GRIN2A mutations cause epilepsy-aphasia spectrum disorders

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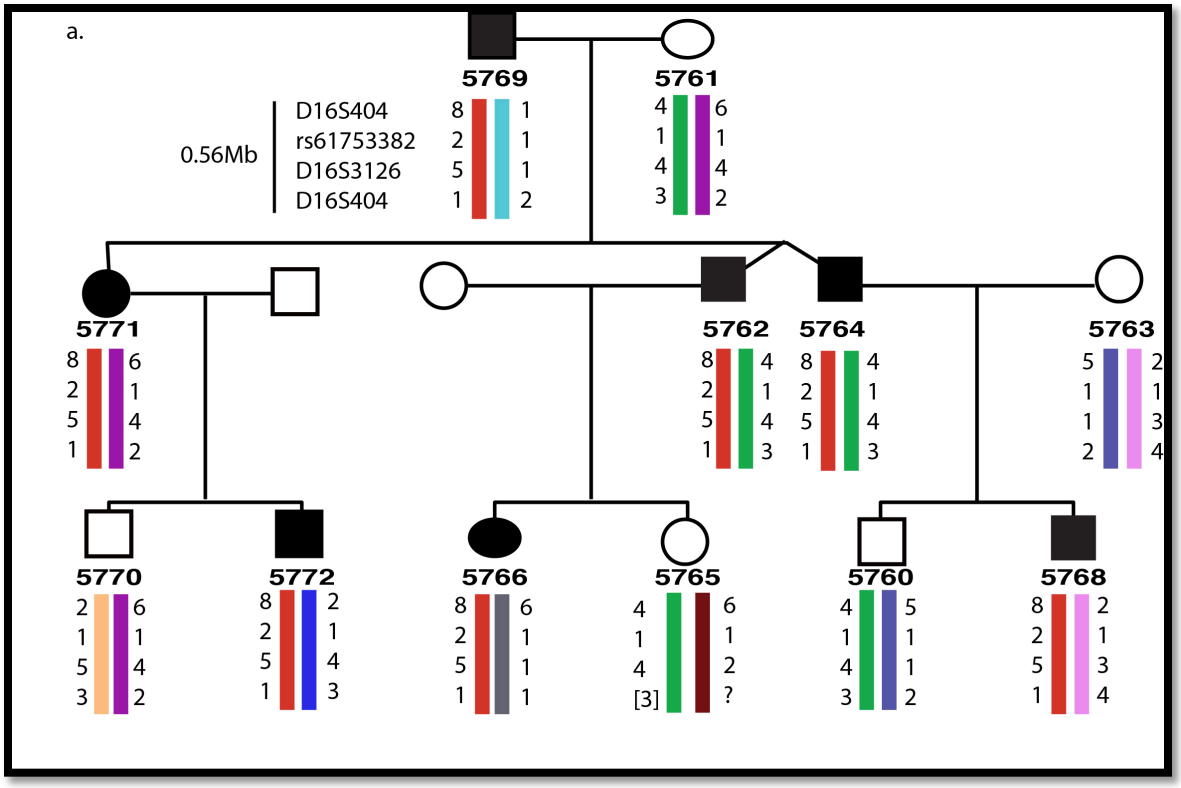


c. Size of microsatellite markers

Allele number	D16S404	D16S3126	D16S407
1	146	204	344
2	156	240	348
3	158	242	350
4	160	246	352
5	162	248	
6	164	250	
7	166		
8	174		

rs61753382 alleles: G (1), A (2)
MAF = 0.015

Supplementary Figure 1: Genotyping in two EAS families reveals a common haplotype for the c.1005-1C>T splice-site mutation. Genotyping of three microsatellite markers and a rare exonic SNV (rs61753382) spanning a 0.56Mb region flanking *GRIN2A* was performed in both families carrying the c1005-1C>T mutation, missing genotypes are designated with '?' and inferred genotypes with the allele in [], allele sizes are given for each marker in c. Haplotype phasing in a) revealed the presence of a common haplotype (indicated in red) in all affected individuals in family A, this haplotype was identical to that in affected members of family C (b), consistent with a common founder effect for these two Australian families of European descent.

