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Mutations in *DEPDC5* cause familial focal epilepsy with variable foci

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The majority of epilepsies are focal in origin, with seizures emanating from one brain region. Although focal epilepsies often arise from structural brain lesions, many affected individuals have normal brain imaging. The etiology is unknown in the majority of individuals, although genetic factors are increasingly recognized. Autosomal dominant familial focal epilepsy with variable foci (FFEVF) is notable because family members have seizures originating from different cortical regions[1]. Using exome sequencing, we detected *DEPDC5* mutations in two affected families. We subsequently identified mutations in five of six additional published large families with FFEVF. Study of families with focal epilepsy that were too small for conventional clinical diagnosis with FFEVF identified *DEPDC5* mutations in approximately 12% of families (10/82). This high frequency establishes *DEPDC5* mutations as a common cause of familial focal epilepsies. Shared homology with G protein signaling molecules and localization in human neurons suggest a role of DEPDC5 in neuronal signal transduction.

FFEVF (MIM 604364) was initially described in a large family in which affected family members had electroclinical seizures arising from different brain cortical regions[1]. Seven further families with FFEVF have since been reported. Affected family members have seizures arising from the frontal, temporal, frontotemporal, parietal and occipital cortical regions. The time of seizure onset varies from infancy to adult life. Affected individuals typically have normal intellect, although some family members also have intellectual disability, psychiatric disorders (such as schizophrenia) or autism spectrum disorders (ASD)[2–5]. Structural magnetic resonance imaging (MRI) studies are usually unremarkable. Families with FFEVF show an autosomal dominant inheritance pattern of focal epilepsy with marked intrafamilial variation in severity[1–6]. Linkage studies have mapped FFEVF to a 5.3-Mb region on chromosome 22q12 (refs. 2–6).

To identify the genetic cause of FFEVF, we applied exome sequencing to one Australian family (A1) and one Dutch family (D1) . In both families, the causative locus for FFEVF had previously been mapped to chromosome 22q12 (refs. 3,5). A novel heterozygous nonsense mutation in the *DEPDC5* gene (encoding Dishevelled, Egl-10 and Pleckstrin (DEP) domain–containing protein 5; reference transcript, NM_001242896.1; reference protein, NP_001229825.1) was identified in each family, including c.21C>G (p.Tyr7*) in family A1 and c.1663C>T (p.Arg555*) in family D1. The *DEPDC5* mutations

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resided within the 22q12 linkage interval and were prioritized for validation and follow-up analyses. Other novel sequence variants identified in the 22q linkage interval are listed in **Supplementary Table 1**. Subsequent Sanger sequencing of *DEPDC5* in the affected members of six additional families showing linkage of pathology to 22q12 (refs. 2,4,6) identified *DEPDC5* mutations in five. Nonsense mutations were present in two families (c.4107G>A (p.Trp1369*) in family S1 and c.4606C>T (p.Gln1536*) in family S2), whereas the same deletion mutation (c.488_490delTGT; p.Phe164del) was found in three French-Canadian families (families FC1, FC2 and FC3). Haplotype and genealogical analyses were compatible with a shared ancestor for these three families[2,4].

Of the eight large families with FFEVF, only one (family A in Klein *et al.*[5]) did not have a *DEPDC5* mutation. Each detected *DEPDC5* mutation segregated with the FFEVF phenotype in the respective family (**Fig. 1a**) and was absent in both dbSNP135 and an in-house exome sequencing database of 710 chromosomes (data not shown). The identification of mutations in seven of eight families with FFEVF linked to 22q firmly establishes *DEPDC5* as the major gene for this disorder.

We postulated that *DEPDC5* might also contribute to non-lesional focal epilepsy in families that were too small to clinically diagnose with FFEVF. We scanned *DEPDC5* for sequence variation by high-resolution melt curve analysis in 82 unrelated probands from families with at least 2 individuals with focal epilepsy without a detectable structural etiology. Ten of 82 probands (12.2%) had a *DEPDC5* mutation, showing that mutations in *DEPDC5* are an important cause of familial focal epilepsy (**Fig. 1b** and **Table 1**). The FFEVF syndrome could not be confidently diagnosed in these smaller families owing to the low numbers of affected individuals and the pedigrees being too small to demonstrate clear autosomal dominant inheritance. The identification of a *DEPDC5* mutation in these smaller families enables molecular diagnosis with FFEVF.

In the ten small families, six additional nonsense or splice-site mutations and three missense mutations (encoding p.Ser1104Leu, p.Ser1073Arg and p.Ala452Val alterations) were identified, with one nonsense mutation (encoding p.Trp1466*) found in two unrelated families (**Table 1**). All mutations were absent in dbSNP135 and an in-house exome variant database (710 chromosomes), consistent with their likely pathogenicity. The c.3311C>T (p.Ser104Leu) change is present in the 1000 Genomes Project database with a minor allele frequency of 0.002, which raises uncertainty as to its pathogenicity, although approximately 2% of the general population has epilepsy.

