]Department of Genome Sciences, University of Washington, Seattle, Washington, 98195, USA [4]Mediterranean Institute of Neurobiology (INMED), Marseille, France [5]INSERM UMR_S901, Marseille, France [6]Aix-Marseille University, Marseille, France [7]INSERM UMR_S663, Paris Descartes University, Paris, France [8]French EPILAND network on Epilepsy, Language and Development [9]Department of Pediatrics, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand [10]Department of Pediatrics, University of Melbourne, Royal Children's Hospital, Australia [11]T.Y. Nelson Department of Neurology and Neurosurgery, The Children's Hospital at Westmead, Sydney, New South Wales, Australia [12]Florey Institute, Melbourne, Australia

Letter to the editor

The epilepsy-aphasia syndromes (EAS) are a group of rare, severe epileptic encephalopathies of unknown etiology with a characteristic EEG pattern and developmental regression, particularly affecting language. Rare pathogenic deletions that include *GRIN2A* have been implicated in neurodevelopmental disorders. We sought to delineate the

Correspondence to: Dr. Heather Mefford: hmefford@u.washington.edu and Prof Ingrid Scheffer: scheffer@unimelb.edu.au.
*These authors contributed equally to this work.

Author Contributions

GLC, HCM and IES designed the study and wrote the manuscript. HCM and IES supervised the study. GLC constructed libraries, developed the variant calling pipeline (assisted by JC), analyzed the sequence data, conducted RNA transcript analysis (assisted by EG) and performed haplotyping. JC and GLC performed array CGH. AK performed mutation segregation analysis. BJO and JS developed the MIPs methodology and analysis pipeline. BMR, SCY, LGS, SJT, MT, RW performed phenotypic analysis. RO, JAD, MSH conducted mutation screening in BECTS cohort. BMR, SFB and IES critically reviewed the manuscript. NL, NBruneau, NBurnashev, PS generated mutant transcripts and performed single-channel recordings and analysis.

URLs

Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [10/2012 accessed]
Polyphen2 (http://genetics.bwh.harvard.edu/pph2/)
SIFT (http://sift.bii.a-star.edu.sg/)
Genome Analysis Toolkit GATK (version 1.6) (http://www.broadinstitute.org/gatk/)
Seattle seq (version 134) (http://snp.gs.washington.edu/SeattleSeqAnnotation134/)

pathogenic role of *GRIN2A* in 519 epileptic encephalopathy probands with diverse epilepsy syndromes. We identified four probands with *GRIN2A* variants that segregated with the disorder in their families. Strikingly, all four families presented with EAS, accounting for 9% of epilepsy-aphasia cases. We did not detect pathogenic variants in other epileptic encephalopathies (n=475), nor in 81 probands with benign childhood epilepsy with centro-temporal spikes. We report the first monogenic cause for EAS. *GRIN2A* mutations are restricted to this group of patients, with important ramifications for diagnostic testing and treatment, and novel insights into the pathogenesis of this debilitating group of conditions.

The epileptic encephalopathies are a severe group of disorders characterized by seizures and abundant epileptiform activity that contribute to cognitive and behavioral impairment[1]. The epileptic encephalopathies comprise a range of electroclinical syndromes with characteristic ages of onset, clinical and EEG manifestations. Two syndromes with overlapping manifestations have the remarkable EEG signature of continuous spike-wave during slow wave sleep (CSWS) in which the non-REM sleep EEG shows virtually continuous (≥85%) high voltage bilateral slow spike wave activity that largely remits on awakening. In Landau-Kleffner syndrome (LKS), children who were previously normal or isolated language delay present with an acquired epileptic aphasia; focal motor seizures occur in 70% of cases and are usually easily controlled. In contrast, in the syndrome of epileptic encephalopathy with continuous spike-wave during slow wave sleep (ECSWS), prior development is delayed in half the children and refractory epilepsy with multiple seizure types is usual. Regression is more global with language, behavior and motor impairment[2]. MRI brain studies are often normal or may show a malformation of cortical development such as perisylvian polymicrogyria.