Supplementary Table 1: *In silico* prediction of the functional consequences of the c1005-1C>T variation on pre-mRNA splicing

	Wildtype	**c.1005-1C>T**
Splice site sequence	cccGTAAGA	cccATAAGA
MAXENT	5.66	-2.52
MDD	10.78	2.60
MM	6.55	-1.63
WMM	6.53	-1.65

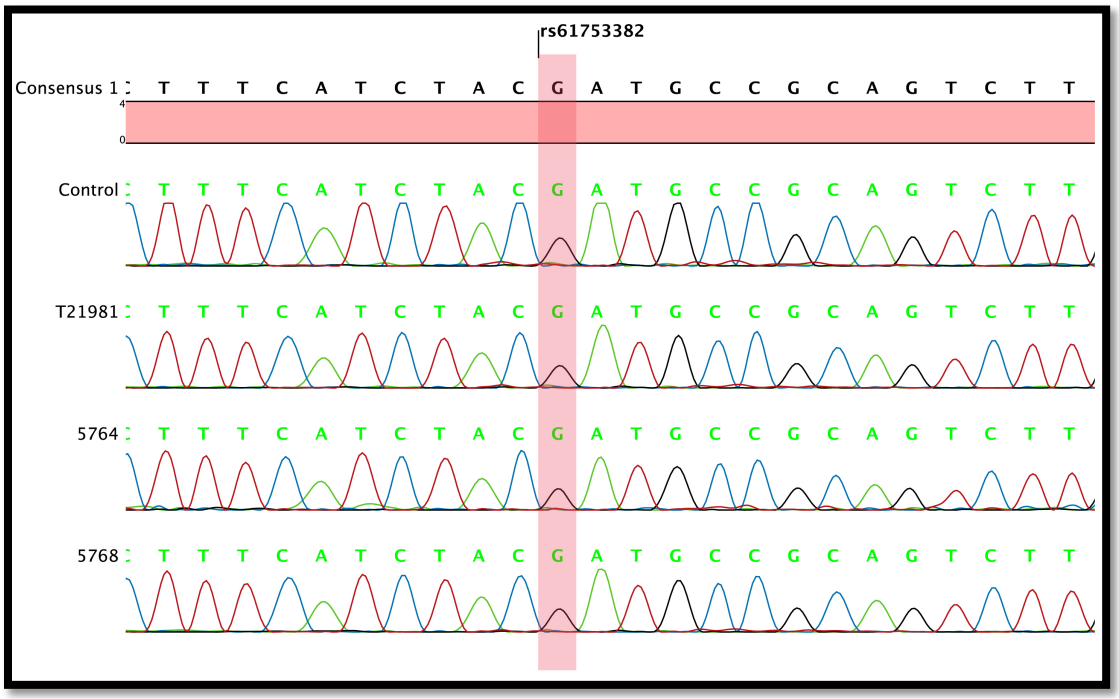
MAXENT, Maximum Entropy Model; MDD, Maximum Dependence Decomposition model; MM, First-order Markov Model; WMM, Weight Matrix Model

The c.1005-1C>T variant disrupts the donor splice-site of exon four of the *GRIN2A* splice-site. *In silico* analysis using MaxEntScan predicted that the c.1005-1C>T variant abolishes the 5' splice recognition site, under all four splice-site prediction models. These models have been shown to accurately predict the functional effect of intronic variants on pre-mRNA splicing [1]. We hypothesized that this mutation resulted in the skipping of exon four in pre-mRNA splicing, resulting in the removal of 593 nucleotides from the transcript and a frameshift mutation, Phe139Ilefs*15 (predicted).

http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html

Reference

1. Wappenschmidt, B. *et al*. Analysis of 30 putative BRCA1 splicing mutations in hereditary breast and ovarian cancer families identifies exonic splice site mutations that escape in silico prediction. *PLoS One* **7**, e50800 (2012).



Supplementary Figure 2: RNA transcript analysis of a rare exonic SNV in three probands carrying the c.1005-1C>T mutation. By haplotype analysis we show that the minor allele (A) of the rs61753382 SNV (reference G) is linked to the c.1005-1C>T change in affected individuals from families A and C. By Sanger sequencing of cDNA transcripts from affected individuals (T21981, 5764, 5768) we show the presence of only the wild-type transcript (G allele), indicated in red, in all affected individuals. Given the absence of the 'A' rs61753382 allele in RNA of affected individuals, we conclude that nonsense mediated decay of the mutant transcript (c.1005-1C>T) occurs due to exon four skipping and a frameshift mutation, Phe139Ilefs*15 (predicted).

Supplementary Table 2 Electro-clinical phenotypes of family members with *GRIN2A* mutations

