Involvement of GATOR complex genes in familial focal epilepsies and focal cortical dysplasia

Sarah Weckhuysen, Elise Marsan, Virginie Lambrecq, Cécile Marchal, Mélanie Morin-Brureau, Isabelle An-Gourfinkel, Michel Baulac, Martine Fohlen, Christine Kallay Zetchi, Margitta Seeck, et al.

Involvement of GATOR complex genes in familial focal epilepsies and focal cortical dysplasia

Sarah Weckhuysen MD, PhD[1,2,3,4,5], Elise Marsan[1,2,3,4], Virginie Lambrecq MD, PhD[1,2,3,4,5], Cécile Marchal MD[6], Mélanie Morin-Brureau PhD[1,2,3,4], Isabelle An-Gourfinkel MD, PhD[1,5], Michel Baulac MD[1,2,3,4,5], Martine Fohlen MD[7], Christine Kallay Zetchi MD[8], Margitta Seeck MD[8], Pierre de la Grange PhD[9], Bart Dermaut MD, PhD[10,11], Alfred Meurs MD, PhD[12], Pierre Thomas MD[13], Francine Chassoux MD[14], Eric Leguern MD, PhD[1,2,3,4,15], Fabienne Picard MD[8] and Stéphanie Baulac PhD[1,2,3,4,15]

[1] INSERM, U1127, ICM, F-75013 Paris, France; [2] CNRS, UMR 7225, ICM, F-75013 Paris, France; [3] Sorbonne Universités, UPMC Univ Paris 06, UMR S 1127, F-75013 Paris, France; [4] Institut du Cerveau et de la Moelle épinière, ICM, F-75013 Paris, France; [5] Centre de référence épilepsies rares, Epilepsy unit, AP-HP Groupe hospitalier Pitié-Salpêtrière, F-75013 Paris, France; [6] CHU de Bordeaux, service d'épileptologie, F33076, Bordeaux, France; [7] Department of Pediatric Neurosurgery, Fondation Rothschild, F-75019 Paris, France; [8] Department of Neurology, University Hospitals (HUG) and Medical School of Geneva, CH-1211 Geneva 14, Switzerland; [9] Genosplice Institut du Cerveau et de la Moelle épinière, ICM, F-75013 Paris, France; [10] Center for Medical Genetics, Ghent University Hospital, B-9000 Gent, Belgium; [11] Inserm U1167, Institut Pasteur de Lille, Université de Lille, 59019 Lille, France; [12] Department of Neurology, Ghent University Hospital, B-9000 Gent, Belgium; [13] Service de Neurologie, Hôpital Pasteur, F-06000 Nice, France; [14] Centre hospitalier Sainte-Anne, Université Paris Descartes, F-75005 Paris, France; [15] Département de Génétique et de Cytogénétique, AP-HP Groupe hospitalier Pitié-Salpêtrière, F-75013 Paris, France.

Corresponding author:

Dr. Stéphanie Baulac, Institut du Cerveau et de la Moelle épinière (ICM), Hôpital de la Pitié-Salpêtrière, 47 bd de l'hôpital, Paris F-75013, France. Tel +331-5727-4339, Email: stephanie.baulac@upmc.fr

Running Title: GATOR complex genes in focal epilepsy

Key Words: NPRL2, NPRL3, DEPDC5, mTOR pathway, genetics, SUDEP

22 text pages; 3249 words (including summary); 5 figures; 1 table; 35 references

Summary

Objective: The discovery of mutations in *DEPDC5* in familial focal epilepsies has introduced a novel pathomechanism to a field so far dominated by ion channelopathies. DEPDC5 is part of a complex named GATOR1, together with the proteins NPRL2 and NPRL3, and acts to inhibit the mTOR complex 1 (mTORC1) pathway. GATOR1 is in turn inhibited by the GATOR2 complex. The mTORC1 pathway is a major signaling cascade regulating cell growth, proliferation and migration. We aimed to study the contribution of GATOR complex genes to the etiology of focal epilepsies and to describe the associated phenotypical spectrum.

Methods: We performed targeted sequencing of the genes encoding the components of the GATOR1 (DEPDC5, NPRL2, NPRL3) and GATOR2 (MIOS, SEC13, SEH1L, WDR24, WDR59*)* complex in 93 European probands with focal epilepsy with or without focal cortical dysplasia. Phospho-S6 immunoreactivity was used as evidence of mTORC1 pathway activation in resected brain tissue of patients carrying pathogenic variants.

Results: We identified four pathogenic variants in *DEPDC5*, two in *NPRL2* and one in *NPRL3*. We showed hyperactivation of the mTORC1 pathway in brain tissue from patients with *NPRL2* and *NPRL3* mutations. Collectively, inactivating mutations in GATOR1 complex genes explained 11% of cases of focal epilepsy, while no pathogenic mutations were found in GATOR2 complex genes. GATOR1-related focal epilepsies differ clinically from focal epilepsies due to mutations in ion channel genes by their association with focal cortical dysplasia and seizures emerging from variable foci, and might confer an increased risk of Sudden Unexplained Death in Epilepsy (SUDEP).