In clinical practice, there are patients who do not meet the criteria on EEG or clinical grounds for LKS and ECSWS, usually because their EEG abnormalities do not occupy 85% of slow sleep, yet they have significant language or learning difficulties which may fluctuate in severity. There is debate whether <85% of bilateral epileptiform activity in non-REM sleep is diagnosable as CSWS or whether it should be regarded as an intermediate epilepsy-aphasia disorder (IEAD)[3]. These disorders can be conceptualized as falling along a spectrum with LKS, ECSWS at the severe end, IEAD in the middle and benign childhood epilepsy with centro-temporal spikes (BECTS) at the mild end of the spectrum[3]. BECTS is the most common focal epilepsy syndrome in childhood and occurs in normal children who present with focal motor rolandic seizures. The EEG shows unilateral or bilateral centro-temporal spikes that are activated by sleep but do not show the almost continuous bilaterally synchronous pattern of CSWS, and the children do not show cognitive decline. The presence of subtle oral dyspraxia has been noted in some patients with BECTS[4].

Until recently there has been scant evidence for a genetic etiology for the disorders of the epilepsy-aphasia spectrum. To date, only four families have been reported with monogenic inheritance of rolandic epilepsy and speech or language difficulties. We reported an autosomal dominant family in 1995 with the syndrome of autosomal dominant rolandic epilepsy with speech dyspraxia (ADRESD)[5]. An additional three-generational family with a strikingly similar phenotype was reported more recently[6]. Finally a family with dysphasia and epilepsy with generalized and focal manifestations was reported[7]. A causal gene has not

been implicated in these families. Conversely, a fourth family, presenting with X-linked rolandic epilepsy, oral and speech dyspraxia and intellectual disability (ID) was identified with a gain-of-glycosylation *SRPX2* mutation[8]. Besides a *SRPX2* mutation in an unrelated proband with perisylvian polymicrogyria and rolandic seizures and female relatives with mild ID, no additional *SRPX2* variants in epilepsy-aphasia phenotypes have been described.

Clinical genetic studies of probands with BECTS or EAS provide little support for genes of major effect. Investigation of relatives up to three degrees of relatedness to probands with BECTS or the epilepsy-aphasia spectrum suggest that complex inheritance is most likely with febrile seizures being the most common phenotype in relatives of probands[3, 9].

While there has been strong contention that the epilepsy-aphasia syndromes have an immune basis, partly due to their resolution with high dose steroids, a genetic etiology is supported by the rare familial forms described. Furthermore, recent evidence for a genetic etiology has come from copy number variant (CNV) studies. An excess of rare CNVs was noted in a cohort of LKS and CSWS probands[10], including a single LKS proband with a 16p13 deletion containing one gene, *GRIN2A* (NM_000833.3)[10]. Furthermore, three children with complex dysmorphic phenotypes were reported with 16p13 deletions that included *GRIN2A*[11]. *GRIN2A* encodes the NR2A subunit of the N-methyl-D-aspartate (NMDA) receptor, a neurotransmitter-gated ion channel that mediates excitatory transmission in the mammalian brain, making it an attractive candidate for epileptogenesis. *GRIN2A* mutation screening in 127 probands with epilepsy or an abnormal EEG and/or ID detected two pathogenic mutations: a nonsense mutation segregating with epilepsy or an abnormal EEG in three family members and a *de novo* missense mutation in a patient with severe early-onset epileptic encephalopathies [12]. Furthermore, a missense mutation was recently reported in a single proband in a large exome sequencing cohort with ID[13]. While these observations strongly support a role for *GRIN2A* in epilepsy and ID, no clear genotype-phenotype correlations have emerged. Therefore, we sought to delineate the phenotypic spectrum of *GRIN2A* mutations by screening a large cohort of patients with epileptic encephalopathy.