Ped Ref	Age studied (yrs), Gender	Seizure onset (yrs)	Seizure offset (yrs)	Seizure type(s)	Early Development	Regression	Intellect	EEG	MRI	Speech / Language Difficulties	Syndrome	Pub Ref
A:I:1	71, M	NA	NA	NA	Normal	No	Normal	Normal	ND	Yes	ADRESD	1
A:II:2	45, F	6	13	Rolandic TCS	Normal	No	Normal	Normal	ND	Yes	ADRESD	1
A:II:4	39, M	6	13	Rolandic TCS	Normal	No	Normal	Normal	Normal	Yes	ADRESD	1
A:II:5	39, M	6	13	Rolandic TCS	Speech delay	No	Normal	Normal	Normal	Yes	ADRESD	1
A:III:2	8, M	1.5	Ongoing	Rolandic TCS	Speech delay	No	Normal	Bilateral independent CTS	ND	Yes	ADRESD	1
A:III:3*	11, F	10	Ongoing	Rolandic TCS	Mild global DD	No	Mild ID	Right CTS activated by sleep	Normal	Yes	ADRESD	1
A:III:5	6, M	2.5	Ongoing	Rolandic TCS NCSE	Mild global DD	Yes	Borderline	Bilateral independent CTS NCSE - continuous CTS discharges awake & sleep	Normal	Yes	ADRESD	1
B:II:3	33, M	Childhood	NK	NK	NK	NK	Normal	NK	NK	Yes	Unclass.	NA
B:III:2	7, F	3.5	Ongoing	Rolandic TCS NCSE	Normal	Yes	Mild ID	Continuous left CTS, multifocal, bi-fronto-central, right occipital discharges	Normal	Yes	LKS	NA
B:III:3	6, F	2	2.5	DA	Normal	Yes	Mild ID	Very frequent bilateral independent CTS continuous in sleep	Normal	Yes	LKS	NA
C:II:2	49, M	5.5	14	FDS TCS	Normal	Yes	Mild ID	Frequent generalized and multi-focal discharges	ND	Yes	ECSWS	NA
C:III:1	19, M	2	16	Rolandic	Mild global DD	Yes	Moderate ID	Very frequent bilaterally synchronous mid and posterior temporal and parietal discharges	Normal	Yes	ECSWS	NA
D:II:2	42, F	NA	NA	NA	Normal	No	Normal	ND	ND	Yes	NA	2
D:III:1	12, M	7	11	FDS	Mild global DD	Yes	Mild ID	Intermittent discharges in clusters from left posterior hemisphere. High voltage bilateral discharges (awake recording only)	Normal	Yes	IEAD	2
D:III:2	11, M	11	11	TCS	Normal	No	Borderline	Bilateral CTS during drowsiness & sleep	ND	Yes	IEAD	2
D:III:6	9, F	6.5	Ongoing	FDS TCS	Mild global DD	Yes	Mild ID	Multifocal discharges from mid parietal and independent bilateral bioccipital regions. Very frequent in awake state and continuous in sleep (CSWS)	Normal	Yes	ECSWS	2

ADRESD = Autosomal Dominant Rolandic Epilepsy and Speech Dyspraxia, CSWS = Continuous Spike-Wave in slow wave Sleep, CTS = centro-temporal spikes, DA = drop attack, DD = developmental delay, ECSWS = Epileptic Encephalopathy with Continuous Spike-Wave in slow wave Sleep, FDS = focal dyscognitive seizure, ID = intellectual disability, IEAD = Intermediate Epilepsy-Aphasia Disorder, LKS = Landau-Kleffner Syndrome, NA = not applicable, NCSE = non-convulsive status epilepticus, ND = not done, NK = not known, Ped Ref = pedigree reference, TCS = tonic-clonic seizure, Unclass = Unclassified, * = mosaic Turner syndrome; Pub Ref = publication reference 1) Tsai, M. *et al*. Clinical genetic study of the epilepsy-aphasia spectrum . *Epilepsia* Accepted 30 October (2012) (2) Scheffer, I. E. *et al*. Autosomal dominant rolandic epilepsy and speech dyspraxia: a new syndrome with anticipation. *Ann. Neurol.* **38**, 633-642 (1995).

Supplementary Table 3 PCR primer pairs for cDNA amplification of rs6173382

Primer pair name	**Sequence (5' – 3')**
rs6173382_ext_Fwd	GGACAGTGCCTAATGGAAGC
rs6173382_ext_Rev	GCTGATGGAGAAGAGCAACC
rs6173382_int_Fwd	AGCACGGAGAGAAACATTCG
rs6173382_int_Rev	ACCCACAAACTGAAGCAAGG

Supplementary Note Human subject recruitment and diagnosis

This study was approved by the Human Research Ethics Committees of Austin Health and the University of Washington. Probands with epileptic encephalopathies were recruited from the epilepsy clinic at Austin Health, the practices of the investigators and by referral for epilepsy genetics research from around Australia and internationally after informed consent. The cohort consisted of 519 patients with a diverse range of epileptic encephalopathy phenotypes. An epileptic encephalopathy was defined as refractory seizures and cognitive slowing or regression associated with frequent, ongoing epileptiform activity [1]. Detailed epilepsy and medical histories were obtained together with the results of investigations including EEG and MRI studies. Epilepsy syndromes were classified according to the Organization of the International League Against Epilepsy Commission on Classification [1].

1. Berg, A. T. *et al*. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* **51**, 676-85 (2010).