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To cite this version:
Sarah Weckhuysen, Elise Marsan, Virginie Lambrecq, Cécile Marchal, Mélanie Morin-Brureau, et al.. Involvement of GATOR complex genes in familial focal epilepsies and focal cortical dysplasia. Epilepsia, Wiley, 2016, 57 (6), pp.994-1003 <10.1111/epi.13391>. <hal-01323850>
Involvement of GATOR complex genes in familial focal epilepsies and focal cortical dysplasia

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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 \textit{DEPDC5} in familial focal epilepsies has introduced a novel pathomechanism to a field so far dominated by ion channelopathies. \textit{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 (\textit{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 \textit{DEPDC5}, two in \textit{NPRL2} and one in \textit{NPRL3}. We showed hyperactivation of the mTORC1 pathway in brain tissue from patients with \textit{NPRL2} and \textit{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. While most affected mutation carriers have a normal MRI, several patients were shown to have focal cortical dysplasia (FCD) type I, IIa or IIb. In vitro and in yeast, DEPDC5 acts as an inhibitor of the mechanistic target of rapamycin complex 1 (mTORC1). mTORC1 has a serine-threonine kinase activity that phosphorylates several downstream proteins regulating essential cellular functions like protein synthesis, cell growth, migration and proliferation. The mTOR pathway is increasingly being recognized as a major player in the pathogenesis of cerebral malformations. 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.

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 (Fig. 1). Recently, mutations in NPRL3 and NPRL2 were described in familial and sporadic focal epilepsies, with or without FCD. 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. The study was approved by the local ethical committee (CCPRB 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.Ile23Asnfs*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)
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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-
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\textsuperscript{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, CHRN2*, *CHRn2*), 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*. *NPRL2* and *NPRL3* mutations were recently described in a few familial and sporadic cases with focal epilepsies, with or without FCD. 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. 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 - 0.9/1000 patient-years) \(^{31}\). Interestingly, a recent article described a family with focal epilepsy caused by a mutation in \textit{DEPDC5} comprising two family members that died of SUDEP, and a whole exome study of 61 SUDEP cases identified 6 probably pathogenic \textit{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.
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**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 hypometabolism 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 coverage 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*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Variant</th>
<th>Sz onset age</th>
<th>Epilepsy type</th>
<th>AED use</th>
<th>Seizure outcome</th>
<th>EEG</th>
<th>MRI brain/Histology</th>
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<td>Family E, <em>NPRL2</em>, c.687_69delCT, p.Ile23Asnfs*6</td>
<td></td>
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<td>E/IV-4 27 y</td>
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<td>3 y</td>
<td>frontal lobe epilepsy</td>
<td>CBZ, VPA VGB, LTG, TPM, LEV, currently LCS, CLZ</td>
<td>after right fronto-orbital resection at 18 y; &gt; 50% seizure reduction</td>
<td>SEEG: right fronto-insular and fronto-orbital onset</td>
<td>MRI normal. PET right fronto-insular hypo-metabolism. Histology: FCD Ia</td>
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<td>yes</td>
<td>unknown</td>
<td>epilepsy with tonic clonic seizures</td>
<td>unknown</td>
<td>unknown</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>E/III-2 deceased</td>
<td>not known</td>
<td>infancy</td>
<td>epilepsy with tonic clonic seizures</td>
<td>unknown</td>
<td>deceased at age 22 y (SUDEP)</td>
<td>unknown</td>
<td>unknown</td>
</tr>
</tbody>
</table>

| 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/I-7 85 y | yes | 5 mo FS | febrile seizures, epilepsy with tonic clonic seizures | VPA | seizure free since age 24 y | diffuse irregular SWC with changing maximum | not done |
| F/I-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)

<table>
<thead>
<tr>
<th>Family</th>
<th>NPRL3</th>
<th>c.1070delC</th>
<th>p.Pro357Hisfs*56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family G, NPRL3, c.1070delC, p.Pro357Hisfs*56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F/III-7 54 y</th>
<th>No</th>
<th>11 mo FS 7 y epilepsy</th>
<th>febrile seizures, generalized epilepsy</th>
<th>PB, PHT, VPA, ZNS</th>
<th>seizure free since age 50 y</th>
<th>generalized SWC, photosensitivity</th>
<th>unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/III-11 43 y</td>
<td>yes</td>
<td>8 y</td>
<td>frontal lobe epilepsy</td>
<td>VGB, CLB, CBZ, LEV, OXC, LTG, VPA, TPM, PMP</td>
<td>weekly nocturnal sz</td>
<td>normal</td>
<td>normal MRI and PET</td>
</tr>
<tr>
<td>F/IV-1 31 y</td>
<td>No</td>
<td>5 y</td>
<td>single tonic clonic seizure</td>
<td>PB</td>
<td>no further episodes after age 5 y</td>
<td>bursts of diffuse SWC during sleep. Normal one year later</td>
<td>unknown</td>
</tr>
<tr>
<td>F/IV-12 14 y</td>
<td>yes</td>
<td>2 mo</td>
<td>frontal lobe epilepsy</td>
<td>VGB, VPA, OXC CNZ</td>
<td>after incomplete FCD resection at 1 y and second surgery at 5 y: Rare seizures when medication errors</td>
<td>frontal central left spikes. SEEG (5y): left mesial temporal onset</td>
<td>4 mo: left frontal FCD 2 y: postoperative MRI with incomplete resection of FCD, left hippocampal atrophy Histology: FCD IIa and hippocampal sclerosis</td>
</tr>
<tr>
<td>F/IV-14 not available</td>
<td>not known</td>
<td>unknown</td>
<td>epilepsy with tonic clonic seizures</td>
<td>unknown</td>
<td>unknown</td>
<td>unknown</td>
<td>hydrocephaly</td>
</tr>
</tbody>
</table>

**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