We performed high-throughput sequence analysis of *GRIN2A* in 519 probands with a range of epileptic encephalopathies (Table 1). As part of a larger study [14] we performed targeted gene capture of 18 genes associated with epilepsy, including *GRIN2A*. Briefly, we re-sequenced all exons and flanking 5 base pairs using molecular inversion probes (MIPs), highly multiplex PCR and next generation sequencing as described previously with minor exceptions (online methods)[15]. Using this approach we achieved, on average, 98% coverage (>25X) across *GRIN2A* for all probands.

We identified four probands with *GRIN2A* mutations, each of which was confirmed by Sanger sequencing. Segregation analysis in additional family members showed that each variant segregated in an autosomal dominant manner (Table 2, Figure 1). These *GRIN2A* variants are not present in 6500 control exomes (see Resources). Two families (A, C) carried the same c.1005-1C>T variant, affecting the highly conserved donor splice site. Genotyping of microsatellite makers and a rare SNV flanking this *GRIN2A* mutation revealed an identical haplotype in these families, suggesting a common founder mutation

(Supplementary Figure 1). The c1005-1C>T variant was predicted *in silico* to cause skipping of exon four during pre-mRNA splicing, resulting in the removal of 593 exonic nucleotides from the mature transcript and thus a frameshift mutation, Phe139Ilefs*15 (predicted) (see Supplementary Table1). We tested for the presence of a rare exonic SNV (rs61753382), encompassed by the common haplotype in affected individuals, in the RNA transcripts of three affected individuals from both families. We detected monoallelic expression of the wild-type variant, suggesting nonsense mediated decay of the mutant transcript (Supplementary Figure 2).

We detected a p. Met1Thr variant in family B. The alteration of this translation start codon is likely to have detrimental effects on *GRIN2A* protein synthesis, resulting in either complete absence of product due to failure of translation initiation at the start codon, or a truncated protein stemming from translation initiation at an alternate start codon. We were unable to test this as proband RNA was unavailable.

Finally we describe a p.Thr531Met variant that affects a highly conserved residue (as predicted by high GERP and Grantham scores), that is predicted to be probably damaging by Polyphen2 and SIFT (Table 2). This variant is located in the extracellular ligand-binding domain of NR2A. Specific sites within this domain are known to influence gating and kinetic properties of NMDA receptors[16, 17]. We assessed the effect of the p.Thr531Met mutation on NR2A function by co-expression with NR1 in COS-7 cells to form a mutant heteromeric NMDA receptor. A resultant shift in NMDA receptor kinetics was observed by single channel recordings with a four-fold increase in mean open time of the mutant channels (36.7±2.5 ms ; n=2299 channel events), as compared to the wild-type channels (9.1±0.2 ms; n=6715 channel events) ($P<0.0001$, Mann Whitney test, two-tailed) (Figure 2). This novel variant displayed similar clinical and functional consequences to missense mutations in the same domain in a parallel study by Lesca and colleagues (this issue).

The c1005-1C>T and Met1Thr variants likely cause disease as a result of haploinsufficiency of the NR2 subunit of the NMDA receptor, possibly by aberrant NMDA receptor composition or distribution in the brain. Furthermore, we show that the p. Thr531Met variant has a profound effect on NMDA receptor kinetics. Given the pathogenic effect of these mutations and their segregation with the disorder, we conclude that the *GRIN2A* mutations ascribe causality in these families.

Remarkably, all four *GRIN2A* positive families presented with EAS, yielding a 9% (4/44) mutation rate in patients with this group of EE. No additional pathogenic variants were detected in the remaining epileptic encephalopathy phenotypes (Table 1). In the 40 remaining EAS patients, we performed array-CGH using a custom microarray with probes spanning *GRIN2A* at an average density of one probe every ~350bp. No copy number alterations were detected.