Significance: GATOR1 complex gene mutations leading to mTORC1 pathway upregulation is an important cause of focal epilepsy with cortical malformations and represents a potential target for novel therapeutic approaches.

Key Words: NPRL2, NPRL3, DEPDC5, mTOR pathway, genetics, SUDEP

Introduction

Germline mutations in disheveled, Egl-10, and pleckstrin (DEP) domain-containing 5 (*DEPDC5)*, have recently been described in a range of focal epilepsy syndromes including familial focal epilepsy with variable foci (FFEVF), autosomal dominant nocturnal epilepsy (ADNFLE), and rare families with familial mesial temporal lobe epilepsy (FMTLE) and rolandic epilepsies[1–6]. While most affected mutation carriers have a normal MRI, several patients were shown to have focal cortical dysplasia (FCD) type I, IIa or IIb [7–10]. *In vitro* and in yeast, DEPDC5 acts as an inhibitor of the mechanistic target of rapamycin complex 1 (mTORC1) [11,12]. mTORC1 has a serine-threonine kinase activity that phosphorylates several downstream proteins regulating essential cellular functions like protein synthesis, cell growth, migration and proliferation [13]. The mTOR pathway is increasingly being recognized as a major player in the pathogenesis of cerebral malformations [14]. Brain somatic mutations in other genes of the pathway, namely *MTOR* itself, *AKT3* and *PIK3CA*, have also been shown to cause cortical malformations, and mTORC1 hyperactivity has been demonstrated in resected brain tissue [9,15–19].

Together with the proteins NPRL2 and NPRL3, DEPDC5 is part of a newly discovered protein complex named GATOR1 (GAP Activity Towards Rags). This complex is in turn inhibited by the GATOR2 complex, consisting of the proteins MIOS, SEC13, SEH1L, WDR24, and WDR59 [11] (Fig. 1). Recently, mutations in *NPRL3* and *NPRL2* were described in familial and sporadic focal epilepsies, with or without FCD [20–22]. In this study, we aimed to verify the hypothesis that loss-of-function mutations in *NPRL2* or *NPRL3*, or gain-of-function mutations in GATOR2 complex genes (the respective mutation types theoretically both leading to mTORC1 hyperactivation) are a cause of focal epilepsies.

Materials and Methods

Patients

We recruited a cohort of 93 unrelated European patients with focal epilepsy, including sporadic patients and probands from families with autosomal dominant nocturnal epilepsy (ADNFLE), mesial temporal lobe epilepsy (FMTLE), focal epilepsy with variable foci (FFEVF), or unclassified focal epilepsies. The cohort consisted of 55 probands with familial focal epilepsy and normal MRI, nine sporadic patients with focal epilepsy and normal MRI, 14 patients with familial focal epilepsy and at least one family member with FCD, and 15 sporadic patients with focal epilepsy and FCD. Familial cases (n= 69) were defined as having at least one first- or second-degree relative with focal epilepsy. Clinical information was collected from medical records and telephone interviews. In 43 of these patients, *DEPDC5* mutations had previously been excluded by massively parallel pyrosequencing [2,3]. The study was approved by the local ethical committee (CCPPRB of Pitié-Salpêtrière Hospital, Paris, no. 69-03, 25/9/2003). Written informed consent was obtained from each participant or parents of minors.

Genetic screening

Genomic DNA extracted from blood was sequenced using a targeted epilepsy gene panel (SeqCapEZ custom design, Roche Nimblegen) including the GATOR1 and GATOR2 complex genes *DEPDC5, NPRL2, NPRL3, MIOS, SEC13, SEH1L, WDR24* and *WDR59*. The panel also included non-mTORC1 pathway genes known to be involved in familial focal epilepsies (*CHRNA4, CHRNA2, CHRNB2, LGI1, KCNT1, GRIN2A*), to exclude alternative genetic causes in patients found to carry potentially pathogenic variants in GATOR1 and GATOR2 genes. GATK best practices were applied to call variants. Variants with low quality (< 20) and low VQSLOD (< 0) were filtered out. Variants were further filtered for quality

using a read depth > 30 X and a variant allele read frequency > 0.30. Probable pathogenic variants were selected applying the following filters: heterozygous non-synonymous variants with frequency < 1% in exome variant server (http://evs.gs.washington.edu/EVS/), affecting the protein-coding sequence (nonsense, missense, frameshift, splice variants) and predicted to be damaging by at least two of three *in silico* prediction tools (SIFT, http://sift.jcvi.org/; MutationTaster, http://www.mutationtaster.org/; PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/). Impact of mutations was assessed with Alamut version 2.7.1 (Interactive Biosoftware, France). All filtered variants were validated by Sanger sequencing, and their segregation was analyzed in all available family members.

RNA sequencing

RNA integrity from total RNA extracted from frozen post-mortem frontal cortex (Brodmann area 9/10) of four control individuals was verified on an Agilent 2100 bioanalyser. Paired-end sequencing libraries were sequenced on the Illumina High Seq 2000. GenoSplice (www.genosplice.com) performed quality control, processing, and further analyses of the data. Mapped reads by STAR [23] were normalized using DESeq [24]. Putative transcripts expressed in brain were predicted using Cufflinks.