The penetrance of mutations in *DEPDC5* associated with FFEVF was estimated at 66% (69/105) in the 7 large families (**Fig. 1a**). The penetrance ranged from 50% in family S2 to 82% in family A1, including one individual with ASD who had not had seizures (**Table 1**). The majority of the *DEPDC5* mutations detected encoded premature termination codons, suggesting that haploinsufficiency is the likely mechanism underlying pathogenesis. One individual in the DECIPHER database has a deletion encompassing 15 genes, including *DEPDC5*, but no phenotype was reported[7], implying that hemizygosity for *DEPDC5* has incomplete penetrance.

One proband, for whom parentage was confirmed, had a *de novo* nonsense mutation (encoding p.Arg1268*) (**Fig. 1b**), implicating *DEPDC5* as a contributor to sporadic as well as to familial focal epilepsies. It remains to be determined how prevalent *DEPDC5* mutations are in large cohorts of sporadic non-lesional focal epilepsy.

Temporal lobe epilepsy and frontal lobe epilepsy were the most common phenotypes in the families, accounting for over 70% of affected individuals. Parietal and occipital epilepsies were infrequent. Rarely, multifocal epilepsies and epileptic spasms were observed (**Table 1**). The mean age of seizure onset with *DEPDC5* mutations was 12.5 years (median of 11 years, range of 6 weeks to 52 years). Of note, there were seven families that included affected individuals who did not have a *DEPDC5* mutation. As the focal epilepsy phenotypes seen in FFEVF are common, this may simply reflect ascertainment bias in family studies.

Mutations in the ion channel subunit genes *CHRNA4*, *CHRNB2* and *CHRNA2* collectively account for approximately a tenth of the cases of the rare focal epilepsy syndrome autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)[8]. Mutations in the potassium channel gene *KCNT1* have recently been shown to contribute to a severe form of ADNFLE[9]. Mutations in *LGI1*, which encodes the synaptic protein leucine-rich glioma inactivated 1, underlie approximately half of the cases of another rare focal epilepsy syndrome, autosomal dominant epilepsy with auditory features[10]. Here, we add to the understanding of the molecular basis of autosomal dominant focal epilepsies by identifying the involvement of *DEPDC5*. Notably, mutations in *DEPDC5* are found in 12% of pedigrees with familial focal epilepsy, suggesting that *DEPDC5* is a highly relevant gene to the common clinical population with non-lesional frontal and temporal lobe epilepsy and a positive family history. It is possible that variation in *DEPDC5* also contributes to sporadic cases of focal epilepsy associated with monogenic or even complex inheritance.

DEPDC5 encodes a full-length protein of 1,604 amino acids of unknown function, with orthologs found in species as divergent as *Anopheles gambiae* (mosquito). The DEPDC5 protein contains an 80-amino-acid Dishevelled, Egl-10 and Pleckstrin (DEP) homology domain (**Fig. 2a**) found in proteins involved in G protein signaling and membrane targeting[11,12]. Proteins containing DEP domains mediate a broad range of cellular functions, including signaling in platelets and neutrophils[13] and Wnt signaling[14]. Notably, the Wnt signaling pathway has an important role in several aspects of neuronal circuit formation, including neuronal polarity, axon guidance, synapse formation and synaptic plasticity[15]. The biological role of *DEPDC5* and how its perturbation leads to focal seizures are yet to be determined.

The occurrence of seizures originating from different brain regions within families is an intriguing feature of FFEVF. Although the reason for this variability within families is not yet known, there are precedents for marked phenotypic variability in other monogenic epilepsies. The syndrome of familial partial epilepsy with pericentral spikes has been reported in a single large family and also features focal epilepsies with variable onset[16]. Another example is the familial epilepsy syndrome of genetic epilepsy with febrile seizures plus in which sodium channel gene mutations can cause generalized or focal epilepsies and are associated with intellectual disability and ASD in some individuals[17]. The presence of ASD and intellectual disability in our families suggests a shared pathogenic mechanism for epilepsy and other neuropsychiatric disorders, as was also observed with other genetic variants, including recurrent copy number variants at 15q13.3 (refs. 18,19).