Given that BECTS lies at the mild end of the EAS, we next screened 81 probands with BECTS for *GRIN2A* variants using Sanger sequencing. No additional pathogenic variants were identified.

There were 16 subjects with *GRIN2A* mutations. Segregation was perfect in the 7 affected members of the original family with autosomal dominant rolandic epilepsy with speech dyspraxia (Family A, Fig. 1)[5]. The same mutation was found in a father-son pair with ECSWS (Family C). Interestingly the *GRIN2A* mutations were associated with a range of EAS phenotypes including LKS, ECSWS and IEAD (Table 3). All individuals with LKS and ECSWS showed CSWS on EEG studies. Individuals with IEAD had not had a sleep EEG performed to detect CSWS. Affected family members had a complex phenotype including epilepsy (14/16), speech and language difficulties (16/16). While intellectual disability occurred in 6/16 mutation carriers, a further two were of borderline intellect (Supplementary Table 2).

Previous cases implicating *GRIN2A* have not identified a consistent epilepsy phenotype but have shared features with our cases. Four cases with 16p13 microdeletions including *GRIN2A* have been reported; one had LKS[10]. The remaining three were more complex with dysmorphic features and moderate to severe ID; two were non-verbal and only one walked independently [3]. All had seizures; one had atypical benign partial epilepsy, which is part of the EAS. One had rolandic seizures without regression and EEG studies were not available. In two patients, eyelid myoclonias were noted which is somewhat atypical for EAS. Two had an EEG pattern suggestive of CSWS. In another study, a three generation family with a translocation disrupting *GRIN2A* was associated with childhood and adolescent onset convulsions in the setting of learning difficulties or ID. There was no suggestion of CSWS on their EEG studies and no epilepsy syndrome was determined.

We conclude that *GRIN2A* mutations are highly predictive of EAS that include LKS, ECSWS and IEAD. Furthermore, in a separate study Lesca and colleagues report *GRIN2A* mutations in 20% of LKS, ECSWS and atypical rolandic epilepsy with speech impairment confirming the importance of *GRIN2A* to the EAS (this issue). Of note, we did not detect any *GRIN2A* variants in 475 probands with other epileptic encephalopathy phenotypes, or in 81 probands with BECTS. Furthermore, in a large series (n= 1703) of autism probands no *GRIN2A* mutations were identified[18]. These results demonstrate that the genetic etiology of EAS may well be distinct, an observation that balks the current trend towards an overlapping etiology for neurodevelopmental disorders. We hypothesize that altered NMDA receptor activity due to *GRIN2A* haploinsufficiency or missense mutations results in aberrant ion flux and disruption of the downstream signaling cascade. The role of NMDA receptor aberration and its potential role in the corticothalamic network disrupted in slow sleep will be an important area of future research. This study is the first to detect a monogenic cause for epilepsy-aphasia syndromes with a mutational rate of 9%. These results strongly suggest that *GRIN2A* diagnostic testing is warranted in patients with epilepsy-aphasia and will enhance prognostic and genetic counseling for families.

Online methods

Data analysis and variant calling

Raw read processing and alignment was performed as previously[14]. However variant (single nucleotide and indel) calling and filtering was performed using the Genome Analysis Tool Kit (GATK) (see URLs version). Variants that did not adhere to the following criteria were

excluded from further analysis: allele balance >0.70, QUAL<30, QD<5, coverage<25X, clustered variants (window size-10) and variants in homopolymer runs (5 bp). Variants were annotated with Seattle seq (see URLS and version) and the ESP6500 dataset (see URLs) used to assess variant frequency in control population. PCR and Sanger sequencing were conducted according to standard methods as described previously.

Array CGH

We performed array CGH using a custom designed 8plex microarray [Agilent], designed to detect copy number alterations in known epilepsy genes. *GRIN2A* was covered at a density of one probe every ~350bp. All experiments were performed as per the manufacturer's instructions and data analysis conducted using Genomic Workbench [Agilent].

