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BRIEF COMMUNICATION

# Familial cortical dysplasia type IIA caused by a germline mutation in *DEPDC5*

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## Abstract

Whole-exome sequencing of two brothers with drug-resistant, early-onset, focal epilepsy secondary to extensive type IIA focal cortical dysplasia identified a paternally inherited, nonsense variant of *DEPDC5* (c.C1663T, p.Arg555\*). This variant has previously been reported to cause familial focal epilepsy with variable foci in patients with normal brain imaging. Immunostaining of resected brain tissue from both brothers demonstrated mammalian target of rapamycin (mTOR) activation. This report shows the histopathological features of cortical dysplasia associated with a *DEPDC5* mutation, confirms mTOR dysregulation in the malformed tissue and expands the spectrum of neurological manifestations of *DEPDC5* mutations to include severe phenotypes with large areas of cortical malformation.

## Introduction

Focal cortical dysplasia (FCD) encompasses a spectrum of lesions from highly localized bottom of the sulcus dysplasias (BOSD) to extensive multifocal, quadrantic or hemispheric malformations. Although the magnetic resonance imaging (MRI) appearance can lead to the suspicion of FCD, definitive diagnosis and classification requires histological analysis. FCD is characterized by cortical dyslamination either in isolation (FCD type I) or with dysmorphic neurons (FCD type IIA) or dysmorphic neurons and balloon cells (FCD type IIB).<sup>1</sup> Most cases of FCD are sporadic; however, rare familial cases are described.<sup>2,3</sup>

Deleterious mutations affecting the gene encoding Dishevelled, Egl-10 and Pleckstrin (DEP) domain-containing protein 5 gene (*DEPDC5*) cause familial focal epilepsies without obvious cortical malformations with variable penetrance and expressivity.<sup>4–6</sup> *DEPDC5* is a component of the GATOR1 complex, a critical negative regulator of the mammalian target of rapamycin (mTOR) pathway.<sup>7</sup> Germline heterozygous mutations in *DEPDC5* have been associated with lesional epilepsies including BOSD type FCD.<sup>3</sup> Notably, there was considerable intra-familial variability in the presence or absence of cortical abnormalities, with only one pedigree showing more than one individual with FCD. Surgery was not required for seizure control, therefore the pathological correlates of these lesions remain unknown. Recently, two studies showed mutations in *DEPDC5* associated with a range of FCD subtypes and hemimegalencephaly,<sup>8,9</sup> yet no evidence of *DEPDC5*-mediated mTOR dysregulation has yet been shown in human brain.

We previously described six families with FCD and related lesions; one family including two brothers with neonatal seizures and extensive type IIA FCD.<sup>2</sup> To determine a genetic etiology for FCD in this family, we performed whole-exome sequencing (WES) of both siblings and identified a heterozygous nonsense mutation in *DEPDC5*.

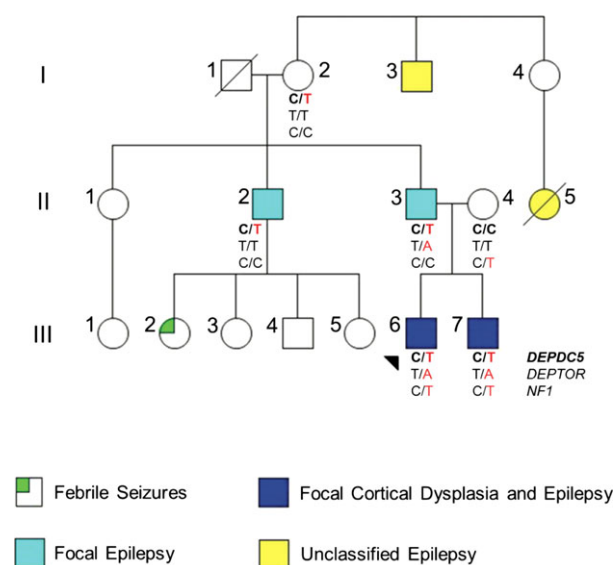
## Patients and Methods

The Royal Children's Hospital Human Research Ethics Committee approved the study and informed consent was obtained from affected individuals or their parents. Clinical details were obtained from parent interview and medical records. Brain MRI was obtained using age-specific epilepsy protocols on 1.5 T and 3 T scanners. Resected tissue was classified by a neuropathologist according to the system of the ILAE Diagnostic Methods Commission.<sup>1</sup> Resected brain tissue was assessed for mTOR activity by phospho-S6 ribosomal protein antibody staining as described previously.<sup>10</sup> Genomic DNA was isolated from