We analyzed *DEPDC5* expression in mouse and human brain tissues. Mouse *Depdc5* transcripts were detected by quantitative RT-PCR (qRT-PCR) at low levels in all brain regions analyzed (**Fig. 2b**) and were detected throughout brain development, including in midgestation embryonic head (11.5 d post-conception (d.p.c.)), neonatal brain (postnatal day 4 (P4)) and whole adult brain (P240). Immunofluorescence analyses in mouse brain showed that Depdc5 was expressed in neurons, identified on the basis of

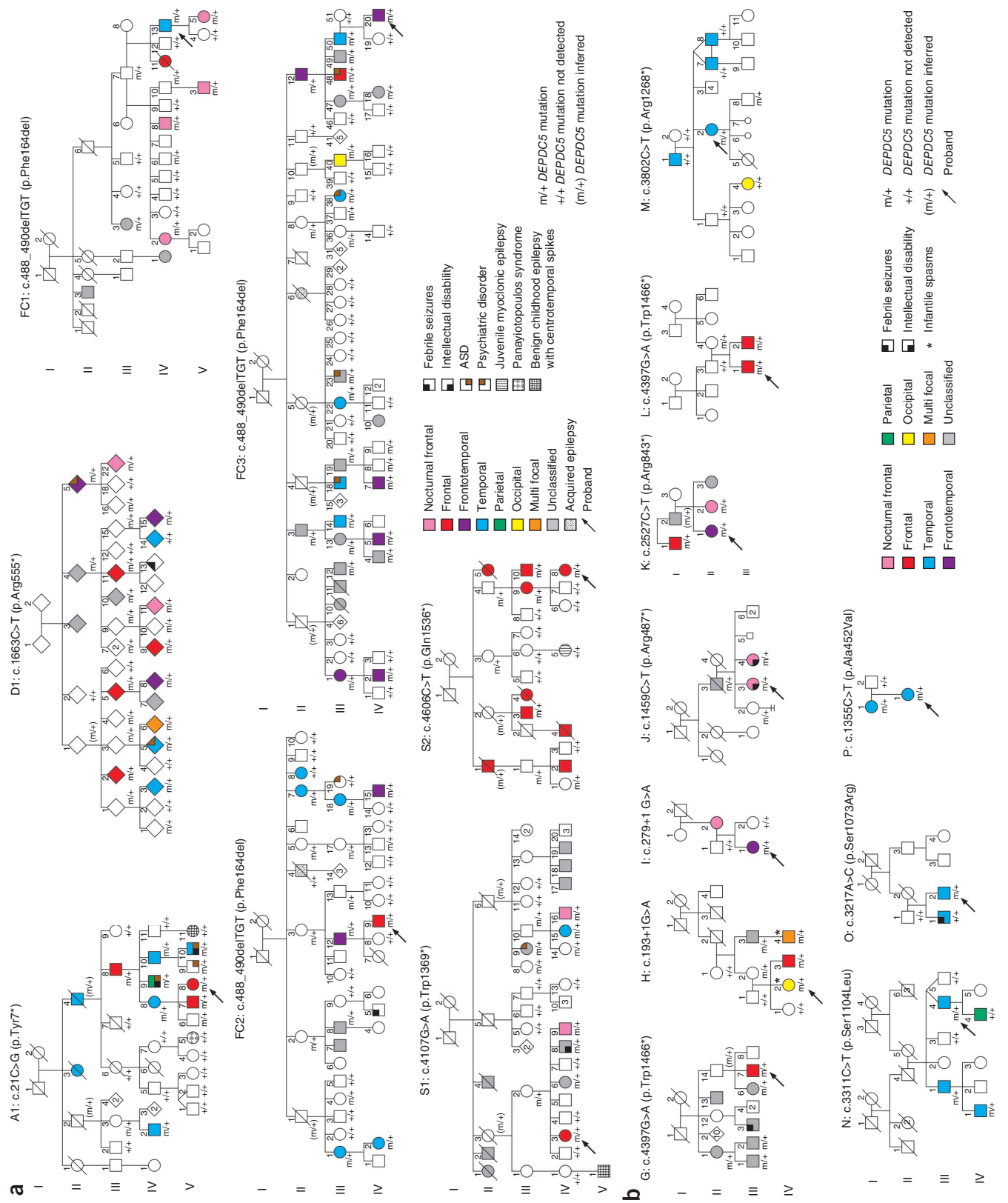

Figure 1 Pedigrees of families with FFEVF. (**a**) Pedigrees of the large families with FFEVF, showing segregation of the *DEPDC5* mutation within each family with disease. (**b**) Pedigrees of the smaller families with FFEVF. Individuals with a mutation in *DEPDC5* are indicated by m/+, and individuals tested for mutations and found to be negative are indicated by +/+. Individuals for whom the presence of a mutation was inferred on the basis of its presence in relatives are indicated by (m/+). Focal epilepsy type is indicated by color. Specific epilepsy syndromes are indicated by patterns, and filled quarters indicate comorbidities. Symbols with slashes are deceased. To maximize confidentiality, information on the sexes of family members, proband identity and alive/deceased status were removed in pedigree D1. Full details of psychiatric disorders are given in **Table 1**.