Genotyping

We performed genotyping in all available affected and unaffected members of Family A and B, who carried the c.1005-1C>T variant. We selected three microsatellite markers, D16S404, D16S3126 and D16S407, spanning a 0.56Mb interval across *GRIN2A*. Fluorescently labeled PCR products were analyzed on an ABI3100 genetic analyzer, and allele size ranges determined with the GS500LIZ size standard [Applied Biosystems] using the PeakScanner V2.0 software [Applied Biosystems]. Furthermore, we genotyped all family members for the rare exonic GRIN2A variant (rs61753382) using Sanger DNA sequencing.

RNA transcript analysis

RNA was isolated from whole blood of affected family members and controls using the PAXgene blood RNA kit [PreAnalytiX]. cDNA synthesis was performed using 1ug of DNA using the iScript Reverse Transcription Supermix kit [Bio-Rad]. Nested PCR and Sanger sequencing was performed for *GRIN2A* RNA transcripts analysis in three affected family members from both c1005-1C>T carrier families. We assessed the presence of the rs6173382 variant, the minor allele was linked to the c.1005-1C>T mutation (see Supplementary Table 3 for primer pairs).

Constructs and transfections

NR1 and NR2A constructs were commercially purchased [Genecopeia]. Site-directed mutagenesis [Agilent Technologies] was used to generate the mutant NR2A (p.Thr531Met) construct, using the following 5′-gtgccctttgtggaaatgggaatcagtgtcatgg and corresponding reverse complement primers. All wild-type and mutant constructs were verified by Sanger sequencing. Monkey kidney fibroblast like COS-7 cells were seeded in six-well plates (10^5 cells/well) one day before transfection. Magnetofection of NR1 and NR2A constructs (1:3 ratio) was performed using the Magnetofectamine transfection kit [OzBiosciences, France]. The presence of the NR1 and NR2A (wt and mutant) subunits at the plasma membrane was verified by immunocytochemistry experiments (data not shown).

Single channel recordings and analysis

Single channel recordings were made from transfected COS-7 cells (ATCC, CRL-1651) in cell-attached patches at holding potential +100 mV using an EPC-10 amplifier (HEKA Elektronik, Germany). Cells were negative for Mycoplasma contamination using the MycoTrace Mycoplasma PCR detection kit (PAA, France). No authentication was made. Cells in the recording chamber were perfused with oxygenated ACSF containing (in mM): 126 NaCl, 3.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 D(+)-glucose, 1.20 NaH_2PO_4, 26 $NaHCO_3$ (oxygenated with 5% CO_2/95% O_2). Recording patch pipettes were pulled from borosilicate glass capillaries (World Precision Instruments Inc., Sarasota, USA) and had resistances of 4 to 7 MΩ when filled with the solution of the following composition (in mM): 140 NaCl, 2.8 KCl, 2 $CaCl_2$, 10 Glucose, 20 HEPES/NaOH, (pH 7.5, 320–330 mOsm). The channels were activated by 50 μM glycine and 1 mM glutamate in the pipette solution. Recordings were performed at room temperature (22–24°C). For analysis, the recordings were filtered at 2 kHz (−3 dB) and digitized at 20 kHz. Lifetime analysis was performed using a Clampfit 10.2 (Molecular Devices, Eugene, OR) and Origin 8.5 (Origin-Lab, Northampton, MA) software. For data analysis by Clampfit, a digital 8-pole Bessel low-pass filter was set at 1 kHz. Average values were expressed as means ± SEM. Statistical significance of the differences was evaluated by nonparametric Mann Whitney test, two-tailed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Berg AT, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia. 2010; 51:676–85. [PubMed: 20196795]
2. Tassinari CA, et al. Encephalopathy with electrical status epilepticus during slow sleep or ESES syndrome including the acquired aphasia. Clin Neurophysiol. 2000; 111 (Suppl 2):S94–S102. [PubMed: 10996561]
3. Tsai M, et al. Clinical genetic study of the epilepsy-aphasia spectrum. Epilepsia. 2012; 54:280–287. [PubMed: 23294109]
4. Deonna TW, Roulet E, Fontan D, Marcoz JP. Speech and oromotor deficits of epileptic origin in benign partial epilepsy of childhood with rolandic spikes (BPERS). Relationship to the acquired aphasia-epilepsy syndrome. Neuropediatrics. 1993; 24:83–87. [PubMed: 7687041]
5. Scheffer IE, et al. Autosomal dominant rolandic epilepsy and speech dyspraxia: a new syndrome with anticipation. Ann Neurol. 1995; 38:633–642. [PubMed: 7574460]
6. Kugler SL, et al. An autosomal dominant genetically heterogeneous variant of rolandic epilepsy and speech disorder. Epilepsia. 2008; 49:1086–1090. [PubMed: 18248446]