peripheral blood using standard methods. SNP genotype data were generated with the Illumina HumanCytoSNP-12v2 SNP chip and linkage (identity-by-descent [IBD] sharing) analysis was performed with MERLIN<sup>11</sup> (v1.1.2). Exonic targets were enriched with the TruSeq whole-exome kit and WES was performed with 100-base pair paired-end reads on a HiSeq2000 (Illumina, San Diego, CA). An in-house pipeline was used for data analysis. Raw sequence data were aligned to the human reference genome (hg19) with Novoalign (v2.08.01 [www.novocraft.com](http://www.novocraft.com)). Local re-alignment was performed with Genome Analysis Toolkit (GATK; v.5-2)<sup>12</sup> and variant detection and annotation utilized GATK's Unified Genotyper (v3.0-0) and ANNOVAR (version dated 2013-05-20).<sup>13</sup> Variants were filtered in step-wise fashion against criteria including minor allele frequency (MAF) <1% within the 1000 Genomes Project (release of November 2010) and Exome Variant Server (ESP6500 release; <http://evs.gs.washington.edu/EVS/>) data. Variants were then filtered with the following inclusion criteria; (1) within a linkage region (IBD = 1 or 2), (2) coding/nonsynonymous or an insertion or deletion, or in close proximity to a splice site, (3) a prediction of at least possibly damaging by either SIFT<sup>14</sup> (v5.1.1) or Polyphen-2<sup>15</sup> (v2.2.2r398) and (4) presence within a list of 483 candidate genes potentially associated with brain malformations (Table S1). Variants of interest were validated in the siblings and genotyped in extended family members by Sanger sequencing. The *DEPDC5* reference sequences NM\_001242896.1 and NP\_001229825.1 were utilized.

## Results

Detailed clinical summaries for the two affected siblings are published as Family 1.<sup>2</sup> The extended pedigree is shown in Figure 1. Both brothers (III:6 and III:7) had intractable neonatal-onset focal epilepsy, successfully treated by surgery in infancy; a right hemispherectomy in III:6 and a right temporo-parietal-occipital resection in III:7. There was no relevant family history on the maternal side, and mother had a normal brain MRI. The father (II:3) had four nocturnal tonic clonic seizures and one daytime seizure beginning at 24 years managed successfully with carbamazepine. Right leg jerking was witnessed at onset on one occasion and post-ictal EEG showed focal slowing over the left hemisphere. 3 T brain MRI at age 49 years was normal. The paternal uncle (II:2) had nocturnal tonic clonic seizures followed by left-sided weakness beginning at 38 years treated successfully with carbamazepine. 3 T brain MRI at age 53 years showed mild ventriculomegaly. Results of EEG were not available. A paternal first cousin (III:2) had a history of febrile seizures. A grand paternal uncle (I:3) had epilepsy with



**Figure 1.** Pedigree structure and genotyping. Pedigree showing the epilepsy phenotypes and the genotypes for the variants identified in *DEPDC5*, *DEPTOR*, and *NF1*.

onset at age 12 years. A paternal second cousin once removed (II:5) died during a seizure at 18 years. Further clinical and imaging details and DNA samples were not available on these three individuals.

Brain MRI, histopathology and phospho S-6 immunostaining are shown in Figure 2A–F. Both brothers had extensive imaging abnormalities of their right hemisphere suggestive of FCD. Histopathology showed cortical dyslamination and dysmorphic neurons but no balloon cells consistent with FCDIIA. Phospho S6 labeling was positive in both.