Table 1 Clinical and genetic data of families with *DEPDC5* mutations

Seven large published families with FFEVF showing linkage to 22q12 with *DEPDC5* mutation

Family	Previous publication	Mean age of seizure onset (range) (*n*)	Phenotypes	Penetrance	Family origin	*DEPDC5* mutation	
						Nucleotide change	Protein change
A1	Scheffer *et al.*[1]; Family B, Klein *et al.*[5]	13.5 years (10 months–40 years) (8)	3 FLE 4 TLE (1 ASD and intellectual disability) 1 PLE (ASD and intellectual disability) 1 ASD 2 unaffected	82% (9/11)	Australian	c.21C>G	p.Tyr7*
D1	Callenbach *et al.*[3]	11.1 years (3 months–24 years) (13)	2 NFLE 4 FLE 3 FTLE (1obsessive-compulsive disorder) 2 TLE (1 unclassified psychiatric disorder) 1 multifocal 2 unclassified 11 unaffected	56% (14/25)	Dutch	c.1663C>T	p.Arg555*
FC1	Family Q, Berkovic *et al.*[4]	19.7 years (10 years–52 years) (6)	4 NFLE 1 TLE 1 unclassified 3 unaffected	67% (6/9)	French Canadian	c.488-490delTGT	p.Phe164del
FC2	Family 14, Xiong *et al.*[2]	13.4 years (5 years–26 years) (7)	1 FLE 2 FTLE 4 TLE 1 unclassified 7 unaffected	53% (8/15)	French Canadian	c.488-490delTGT	p.Phe164del
FC3	Family 22, Xiong *et al.*[2]	11.3 years (18 months–25 years) (15)	1 FLE (personality disorder) 6 FTLE 4 TLE (1 schizophrenia) 1 OLE 9 unclassified (1 schizophrenia) 5 unaffected	81% (21/26)	French Canadian	c.488_490delTGT	p.Phe164del
S1	Spanish family (S), Berkovic *et al.*[4]	15.8 years (4 years–25 years) (6)	2 NFLE 1 FLE 1 TLE 3 unclassified (1 unclassified psychiatric disorder; 1 intellectual disability)	64% (7/11)	Spanish	c.4107G>A	p.Trp1369*
S2	Morales-Corraliza *et al.*[6]	8.3 years (4 years–12 years) (4)	4 FLE 4 unaffected	50% (4/8)	Spanish	c.4606C>T	p.Gln1536*

Ten smaller families with *DEPDC5* mutation

Family	Previous publication	Mean age of seizure onset (range) (*n*)	Phenotype	Family origin	*DEPDC5* mutation	
					Nucleotide change	Protein change
G		10 years (6 years–13 years) (4)	1 FLE 4 unclassified	French Canadian	c.4397G>A	p.Trp1466*
H		6.5 years (6 weeks–21 years) (4)	1 FLE 1 multifocal (spasms) 1 OLE (spasms) 1 unclassified 1 unaffected	Australian	c.193+1G>A	
I		15 years	1 FTLE	Italian	c.279+1G>A	
J		12.7 years (4 years–25 years) (3)	2 NFLE (intellectual disability) 1 unclassified 1 unaffected	Australian	c.1459C>T	p.Arg487*
K		11 years (6 years–15 years) (3)	1 NFLE 1 FLE 1 FTLE	French Canadian	c.2527C>T	p.Arg843*
L		2.5 years (2 years–3 years) (2)	2 FLE 1 unaffected	Israeli	c.4397G>A	p.Trp1466*
M		8 years (1)	1 TLE 1 unaffected	Australian	c. 3802C>T	p.Arg1268*
N	Family 16, Crompton *et al.*[20]	22.7 years (20 years–27 years) (3)	3 TLE	Australian	c.3311C>T	p.Ser1104Leu
O		22 years (1)	1 TLE	Australian	c.3217A>C	p.Ser1073Arg
P		6 years (3.5 years–8 years) (2)	2 TLE	Israeli	c.1355C>T	p.Ala452Val

Only the phenotypes of individuals with a *DEPDC5* mutation are shown. FLE, frontal lobe epilepsy; FTLE, frontotemporal lobe epilepsy; NFLE, nocturnal frontal lobe epilepsy; OLE, occipital lobe epilepsy; PLE, parietal lobe epilepsy; TLE, temporal lobe epilepsy.