7. Michelucci R, et al. Familial epilepsy and developmental dysphasia: description of an Italian pedigree with autosomal dominant inheritance and screening of candidate loci. Epilepsy Res. 2008; 80:9–17. [PubMed: 18508238]
8. Roll P, et al. SRPX2 mutations in disorders of language cortex and cognition. Hum Mol Genet. 2006; 15:1195–1207. [PubMed: 16497722]
9. Vears DF, et al. Clinical genetic studies in benign childhood epilepsy with centrotemporal spikes. Epilepsia. 2012; 53:319–324. [PubMed: 22220564]
10. Lesca G, et al. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. Epilepsia. 2012; 53:1526–1538. [PubMed: 22738016]
11. Reutlinger C, et al. Deletions in 16p13 including GRIN2A in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. Epilepsia. 2010; 51:1870–1873. [PubMed: 20384727]
12. Endele S, et al. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet. 2010; 42:1021–1026. [PubMed: 20890276]
13. de Ligt J, et al. Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability. N Engl J Med. 2012
14. Carvill GL, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. Nat Genet. 2013
15. O'Roak BJ, et al. Multiplex Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders. Science. 2012; 338:1619–1622. [PubMed: 23160955]
16. Talukder I, Borker P, Wollmuth LP. Specific sites within the ligand-binding domain and ion channel linkers modulate NMDA receptor gating. J Neurosci. 2010; 30:11792–11804. [PubMed: 20810899]
17. Traynelis SF, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev. 2010; 62:405–496. [PubMed: 20716669]
18. O'Roak BJ, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. 2012; 485:246–250. [PubMed: 22495309]