Nonsense Mediated Decay Assay (NMD)

Lymphoblastoid cell lines from individual E/IV-4 carrying the frameshift mutation c.68_69del (p.lle23Asnfs*6) in *NPRL2,* and from individuals F/II-7 and F/III-6 carrying the nonsense mutation c.1270C>T (p.Arg424*) in *NPRL3* were treated overnight with 10 mg/mL emetine to inhibit NMD. Total RNA was extracted from treated and untreated cell cultures with the RNeasy Mini kit and RNA was reverse transcribed into complementary DNA (cDNA) with the ThermoScript RT-PCR System (Invitrogen). *NPRL2* and *NPRL3* cDNA was amplified and sequenced using specific primers (available on request) located in exons 1 and

2 for family E, and in exons 11 and 13 for family F. Since no lymphoblast cell culture or fresh blood was available for family G, the presence of NMD could not be tested for the *NPRL3* p.Pro357Hisfs*56 variant.

Histopathology and immunostaining

Paraffin-embedded resected brain tissue of individuals E/IV-4 and F/IV-12 carrying deleterious variants in *NPRL2* and *NPRL3* was examined for the presence of cyto-architectural features of FCD using hematoxylin and eosin (H&E) staining and NeuN immunostaining against neuronal cells. Cortical specimen abnormalities were classified according to the proposed classification of the International League Against Epilepsy (ILAE) Diagnostic Methods Commission [25]. mTORC1 activity was assessed by visualizing the phosphorylation level of its downstream effector, the ribosomal protein S6, a commonly used biomarker in mTORC1 pathway research [17] (Fig. 1). Sections (10 µm) were probed overnight at 4°C with antibody against Ser235/236 phosphorylated S6 (Cell Signaling #4856; 1:200 dilution) or against NeuN (Millipore #MAB377; 1:500 dilution) and visualized using avidin-biotin conjugation (Vectastain ABC Elite; Vector Labs). Images were acquired on a Hamamatsu Nanozoomer.

Results

Genetic results

Coverage of GATOR1 and GATOR2 complex genes was satisfactory and a summary can be found in Supplemental Table 1. A list of all variants retained after filtering can be found in Supplemental Table 2. We found a total of 13 rare variants in GATOR1 and GATOR2 complex genes. All variants were absent from the Exome Aggregation Consortium (ExAC)

database (http://exac.broadinstitute.org) [accessed September 2015] or had an allele frequency ≤ 0.02%.

Mutations in DEPDC5

We identified four premature termination codon variants in *DEPDC5* (NM_001242896) in four out of 50 (8%) not previously screened probands, including three probands with familial focal epilepsy, and one proband with familial focal epilepsy and FCD (Supplemental Fig. 1, Supplemental Table 2): one nonsense (c.1393C>T, p.Gln465*) and three frameshift variants (c.454_455delAT, p.Met152Valfs*6; c.4260delG, p.Glu1421Argfs*153, and c.1750-1756delCATGCTG, p.Leu584Phefs*12). These four variants were considered pathogenic since the majority of reported disease-causing *DEPDC5* variants are also inactivating mutations. One additional rare missense (c.3194A>G, p.Lys1065Arg) was identified, but since it was not present in the affected sib with similar phenotype, it was considered to be a variant of unknown significance.

Mutations in NPRL2 and NPRL3

We identified five rare variants in *NPRL2* (NM_006545) and *NPRL3* (NM_001077350) (Supplemental Table 1). Two missense variants (*NPRL2*: c.593C>A, p.Pro198His; *NPRL3*: c.745G>A , p.Glu249Lys) were not present in the affected sib with similar phenotype, and we concluded there was insufficient evidence to classify them as pathogenic. We further detected three premature termination codon variants in three families with familial focal epilepsy and FCD. The frameshift variants (c.68_69delCT, p.Ile23Asnfs*6) in *NPRL2* and (c.1070delC, p.Pro357Hisfs*56) in *NPRL3* were present in all affected family members for whom DNA was available in family E and G respectively (Fig. 2). The nonsense variant (c.1270C>T, p.Arg424*) in *NPRL3* was present in five of seven individuals with seizures of family F for whom DNA was available (Fig. 2). Two subjects not carrying the variant were considered phenocopies: III-7, who had a single febrile seizure followed by generalized epilepsy, and IV-

1 who had one episode of loss of consciousness and convulsions (see clinical information below). In two families we noticed a reduced penetrance, as reported in the other families with *NPRL2* or *NPRL3* mutations[20–22].

Together, *NPRL2* and *NPRL3* loss-of-function mutations account for 3.2% (3/93) of focal epilepsy patients in our cohort, and 21% of cases with familial focal epilepsy with FCD (3/14) (Fig. 3, Supplemental Table 3).

All three pathogenic variants identified in *NPRL2* and *NPRL3* were nonsense or frameshift variants, predicted to be degraded by Nonsense-mediated decay (NMD). To prove these variants lead to loss of one functional allele (haploinsufficiency), we sequenced *NPRL2* and *NPRL3* cDNA from lymphoblasts of subjects from family E and F. We did not detect the *NPRL3*: c.1270C>T/p.Arg424* mutation in the cDNA of individuals F/II-7 and F/III-6. Emetine treatment, an NMD inhibitor, rescued the transcript expression, indicating that the mutated transcript is indeed degraded by NMD (Fig. 4). In contrast, the *NPRL2*: c.68_69del/p.Ile23Asnfs*6 frameshift variant was present in cDNA of individual E/IV-4 regardless of treatment with emetine, showing that no NMD of the mRNA transcript occurs in lymphoblasts. Given that the frameshift mutation occurs early in exon 1, it is predicted to lead to a premature stop after six amino acids and thus result in a very short aberrant protein.