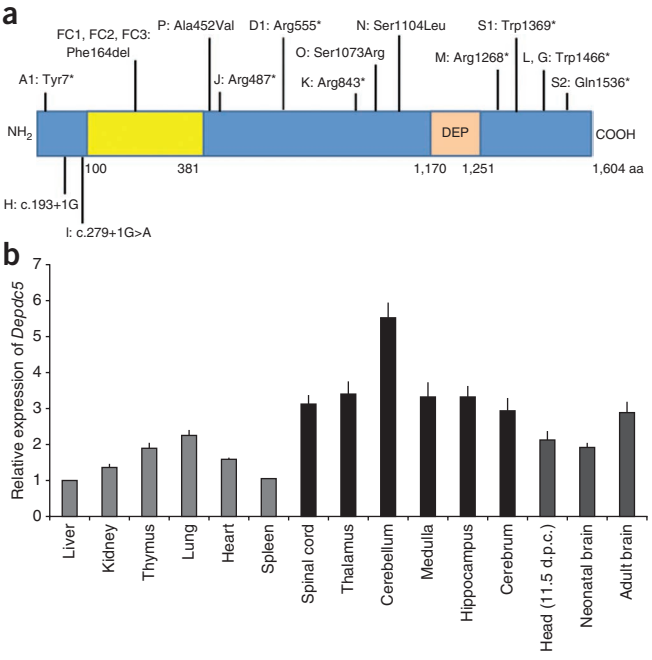

Figure 2 Location of the alterations in DEPDC5 detected in families with FFEVF and expression analysis of *Depdc5* transcripts in mouse neural tissues. (**a**) Diagram of the DEPDC5 protein showing the positions of the alterations found in each family with FFEVF (letter coded). The positions of a highly evolutionarily conserved but functionally uncharacterized protein domain (yellow box) and of the DEP domain (pink box) are also indicated. The splice-site mutations found in families H and I are indicated below the diagram. (**b**) qRT-PCR on RNA extracted from various mouse tissues shows that *Depdc5* is widely expressed in a series of adult organs (light gray), central nervous system tissues (black), the developing brain at 11.5 d.p.c. and neonatal and adult brain (dark gray). Gene expression is shown relative to that in the least abundant tissue (liver) and is normalized to that of the low-abundance reference gene *Rpl38*. PCR products were sequence verified, and reverse transcriptase–negative and water controls showed no amplification (data not shown), confirming the specificity of the PCR products. The experiment was repeated three times on the same cDNA series; error bars represent 1 s.d. from the mean. Similar results were obtained using *Eef2* as the reference gene.

their morphology and NeuN staining, and was absent in non-neuronal cells, including astrocytes (**Fig. 3**). GABAergic interneurons, identified by glutamic acid decarboxylase (Gad67) staining, also expressed Depdc5. Immunofluorescence was localized to the cytosol of the neuronal cell body and was mostly perinuclear, with little or no extension into neuronal processes. This subcellular localization was confirmed by immunofluorescence in human neurospheres derived from induced pluripotent stem (iPS) cells from control individuals (**Supplementary Fig. 1**), as well as in mouse brain (**Supplementary Fig. 2**) and SH-SY5Y human neuroblastoma cell protein extracts by protein blot analysis (**Supplementary Fig. 3**). The methods used here provide preliminary data on the localization of DEPDC5. Further analyses using approaches such as gene knockdown and animal models are warranted. The localization of DEPDC5 in neurons and its homology to proteins involved in G protein signaling pathways suggest a role in neuronal signal transduction.

We report *DEPDC5* mutations in seven large families with FFEVF and in approximately 12% of smaller families with focal epilepsy and a *de novo* mutation in an individual with focal epilepsy. These findings establish *DEPDC5* mutations as the most common known cause of familial focal epilepsy. The identification of *DEPDC5* as the gene underlying FFEVF substantially advances understanding of the pathogenesis of epilepsy by implicating another new gene pathway. Apart from enabling the diagnosis of FFEVF through molecular testing, these findings also enable strategies to be devised to improve prognosis through tailored treatment targeting *DEPDC5*.