Analysis of the SNP-chip genotypes for the two siblings confirmed sibling relatedness and excluded consanguinity. Linkage analysis identified 69.4% of the siblings' genomes was shared (IBD = 1 or 2), in broad agreement with the expectation for two siblings (25% IBD = 0, 75% IBD = 1/2). Bioinformatic analysis of WES data identified a total (union) of 441,161 variants, of which three (Table 1) fulfilled the inclusion criteria. The nonsense variant in *DEPDC5* (c.C1663T, p.Arg555\*) and the missense variant in the gene encoding DEP domain-containing mTOR-interacting protein (*DEPTOR*, c.T338A, p.Leu113His) were novel while the missense variant in the gene encoding Neurofibromin 1 (*NF1*, c.C2159T, p.Ala720Val, rs148154172) had a reported MAF of 0.5%. Sanger sequencing confirmed the siblings carried the *DEPDC5*, *DEPTOR* and *NF1* variants in the heterozygous state. Sequencing showed the father, uncle and paternal grandmother carried the *DEPDC5* nonsense variant (Fig. 1). The father was heterozygous for the *DEPTOR*

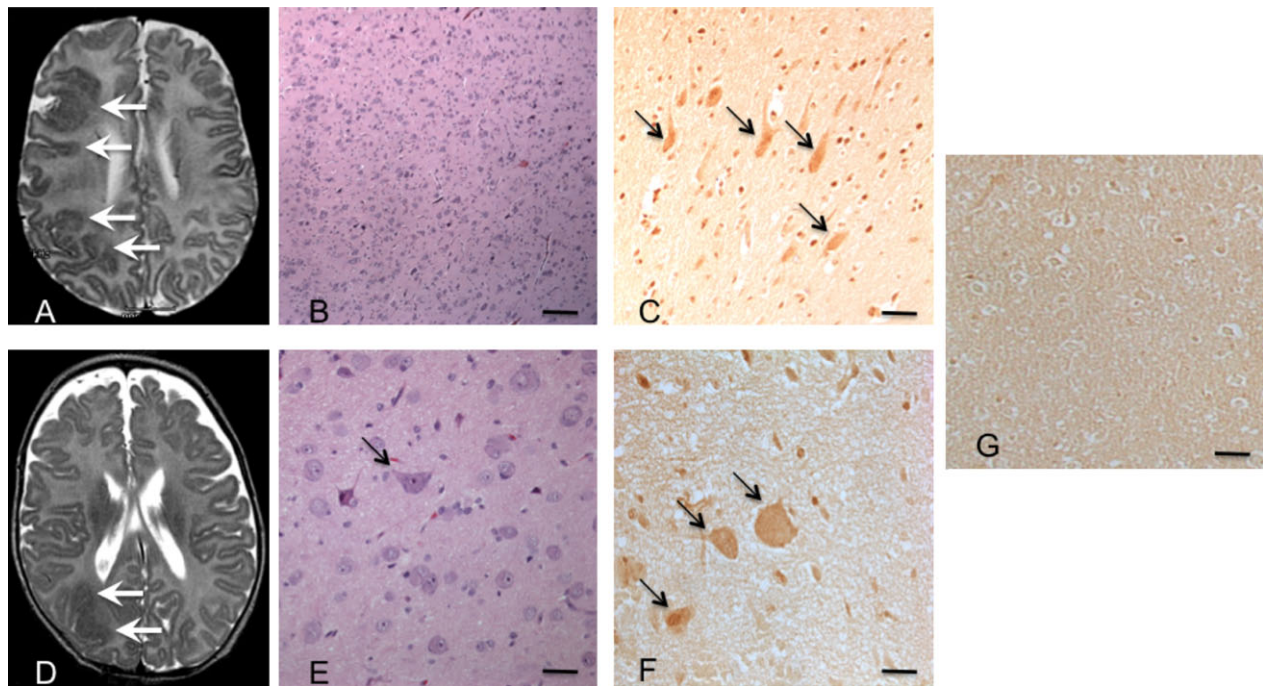
variant, while the mother was heterozygous for the *NF1* variant. This *DEPDC5* nonsense variant was previously found in a Dutch family (D1) with familial focal epilepsy and is not reported in the 1000 genome, ESP6500 or ExAC databases.<sup>4</sup> Family 1 also has Dutch ancestry and haplotype analysis demonstrated that the *DEPDC5* nonsense mutation arose on a rare haplotype carried by both our Family 1 and family D1, suggesting shared ancestry.

## Discussion

Disruption of the mTOR signaling pathway is increasingly recognized in the etiology of malformations of cortical development, with both germline and somatic mutations in mTOR pathway genes contributing to a range of phenotypes.<sup>3,16–18</sup> Mutations in *DEPDC5*, a negative regulator of mTOR activity, cause focal epilepsy with or without a cortical malformation visible on MRI.<sup>3,4,8,9</sup> Here, we show a *DEPDC5* mutation in two brothers with extensive FCD type IIA, and a paternal family history of nonlesional epilepsy.