We next asked whether *NPRL2* and *NPRL3* transcripts are expressed in the human brain. RNA sequencing of frontal lobe cortex of four control individuals showed that both *NPRL2* and *NPRL3* are highly expressed in brain cortex under the form of different transcripts, including the RefSeq transcripts NM_006545 and NM_001077350, and transcripts not previously described in public databases. The three pathogenic variants in *NPRL2* and *NPRL3* identified in this study were located in all brain-expressed transcripts (Supplemental Fig. 2).

Involvement of GATOR2 complex genes

Three novel missense variants were identified in the GATOR2 complex genes *WDR24* (NM_032259) and *SEC13* (NM_183352). While they were predicted disease-causing and probably damaging by MutationTaster and PolyPhen-2 respectively, they did not fully segregate with the epilepsy phenotype in the family. We concluded there was insufficient support for their pathogenicity.

Clinical description of families with pathogenic variants in NPRL2 or NPRL3

An extensive clinical description of family E, F and G can be found in Table 1 and the Supplement. Families E and F are of French origin, and family G is of Swiss origin.

NPRL2 family

In brief, family E (*NPRL2*, c.68_69delCT, p.Ile23Asnfs*6) had a phenotype of FFEVF including family members with temporal or frontal lobe epilepsy. One family member (E/IV-4), whose MRI was normal, had a PET-MRI evocative of an FCD II (Fig. 5A). Histopathological examination of resected brain tissue was compatible with FCD Ia (Fig. 5B, C), but since the patient was not seizure free post-operatively, and given the PET-MRI results, a residual FCD II could not be excluded. One individual in this family (F/III-2) died of probable Sudden Unexplained Death in Epilepsy (SUDEP).

NPRL3 families

Family F (*NPRL3*: c.1270C>T, p.Arg424*) is a large French family comprising 10 individuals with febrile seizures, focal epilepsy with variable foci, generalized epilepsy, or undefined epilepsy with tonic clonic seizures, and one individual (F/IV-12) with focal epilepsy and FCD IIa (Fig. 5E). One individual (F/III-1) with focal epilepsy died of probable SUDEP. One family member (F/IV-1) with a single convulsive seizure at the age of five years and diffuse spike and wave complexes on EEG, and one individual (F/III-7) with generalized

epilepsy did not carry the pathogenic variant. We concluded that the *NPRL3* mutation is responsible for the focal epilepsy and FCD trait, while a more complex trait probably underlies the generalized epilepsy.

Family G (*NPRL3*, c.1070delC, p.Pro357Hisfs*56) also had a phenotype of FFEVF with seizures arising from either the frontal or temporal lobe in different family members. Brain MRI of one family member (G/III-2) was evocative of a FCD IIb (Fig. 5I).

Histopathology and immunostaining

Postoperative brain tissue from the fronto-insular cortex of individual E/IV-4 (*NPRL2*, c.68_69delCT, p.Ile23Asnfs*6) and the frontal cortex from individual F/IV-12 (*NPRL3*: c.1270C>T, p.Arg424*) was available for further study. In individual E/IV-4, histopathology showed a FCD Ia with abnormal radial cortical lamination with microcolumns on NeuN immunostaining, and absence of dysmorphic neurons on H&E staining (Fig. 5B, C). Individual F/IV-12 had a FCD IIa with cortical dyslamination illustrated by NeuN immunostaining, and abundant enlarged and dysmorphic neurons (but no balloon cells) on H&E staining (Fig. 5F, G). We assessed mTORC1 activity by visualizing the phosphorylation level of its downstream effector S6 (pS6 on Ser235/236). Hyperactivation of the mTORC1 pathway was observed in neurons of normal appearance in E/IV-4 (Fig. 5D), and in normal and dysmorphic neurons in tissue from F/IV-12 (Fig. 5H).

Discussion

The familial focal epilepsies constitute a genetically heterogeneous group of epilepsy syndromes, ranging from epilepsies due to ion channel mutations in the acetylcholine nicotinic receptors (*CHRNA4*, *CHRNB2*, *CHRNA2*), the glutamate receptors (*GRIN2A*), or the sodium-gated potassium channels (*KCNT1*); to mutations in secreted proteins such as *LGI1* or *RELN;* and recently to mutations in repressors of the mTORC1 pathway, *DEPDC5, NPRL3* and *NPRL2* [26–28]. *NPRL2* and *NPRL3* mutations were recently described in a few familial and sporadic cases with focal epilepsies, with or without FCD [20–22] (Supplemental Fig. 3). In this study, we validate the role of both genes in focal epilepsies through the identification of three additional families with premature termination codon mutations in *NPRL2* and *NPRL3*. We also evaluated the involvement of GATOR2 complex genes, and while we cannot definitively exclude a role in focal epilepsy and/or FCD yet, the frequency of mutations at least seems to be low.