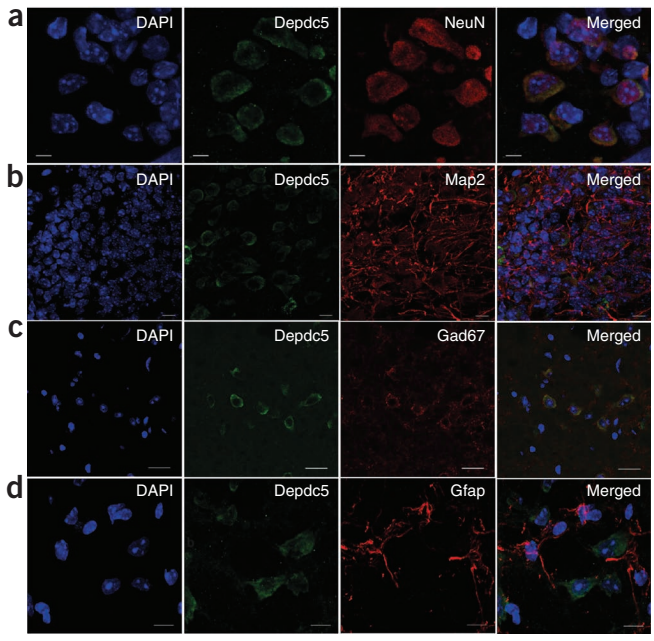

Figure 3 Depdc5 protein localization in adult mouse brain. (**a**–**d**) Confocal images of immunofluorescence analyses. (**a**) Double immunostaining with D19 antibody to DEPDC5 (green) and a neuron-specific antibody to NeuN (red). The merged image shows colocalization of Depdc5 with NeuN in neuronal cells. (**b**) Double immunostaining with D19 antibody to Depdc5 (green) and an antibody to Map2 (red). The merged image shows the absence of Depdc5 signal in Map2-positive neuronal processes. (**c**) Double immunostaining with D19 antibody to Depdc5 (green) and an antibody to Gad67 (red). The merged image shows the presence of Depdc5 signal in Gad67-positive cell bodies. (**d**) Double immunostaining with D19 antibody to Depdc5 (green) and an antibody to Gfap (red). The merged image shows the absence of Depdc5 signal in Gfap-positive astrocytes. Nuclei are stained with DAPI (blue). Scale bars, 10 μm.

URLs. dbSNP135, http://www.ncbi.nlm.nih.gov/projects/SNP/; 1000 Genomes Project, http://browser.1000genomes.org/index.html; DECIPHER Consortium, http://decipher.sanger.ac.uk/; SeattleSeq, http://snp.gs.washington.edu/SeattleSeqAnnotation137/.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

L.M.D. designed and oversaw the molecular genetic aspects of the study, coordinated the study and wrote the first draft of the manuscript. B.d.V. and A.M.J.M.v.d.M. designed and carried out molecular genetics analyses and contributed to writing the manuscript. C.M.W. analyzed whole-exome sequencing data. S.E.H. analyzed molecular data, including whole-exome sequencing data. D.E.C., S.T.B., K.M.K., P.M.C.C., S.K., B.M.R., R.G.-L., D.C., T.J.O., F.D., L.L., F.B., P.C., J.S., O.F.B., F.A., E.A., M.P., S.F.B. and I.E.S. performed clinical phenotyping. B.L.H. and X.I. performed molecular studies and interpreted that data. J.C.M., A.E.G., M.A.C. and J.G. assisted with whole-exome sequencing analysis. P.Q.T. and J.N.H. performed and interpreted quantitative gene expression analyses. M.P., S.D. and S.C. performed molecular genetics analyses and designed and performed cell biology and immunohistochemistry analyses. I.E.S. and S.F.B. designed the study, oversaw the collection and clinical characterization of families and jointly wrote the manuscript. All authors contributed to the editing of the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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ONLINE METHODS

Affected individuals and controls. Informed written consent was obtained from participants. The study was approved by the Human Research Ethics Committees of Austin Health and other collaborating centers. Subjects were recruited from epilepsy clinics and private practices and by referral to our epilepsy genetics research program. Individuals underwent phenotyping using a validated seizure questionnaire[21]. All medical records, electroencephalography (EEG) and neuroimaging data were obtained where available. Australian control samples were from anonymous blood donors.

Exome sequencing, validation of variants and mutation screening. The exomes of individuals from Australian family A1 and Dutch family D1 were independently sequenced in the Princess Alexandra Hospital (Brisbane, Queensland, Australia) and the Leiden Genome Technology Centre (LGTC) (Leiden, The Netherlands). The coding sequences were enriched using the SureSelect Human All Exon 50Mb kit (Agilent Technologies). After sequence capture and amplification, fragments were sequenced using a SOLiD v4 instrument (Applied Biosystems) in Australia and using the HiSeq 2000 platform from Illumina at LGTC. Sequence reads were aligned to the UCSC Genome Browser hg18 reference sequence using Burrows-Wheeler Aligner (BWA)[22]. Sequence variants were reported with SAMtools and annotated using SeattleSeq. Chromosome 22 linkage interval variants between D22S1163 (chr. 22: 26,148,651) and D22S280 (chr. 22: 31,639,700) were extracted from the annotation file. Variants reported in dbSNP and intronic and intergenic variants were filtered out.