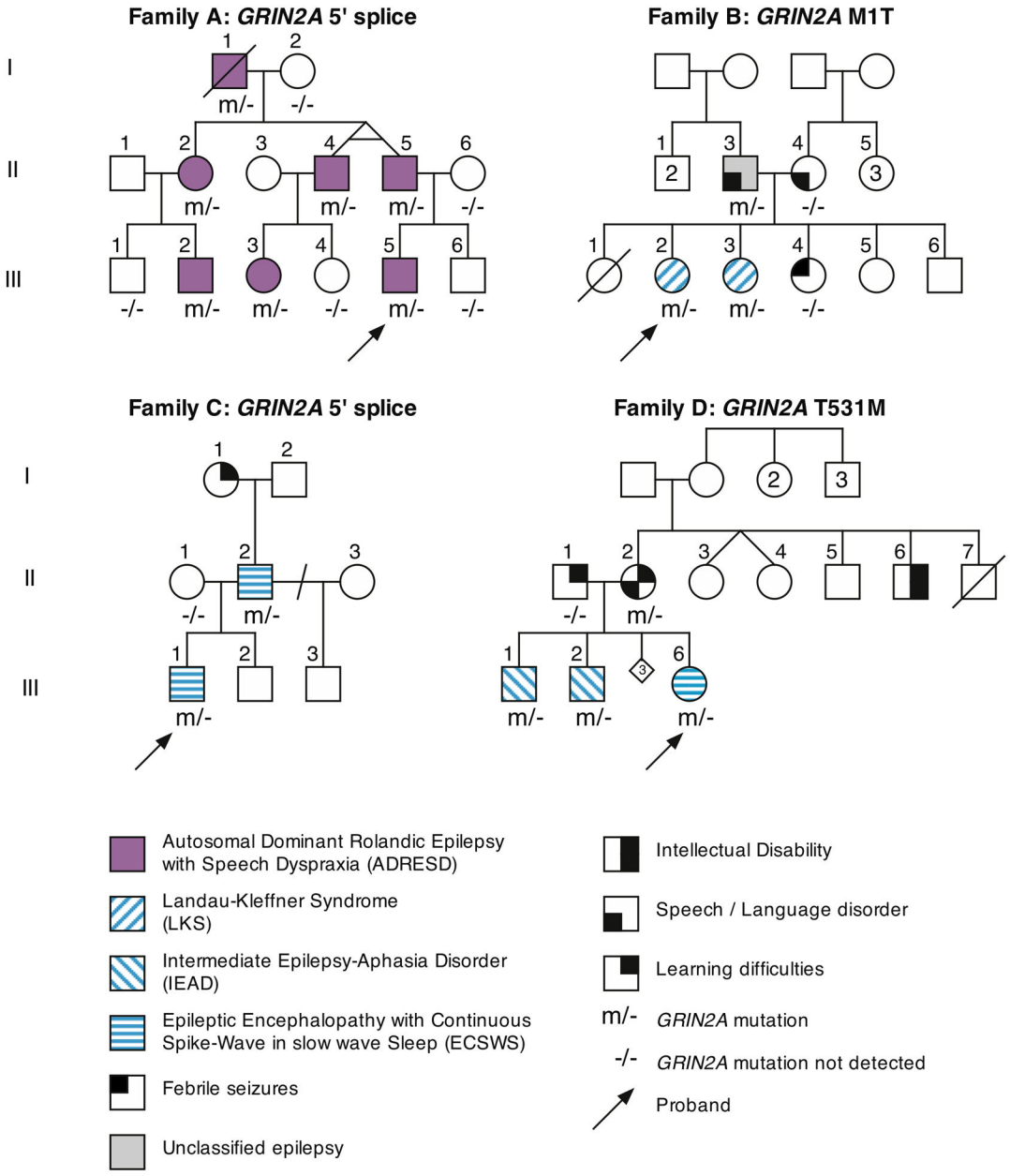

Figure 1.

Phenotypes and segregation of *GRIN2A* mutations in four families with epilepsy-aphasia syndromes