The vast majority of mutations in GATOR1 complex genes are inactivating variants. We provided proof for a loss-of-function mechanism of GATOR1 complex mutations by showing that the *NPRL3* nonsense variant leads to NMD. We further showed that *NPRL2* and *NPRL3* mutations lead to hyperactivation of mTORC1 in resected brain tissue.

In our cohort, *DEPDC5* mutations were present in 8% of individuals with focal epilepsy, including one individual with FCD. Mutations in *NPRL2* and *NPRL3* were identified in an additional 3,2% of patients, showing that collectively mutations in GATOR1 complex genes are an important cause of focal epilepsies, explaining more than 11% of cases (Supplemental Table 3). Individuals with mutations in the three GATOR1 complex genes have a comparable phenotypic spectrum. As a group, their clinical characteristics distinguish them from focal epilepsies due to mutations in non-mTORC1 pathway genes. First of all, there is a clear

enrichment of GATOR1 complex gene mutations in families comprising individuals with FCD (Fig. 3), illustrating the tight link between mTORC1 pathway dysfunction and malformations of cortical development. Secondly, seizures in GATOR1-related epilepsies often originate from variable foci in larger families. In contrast, non-mTORC1 pathway linked epilepsies have a preferential seizure onset in a specific lobe (frontal lobe epilepsy in case of nicotinic acetylcholine receptor genes and *KCNT1*, and lateral temporal lobe epilepsy in case of *LGI1* and *RELN*) [26,27]. Together with the strongly reduced penetrance reported for all GATOR1 complex genes, this variable foci onset adds to the phenotypical intrafamilial variability associated with GATOR1 mutations, ranging from asymptomatic carriers, over apparently non-lesional focal epilepsy, to FCD. Possibly, individuals with normal MRI have microscopic cortical abnormalities that remain undetected with routine imaging, as in patient E/IV-4, in whom initial MRI was negative and an FCD was diagnosed by histological analysis of resected brain tissue. Alternatively, dysplastic lesions might only occur in patients carrying a second-hit somatic mutation in brain tissue, as shown previously by our team for one patient with FCD and both a germline and a somatic mutation in *DEPDC5* [10]. The insufficient quality of brain DNA isolated from formalin-fixed, paraffin-embedded tissue from the patients presented in this study did not permit sequencing to search for somatic mutations.

Despite the ubiquitous expression of *DEPDC5*, *NPRL2* and *NPRL3*, all patients described so far with mutations in GATOR1 complex genes seem to have a purely neurological phenotype. In contrast, patients with tuberous sclerosis due to mutations in the mTORC1 pathway genes *TSC1* or *TSC2*, have multisystemic pathology [29]. Of note, two families with *NPRL2* or *NPRL3* mutations reported in the present paper comprised a family member who died of probable SUDEP [30]. DNA of neither individual was available, but the epilepsy phenotype of the deceased individuals was very similar to that of other family members

carrying the mutation, making it highly likely that they also carried the same mutations. The recurrence of SUDEP in two unrelated families with GATOR1 complex mutations is at least remarkable given the relatively low incidence of SUDEP in the general epilepsy population (0,3 - 9/1000 patient-years) [31]. Interestingly, a recent article described a family with focal epilepsy caused by a mutation in *DEPDC5* comprising two family members that died of SUDEP, and a whole exome study of 61 SUDEP cases identified 6 probably pathogenic *DEPDC5* mutations [32,33]. Further studies are needed to determine whether GATOR1 complex mutations indeed lead to an increased risk of SUDEP, and if so what the underlying mechanism is. Evaluation of a possible increased risk of SUDEP in patients with GATOR1 mutations is important, as it might advocate the use of preventive measures or earlier referral for presurgical evaluation [34,35].

This study underlines the importance of all three GATOR1 proteins in the pathophysiology of focal epilepsy and FCD. Altogether they explain around 11% of patients with focal epilepsy, and are enriched in epilepsies associated with FCD. The identification of an increasing number of patients with GATOR1 complex mutations is an additional incentive for the clinical evaluation of drugs specifically targeting the defective mTOR pathway.

Acknowledgements

We thank patients and families for their participation in the study, and clinicians for referring patients. We thank the ICM sequencing platform (Y. Marie), the ICM bioinformatics/biostatistics platform (J. Guegan), the ICM DNA and cell bank (C. Dussert, S. Forlani, P. Couarch), the ICM imaging platform, the ICM histomics platform (A. Prigent) and the ENS platform (IBENS) for RNAseq technology (S. Le Crom). We also thank E. Noé, G. Muraca, A. Camuzat and S. Lecas of ICM for providing technical assistance.

This study was funded by the program "Investissements d'avenir" ANR-10-IAIHU-06, Fondation Française pour la Recherche sur l'Epilepsie (FFRE), and Fondation pour la Recherche Médicale (team FRM DEQ2015033). None of the authors have any conflicts of interest to report.

Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Websites

ExAC database: http://exac.broadinstitute.org

Cufflinks: http://cole-trapnell-lab.github.io/cufflinks/

Genosplice: www.genosplice.com

Key Point Box

- Mutations in the GATOR1 complex genes *DEPDC5, NPRL2* and *NPRL3* leading to mTORC1 hyperactivation explain 11% of cases of focal epilepsy
- GATOR1-related epilepsies differ from epilepsies due to ion channel mutations by their association with FCD and seizures emerging from variable foci
- The recurrence of SUDEP in multiple families with GATOR1 mutations deserve further study to determine a possibly increased incidence of SUDEP

References

1. Dibbens LM, de Vries B, Donatello S, et al. Mutations in DEPDC5 cause familial focal epilepsy with variable foci. Nat Genet. 2013 May;45(5):546–51.
2. Ishida S, Picard F, Rudolf G, et al. Mutations of DEPDC5 cause autosomal dominant focal epilepsies. Nat Genet. 2013 May;45(5):552–5.
3. Picard F, Makrythanasis P, Navarro V, et al. DEPDC5 mutations in families presenting as autosomal dominant nocturnal frontal lobe epilepsy. Neurology. 2014 Jun 10;82(23):2101–6.
4. Martin C, Meloche C, Rioux M-F, et al. A recurrent mutation in DEPDC5 predisposes to focal epilepsies in the French-Canadian population. Clin Genet. 2014 Dec;86(6):570–4.
5. Striano P, Serioli E, Santulli L, et al. DEPDC5 mutations are not a frequent cause of familial temporal lobe epilepsy. Epilepsia. 2015 Oct;56(10)
6. Lal D, Reinthaler EM, Schubert J, et al. DEPDC5 mutations in genetic focal epilepsies of childhood. Ann Neurol. 2014 May;75(5):788–92.
7. Scheffer IE, Heron SE, Regan BM, et al. Mutations in mammalian target of rapamycin regulator DEPDC5 cause focal epilepsy with brain malformations. Ann Neurol. 2014 May;75(5):782–7.
8. Scerri T, Riseley JR, Gillies G, et al. Familial cortical dysplasia type IIA caused by a germline mutation in DEPDC5. Ann Clin Transl Neurol. 2015 May;2(5):575–80.
9. D'Gama AM, Geng Y, Couto JA, et al. Mammalian target of rapamycin pathway mutations cause hemimegalencephaly and focal cortical dysplasia. Ann Neurol. 2015 Apr;77(4):720–5.
10. Baulac S, Ishida S, Marsan E, et al. Familial focal epilepsy with focal cortical dysplasia due to DEPDC5 mutations. Ann Neurol. 2015 Apr;77(4):675–83.
11. Bar-Peled L, Chantranupong L, Cherniack AD, et al. A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science. 2013 May 31;340(6136):1100–6.
12. Panchaud N, Péli-Gulli M-P, De Virgilio C. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. Sci Signal. 2013 May 28;6(277):ra42.
13. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012 Apr 13;149(2):274–93.

14. Lim K-C, Crino PB. Focal malformations of cortical development: new vistas for molecular pathogenesis. Neuroscience. 2013 Nov 12;252:262–76.
15. Jansen LA, Mirzaa GM, Ishak GE, et al. PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. Brain. 2015 Jun;138(Pt 6):1613–28.
16. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. Nat Genet. 2012 Aug;44(8):941–5.
17. Lim JS, Kim W, Kang H-C, et al. Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. Nat Med. 2015 Apr;21(4):395–400.
18. Nakashima M, Saitsu H, Takei N, et al. Somatic Mutations in the MTOR gene cause focal cortical dysplasia type IIb. Ann Neurol. 2015 Sep;78(3):375–86.
19. Poduri A, Evrony GD, Cai X, et al. Somatic activation of AKT3 causes hemispheric developmental brain malformations. Neuron. 2012 Apr 12;74(1):41–8.
20. Sim JC, Scerri T, Fanjul-Fernández M, et al. Familial cortical dysplasia caused by mutation in the mTOR regulator NPRL3. Ann Neurol. 2016 Jan;79(1):132-7
21. Ricos MG, Hodgson BL, Pippucci T, et al. Mutations in the mTOR pathway regulators NPRL2 and NPRL3 cause focal epilepsy. Ann Neurol. 2016 Jan;79(1):120-31
22. Korenke G-C, Eggert M, Thiele H, Nürnberg P, Sander T, Steinlein OK. Nocturnal frontal lobe epilepsy caused by a mutation in the GATOR1 complex gene NPRL3. Epilepsia. 2016 Mar;57(3):e60-3
23. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013 Jan 1;29(1):15–21.
24. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010 Jan;11(10):R106.
25. Blumcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. 2011 Jan;52(1):158-74.
26. Steinlein OK. Genetic heterogeneity in familial nocturnal frontal lobe epilepsy. Prog Brain Res. 2014 Jan;213:1–15.
27. Baulac S. Genetics advances in autosomal dominant focal epilepsies: focus on DEPDC5. Prog Brain Res. 2014 Jan;213:123–39.
28. Kingwell K. Epilepsy: GRIN2A mutations identified as key genetic drivers of

epilepsy-aphasia spectrum disorders. Nat Rev Neurol. 2013 Oct;9(10):541.