Mutation analysis of the ORF of *DEPDC5* was performed by high-resolution melting (HRM) analysis using a LightScanner (Idaho Technology). Sequence variants were validated, and family members were analyzed for mutations by Sanger sequencing. Control DNA was analyzed for each mutation by HRM analysis. *DEPDC5* primer sequences and details on the PCR conditions used are available from the authors upon request.

Detection of the *DEPDC5* mutation encoding p.Phe164del by allele-specific PCR. To screen for the *DEPDC5* mutation encoding p.Phe164del, we designed two allele-specific PCR primers that were identical except for the last 3′ nucleotide. The first primer was used to detect mutated alleles, and the second primer was used to detect wild-type alleles. These forward primers were used with the same reverse primer to give a 117-bp PCR product. Primer sequences are given in **Supplementary Table 2**. PCR was performed with the HotStarTaq Plus Master Mix kit (Qiagen Benelux).

qRT-PCR analysis of mouse *Depdc5*. RNA was extracted using TRIzol from various tissues of a P90 mouse as well as a pool of 11.5 d.p.c. heads, a P4 brain and a P240 brain. We used 1 mg of each RNA sample for reverse transcription with the ABI High-Capacity RNA-cDNA kit (Life Technologies). Quantitative PCR was performed using ABI Fast SYBR Master Mix (Life Technologies) on an ABI 7500 StepOnePlus (Life Technologies). Primer sequences and amplicon lengths are given in **Supplementary Table 2**. *Depdc5* expression was normalized to that of *Rpl38*, a low-abundance reference gene with stable expression levels across multiple tissues[23], and expressed as relative quantity using ABI software (Life Technologies).

Immunofluorescence analysis of Depdc5 in mouse brain. Adult FVB/N mice (12 weeks old) were anesthetized with Avertin (administered intraperitoneally) and transcardially perfused with PBS followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brain was isolated and left in 4% PFA for 2 h and was then transferred to 20% sucrose for 24 h. We mounted 18-µm sagittal cryostat sections on superfrost plus slides (Menzel-Glaser). D19 rabbit polyclonal antibody (Santa Cruz Biotechnology, sc-86116) was raised against a peptide mapping near the N-terminal portion of the longest isoform of human DEPDC5. Frozen brain sections were washed with PBS and then incubated in blocking solution containing 5% donkey serum with 0.3% Triton X-100 for 2 h at room temperature. Slides were then incubated with primary antibodies overnight at 4 °C. Primary antibodies included D19 and mouse monoclonal antibodies against NeuN (Neuronal Nuclei; Merck Millipore, MAB377), MAP-2 (microtubule-associated protein 2; Merck Millipore, MAB3418) to detect mature neurons, GAD67 (glutamic acid decarboxylase 67-kD isoform; Merck Millipore, MAB5406) to detect GABAergic neurons and GFAP (glial fibrillary acidic protein; Sigma-Aldrich, G3893) to detect astrocytes. Secondary antibodies included Alexa Fluor 488–conjugated donkey antibody to rabbit immunoglobulin G (IgG) and Cy3-conjugated donkey antibody to mouse IgG. Tissues were washed and incubated with DAPI 4′,6-diamidino-2-phenylindole) to stain cell nuclei and mounted with fluorSave (Merck Millipore). Controls included samples with no primary antibody and, for D19, samples preincubated with a blocking (neutralizing) peptide for 2 h at room temperature.

Immunofluorescence analysis of DEPDC5 in human neural cells derived from iPS cells. Skin fibroblasts from healthy volunteers were reprogrammed to generate iPS cells using the Thomson protocol[24]. For neural induction, iPS cell colonies were treated with Noggin for 14 d as described previously[25] and then mechanically transferred and cultured in NBN (Neurobasal media A+B27+N2) medium in the presence of fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) (Peprotech; both 20 ng/ml), which allowed the formation of neurospheres-containing neural precursors. To detect DEPDC5 subcellular localization in human neural precursors, neurospheres were plated overnight on glass coverslips coated with Matrigel (BD Biosciences) and fixed with 4% PFA. Cultures permeabilized with Triton X-100 were incubated with rabbit polyclonal D19 antibody to DEPDC5 (Santa Cruz Biotechnology) and with an antibody to SOX2 (Merck Millipore, MAB4343). Staining was achieved with Alexa Fluor 488–conjugated donkey secondary antibody to rabbit IgG and Cy3-conjugated secondary antibody to mouse IgG. For neuronal differentiation, neurospheres were plated on laminin-coated glass coverslips in NBN medium without proliferation factors. To promote neuronal differentiation, BDNF (brain-derived neurotrophic factor) and NT3 (neurotrophin-3) (both 100 ng/ml; Peprotech) were added in differentiation medium and cultured for 4 weeks. To detect DEPDC5 in differentiated cells, immunofluorescence was performed on paraformaldehyde-fixed and Triton X-100–permeabilized cultures. Mature neurons were labeled using NeuN (Merck Millipore), and astrocytes were labeled using GFAP (Sigma-Aldrich). Signal was detected using secondary antibodies as described above. DAPI was used to stain cell nuclei. Slides were mounted with fluorSave.