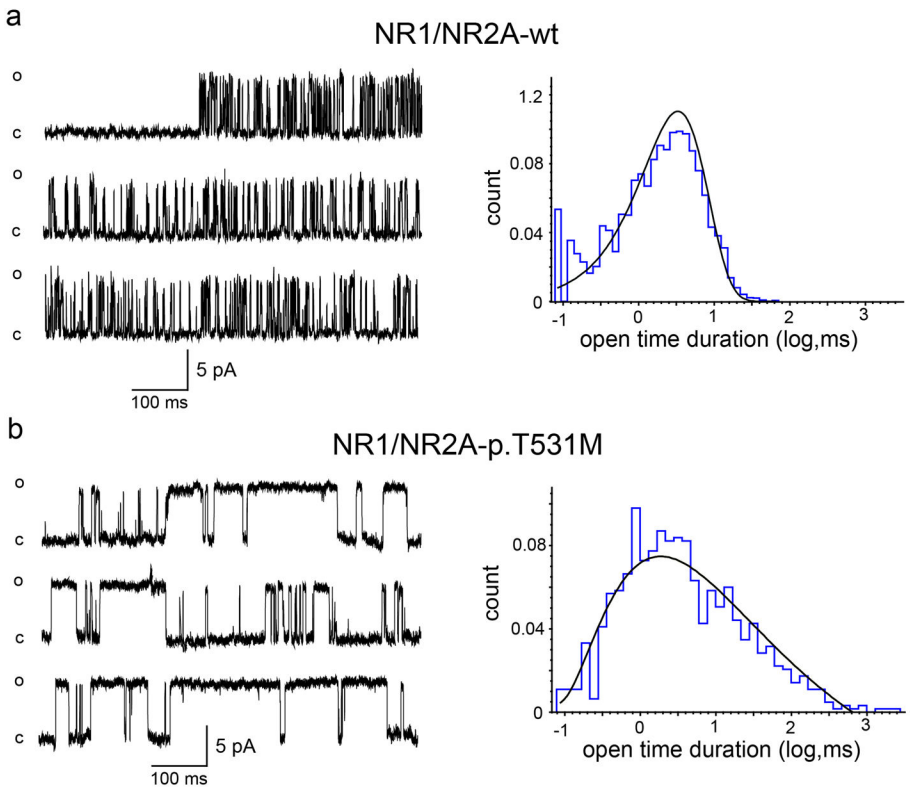

Figure 2.

NR2A-p.Thr531Met mutation increases mean open time of NMDA receptors. Left panels: representative steady-state unitary currents through (a) NR1/NR2A-wt and (b) NR1/NR2A-p.Thr531Met channels, recorded in cell-attached patches from transiently transfected COS-7 cells (n=5 to 7 cells). Holding potential +100 mV. Channels are activated by 50 μM glycine and 1 mM glutamate. o: open state; c: closed state; ms: milliseconds; pA: picoAmpere. Note that single channel amplitudes were not significantly affected by the mutation. Right panels: open duration histograms for the same single channel patches. The histograms were well fitted with single exponentials.

Table 1

Epileptic encephalopathy cohort screened for *GRIN2A* mutations

	N	*GRIN2A* mutations
Epilepsy-aphasia	44	4
Focal epilepsy, Symptomatic focal epilepsy	50	0
Epileptic Encephalopathies (other)	87	0
Infantile Spasms	84	0
Epilepsy with myoclonic-atonic seizures	85	0
Symptomatic Generalized Epilepsies	85	0
Febrile Infection-Related Epilepsy Syndrome	12	0
Dravet syndrome	17	0
Lennox Gastaut syndrome	34	0
Ohtahara syndrome	8	0
Epilepsy of Infancy with Migrating Focal Seizures	7	0
Progressive Myoclonic Epilepsies	6	0
TOTAL	**519**	**4**

Table 2

Pathogenic *GRIN2A* mutations in four epilepsy-aphasia families.

Family	Chr	Pos	cDNA change	GERP	Grantham score	Polyphen score	SIFT	Protein change	Phenotype
A	16	10031815	c.1005-1C>T	5.2	NA	NA	NA	Phe139Ilefs *15 (predicted)	Autosomal Dominant Rolandic Epilepsy with Speech Dyspraxia
B	16	10274267	c.2A>G	4.5	81	0.213 (benign)	0 (damaging)	Met1Thr	Landau-Kleffner Syndrome
C	16	10031815	c.1005-1C>T	5.2	NA	NA	NA	Phe139Ilefs *15 (predicted)	ECSWS
D	16	9934563	c.1592G>A	5.1	81	1.000 (probably damaging)	0 (damaging)	Thr531Met	ECSWS, Intermediate Epilepsy-Aphasia Disorder

Chromosomal coordinates as per hg19 genome build, cDNA position as per NM_000833.3 and protein change as per NP_000824.1. unk, unknown; GERP, genomic evolutionary rate profiling; SIFT, Sorting Intolerant From Tolerant; ECSWS = Epileptic Encephalopathy with Continuous Spike-Wave in slow wave Sleep