29. Curatolo P, Maria BL. Tuberous sclerosis. Handbook of clinical neurology. Elsevier; 2013. 323-31 p.
30. Nashef L, So EL, Ryvlin P, Tomson T. Unifying the definitions of sudden unexpected death in epilepsy. Epilepsia. 2012 Feb;53(2):227–33.
31. Surges R, Sander JW. Sudden unexpected death in epilepsy: mechanisms, prevalence, and prevention. Curr Opin Neurol. 2012 Apr;25(2):201–7.
32. Nascimento FA, Borlot F, Cossette P, Minassian BA, Andrade DM. Two definite cases of sudden unexpected death in epilepsy in a family with a DEPDC5 mutation. Neurol Genet. 2015 Dec 1;1(4):e28.
33. Bagnall RD, Crompton DE, Petrovski S, et al. Exome-based analysis of cardiac arrhythmia, respiratory control and epilepsy genes in sudden unexpected death in epilepsy. Ann Neurol. 2015 Dec 24; Epub
34. Ryvlin P, Nashef L, Tomson T. Prevention of sudden unexpected death in epilepsy: a realistic goal? Epilepsia. 2013 May;54 Suppl 2:23–8.
35. Guerrini R, Duchowny M, Jayakar P, et al. Diagnostic methods and treatment options for focal cortical dysplasia. Epilepsia. 2015 Nov;56(11):1669–86.

Figure Titles and Legends

Figure 1: Schematic representation of the GATOR-mTORC1 pathway. The GATOR1 complex, containing the proteins DEPDC5, NPRL2 and NPRL3, inhibits mTORC1 through its GAP activity toward the GTPases RagA/C. The GATOR2 complex, containing the proteins MIOS, SEH1L, WDR24, WDR59 and SEC13, is thought to inhibit the GATOR1 complex

Figure 2: Pedigrees of families with pathogenic variants in GATOR1 genes. Family E (French), F (French) and G (Swiss) each include several family members presenting focal epilepsy. All three families comprise an individual with focal epilepsy and an FCD.

Figure 3: Proportion of mutations in GATOR1 complex genes in focal epilepsies versus familial focal epilepsies with FCD. The diagram shows a clear enrichment of mutations in patients with familial focal epilepsy with FCD.

Figure 4: Sequence chromatograms of two affected members of family F (II-7 and III-6) and one affected member of family E (IV-4). The *NPRL3* c.1270C>T mutation encoding p.Arg424* is only present in the cDNA of lymphoblasts of individuals from family F when pretreated with emetine, indicating the occurrence of NMD. The c.68_69delCT frameshift mutation in *NPRL2* is present regardless of treatment.

Figure 5: Imaging, histopathology and mTORC1 pathway activity. (A, B, C, D) FDG-PET-MRI and histology of resected brain tissue of E/IV-4. (A) FDG-PET-MRI shows hypo-metabolism in the right fronto-insular area, co-localizing with an area of increased cortical thickness and blurring of grey-white matter border. (B) NeuN immunostaining shows abnormal radial cortical lamination with microcolumns in the upper layers of the cortex. These histological features are compatible with a FCD Ia. Scale bar: 500µm. (C) H&E staining only shows neurons of normal size and morphology. Scale bar: 50µm. (D) Ser235/236 phosphorylated S6 (pS6 235/6) immunostaining is positive in neurons of normal

size and morphology. Scale bar: 50µm. (E, F, G, H) T1-weighted brain MRI and histology of resected brain tissue of subject F/IV-12. (E) MRI at the age of 4 months shows left frontal abnormal gyration, thickened cortex and blurring of the grey-white matter border (arrow). (F) NeuN immunostaining shows severe cortical dyslamination without distinguishable layer formation. Scale bar: 500µm. (G) H&E staining shows enlarged dysmorphic neurons with enlarged nuclei. Balloon cells are not seen, in favor of FCD IIa. Scale bar: 50µm. (H) pS6 235/6 immunostaining shows intense labeling of normal and dysmorphic neurons. Scale bar: 50µm. (I) FLAIR-weighted brain MRI of subject G/III-2. MRI at the age of 4.5 years shows right frontal cortical thickening, abnormal gyration and linear hyperintensity of the grey-white matter junction, characteristic of FCD IIb (arrow).

Supporting Information

Supplemental Results: Clinical description of families E-F-G carrying pathogenic variants in *NPRL2* and *NPRL3*

Supplemental Table 1: Sequencing **c**overage of GATOR1 and GATOR2 complex genes

Supplemental Table 2: List of rare non-synonymous variants in GATOR1 and GATOR2 complex genes

Supplemental Table 3: Frequency of pathogenic variants in GATOR1 complex genes in different clinical subgroups

Supplemental Figure 1: Pedigrees of families with mutations in *DEPDC5*

Supplemental Figure 2: Visualization of *NPRL2* and *NPRL3* transcripts expressed in the frontal lobe tissue of four control individuals as predicted by Cufflinks

Supplemental Figure 3: Schematic representation of all reported and newly identified pathogenic variants in *NPRL2* and *NPRL3*