Immunofluorescence signal for both mouse and human samples was visualized by a laser scanning confocal microscope (LSM 510 META Zeiss), and exported tif images from LSM were assembled in Adobe Illustrator. Fluorescence intensity values obtained during profile analysis were exported, and histograms were generated using Sigmaplot 12.

Protein extraction from adult mouse brain. One FBB/N wild-type mouse was sacrificed by cervical dislocation, and the brain was promptly transferred to cold PBS containing protease inhibitors. Only one hemisphere of the brain was used for protein extraction according to an adapted protocol[26]. Freshly isolated brain tissue was finely minced on ice in 1 ml of hypotonic buffer (10 mM Tris-Cl, pH 7.8, 5 mM $MgCl_2$, 10 mM KCl, 0.1 mM EDTA, 300 mM sucrose, 0.5 mM dithiothreitol and protease inhibitor cocktail). NP-40 was added to a final concentration of 0.125%. Lysate was triturated through a 20-gauge needle 25 times, incubated for 10 min on ice and centrifuged briefly at low speed at 4 °C to pellet the remaining pieces. Supernatant was collected and centrifuged at 4,000*g* for 5 min. The supernatant (cytoplasmic fraction) was collected, and the pellet (nuclei) was washed once with hypotonic buffer, dissolved in hypertonic buffer (20 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 320 mM KCl, 2 mM EDTA, 25% glycerol, 0.5 mM dithiothreitol and protease inhibitor cocktail) and incubated for 15 min on ice. Lysate was centrifuged at 16,000*g* for 10 min, and the supernatant was collected (nuclear fraction). Similarly, proteins were isolated from SH-SY5Y human neuroblastoma cells. Cells were lysed in 100 µl of hypotonic buffer and incubated for 10 min on ice. After NP-40 addition, lysates were vortexed. The suspension was centrifuged at 4,000*g* for 5 min at 4 °C, and the supernatant (cytoplasmic fraction) was collected, while pellets (nuclei) were washed once with hypotonic buffer. Nuclei were lysed in 50 µl of hypertonic buffer for 10 min and centrifuged at 16,000*g* for 10 min, and the supernatant was collected (nuclear fraction).

SDS-PAGE and protein blotting. Proteins were quantified by BCA assay (Pierce), and 40 µg of protein per lane was separated on a 7% acrylamide gel at 40 mA per gel. Proteins were transferred to 0.45-µm nitrocellulose

membranes at a constant voltage of 100 V for 2 h at 4 °C in transfer buffer supplemented with 0.25% SDS. Membranes were incubated overnight with antibody to DEPDC5 (D19, Santa Cruz Biotechnology) diluted 1:500 at 4 °C after a 2-h preincubation at room temperature with or without the D19 blocking peptide that was five times more concentrated than the antibody to D19. Membranes were incubated overnight with antibody to α-tubulin (Sigma, T5168) diluted 1:7,000 at 4 °C. Membranes were incubated with rabbit and mouse secondary antibodies (IRDye secondary antibodies, LI-COR) diluted 1:15,000 for 1 h at room temperature in the dark. Each step was followed by four 5-min washes alternating PBS–0.1% Tween-20 and PBS. Fluorescent signal was detected using an Odyssey Infrared Imaging System (LI-COR).

Confocal laser scanning microscopy. An LSM510 NLO multiphoton confocal microscope fitted on an Axiovert M200 inverted microscope equipped with C-Apochromat 40×/1.2 N.A. and 63×/1.2 N.A. water-immersion objectives (Zeiss) was used for visualizing immunohistochemistry analyses. The 488-nm excitation wavelength of the Argon/2 laser, a main dichroic HFT 488 and a band-pass emission filter (BP 500–550) were used for selective detection of the green fluorochrome. The 543-nm excitation wavelength of the HeNe1 laser, a main dichroic HFT 488/543/633 and a long-pass emission filter (LP 560) were used for selective detection of the red fluorochrome. The nuclear stain DAPI was excited in multiphoton mode at 760 nm with a Mai Tai tunable broad-band laser (Spectra-Physics) and detected using a main dichroic HFT KP650 and a band-pass emission filter (BP 435-485). Optical sections, 1-μm thick, 1024 × 1024 pixels, were collected sequentially for each fluorochrome.

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