Table 1: Clinical features of patients with mutations in *NPRL3* and *NPRL2*

Subject Age	Variant	Sz onset age	Epilepsy type	AED use	Seizure outcome	EEG	MRI brain/Histology
Family E, *NPRL2*, c.68_69delCT, p.Ile23Asnfs*6							
E/IV-4 27 y	yes	3 y	frontal lobe epilepsy	CBZ, VPA VGB, LTG, TPM, LEV, currently LCS, CLZ	after right fronto-orbital resection at 18 y: > 50% seizure reduction	SEEG: right fronto-insular and fronto-orbital onset	MRI normal. PET right fronto-insular hypo-metabolism. Histology: FCD Ia
E/IV-6 20 y	yes	13 y	temporal lobe epilepsy	OXC	seizure free	left temporal discharges	normal
E/II-2 76 y	yes	unknown	epilepsy with tonic clonic seizures	unknown	unknown	unknown	unknown
E/III-2 deceased	not known	infancy	epilepsy with tonic clonic seizures	unknown	deceased at age 22 y (SUDEP)	unknown	unknown
Family F, *NPRL3*, c.1270C>T, p.Arg424*							
F/I-1 deceased	not known	12 y	episodes with loss of consciousness	probably none	no episodes since age 15 y	unknown	not done
F/II-7 85 y	yes	5 mo FS 13 y epilepsy	febrile seizures, epilepsy with tonic clonic seizures	VPA	seizure free since age 24 y	diffuse irregular SWC with changing maximum	not done
F/II-10 deceased	not known	~ 40 y	focal epilepsy due to cerebral tumor	unknown	died two years after the diagnosis of a cerebral tumor	unknown	unknown
F/II-12 not available	not known	infancy	single febrile seizure	none	not applicable	not done	not done
F/III-1 deceased	not known	10 mo	fronto-temporal epilepsy	PHT, PB, VPA, ETX	died at age 23 y (SUDEP)	unknown	not done
F/III-4 56 y	yes	26 y	epilepsy with tonic clonic seizures	PB, VPA, GBP, LTG	seizure free after last pregnancy. At 54 y intracerebral hematoma and tonic-clonic seizure (unclear what was initial event)	SWC, predominant left temporal	normal
F/III-6 55 y	yes	10 mo	fronto-temporal epilepsy	PHT, PB, ETX, CBZ, VPA, CLB	last tonic clonic seizure at 27 y. Still rare focal motor seizures	sharp waves, predominantly left temporal	unavailable

Table 1: Clinical features of patients with mutations in *NPRL3* and *NPRL2* (continued)

F/III-7 54 y	No	11 mo FS 7 y epilepsy	febrile seizures, generalized epilepsy	PB, PHT, VPA, ZNS	seizure free since age 50 y	generalized SWC, photosensitivity	unknown
F/III-11 43 y	yes	8 y	frontal lobe epilepsy	VGB, CLB, CBZ, LEV, OXC, LTG, VPA, TPM, PMP	weekly nocturnal sz	normal	normal MRI and PET
F/IV-1 31 y	No	5 y	single tonic clonic seizure	PB	no further episodes after age 5 y	bursts of diffuse SWC during sleep. Normal one year later	unknown
F/IV-12 14 y	yes	2 mo	frontal lobe epilepsy	VGB, VPA, OXC CNZ	after incomplete FCD resection at 1 y and second surgery at 5 y: Rare seizures when medication errors	frontocentral left spikes. SEEG (5y): left mesial temporal onset	4 mo: left frontal FCD 2 y: postoperative MRI with incomplete resection of FCD, left hippocampal atrophy Histology: FCD IIa and hippocampal sclerosis
F/IV-14 not available	not known	unknown	epilepsy with tonic clonic seizures	unknown	unknown	unknown	hydrocephaly
Family G, *NPRL3*, c.1070delC, p.Pro357Hisfs*56							
G/I-1 75 y	yes	51 y	temporal lobe epilepsy	CBZ	seizure free and without AED since age 60 y	unknown	not done
G/II-1 30 y	not known	14 y	epilepsy with tonic clonic seizures	OXC	ongoing seizures	left temporal discharges	not done
G/ II-2 37 y	yes	3 y 6 mo	frontal lobe epilepsy	VPA, LEV, CBZ	ongoing seizures	right frontocentral discharges	normal
G/III-1 6 y	yes	4 y 6 mo	epilepsy with tonic clonic seizures	VPA	seizure free	normal	not done
G/III-2 5 y	yes	2 y	frontal lobe epilepsy	VPA, CBZ	seizure free for 6 months	bilateral frontal spikes. Fast left frontal ictal activity	right fronto-parietal FCD

Abbreviations: AED: anti-epileptic drugs; CBZ: carbamazepine; CLB: clobazam; CLZ: clonazepam; ETX: ethosuximide; FCD: focal cortical dysplasia; GBP: gabapentine; LCS: lacosamide; LEV: levetiracetam; LTG: lamotrigine; mo: months; OXC: oxcarbazepine; PB: phenobarbital; PHT: phenytoin; PMP: perampanel; SEEG: Stereo-EEG; SUDEP: Sudden Unexplained Death in Epilepsy Patients; SWC: spike wave complexes; TPM: topiramate; VGB: vigabatrin; VPA: valproic acid; y: years; ZNS: zonisamide