WES identified three predicted damaging variants affecting *DEPDC5*, *NF1*, and *DEPTOR*, which encode components of the mTOR pathway. *DEPDC5* encodes a subunit of the GATOR1 complex which suppresses mTORC1 activity in response to amino acid deprivation.<sup>7</sup> A key step in the activation of mTORC1 is its recruitment to the lysosomal surface. shRNA-mediated downregulation of *DEPDC5* in vitro was associated with constitutive localization of mTOR to the lysosomal surface and dysregulated activity.<sup>7</sup> Consistent with these in vitro studies, we demonstrate for the first time mTOR dysregulation in brain tissue of individuals with *DEPDC5* mutations.

These siblings represent the severe end of the spectrum of clinical and imaging phenotypes thus far reported in *DEPDC5* mutations. Both brothers had drug-resistant, early-onset focal epilepsy and imaging showed extensive FCD, being multifocal hemispheric in one and posterior quadrant in the other. The Dutch family reported to have focal epilepsy and an identical mutation in *DEPDC5* all had normal brain MRI.<sup>4</sup> It is possible that additional variants in other genes encoding components of the mTOR pathway could contribute to the phenotypic variability associated with *DEPDC5*, which encompasses both lesional and nonlesional epilepsies. We demonstrated that both siblings also carry heterozygous missense alleles affecting *DEPTOR* and *NF1*, the former paternally inherited and the latter maternally inherited. *DEPTOR* appears to play a key role in mTOR signaling and directly inhibits mTOR activity by binding to the FAT domain.<sup>19</sup> Similarly, while mutations in *NF1* predispose individuals to neurofibromatosis type I, NF-1 can potentially dysregulate *DEPTOR* activity via a cascade of interactions that



**Figure 2.** Brain MRI, histopathology, and phospho-S6 immunostaining. Top row is patient III:6 and bottom row is patient III:7. (A and D) are axial T2-weighted MRIs at age 6 and 14 weeks, respectively, showing multiple areas of cortical thickening and blurring of the gray white junction often maximal at the bases of deep abnormal sulci throughout the right hemisphere in patient III:6 and restricted to the right posterior quadrant in patient III:7 (white arrows). (B and E) are low- and high-power hematoxylin- and eosin-stained images, respectively, of resected cortex showing cortical dyslamination with clusters of dysmorphic cytomegalic neurons (black arrow) consistent with FCDIIA. (C and F) are images showing positive phospho S6 immunostaining (Ser235/236, Cell Signaling #2211, rabbit polyclonal, 1:200) in dysmorphic cytomegalic neurons (black arrows), consistent with mTOR pathway activation. In comparison, (G) shows virtually absent phospho S6 immunostaining in control post mortem human cortex (scale bar B, 400  $\mu$ m; F, 80  $\mu$ m; C, E, and G, 200  $\mu$ m).

**Table 1.** Description of candidate variants identified in Family 1.

| Chr | Position    | Ref allele | Alt allele | Gene   | Transcript | Exon | Coding change | Protein change | Damaging? <sup>1</sup><br>PPH-2/SIFT |
|-----|-------------|------------|------------|--------|------------|------|---------------|----------------|--------------------------------------|
| 8   | 121,013,800 | T          | A          | DEPTOR | uc011lid.2 | 3    | c.T338A       | p.L113H        | prob/prob                            |
| 17  | 29,679,412  | C          | T          | NF1    | uc010cso.3 | 16   | c.C2159T      | p.A720V        | prob/tol                             |
| 22  | 32,211,195  | C          | T          | DEPDC5 | uc011alu.2 | 21   | c.C1663T      | p.R555X        | –/prob                               |

List of variants that satisfy the primary inclusion criteria of minor allele frequency <0.01, predicted damaging effects to protein function and presence in the candidate gene list (Table S1). The full criteria are detailed in the methods.

<sup>1</sup>Predicted pathogenicity by Polyphen-2 (PPH-2) and SIFT with the results “probably damaging” (prob), “tolerated” (tol) and “unscored” (–).

includes the suppression of v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF).<sup>20</sup> Neither brother nor their mother had clinical or imaging features of neurofibromatosis making the *NF1* variant of questionable significance.

These data expand the understanding of *DEPDC5*-associated epilepsies by showing pathologically proven cortical dysplasia with associated mTOR activation. It remains unclear whether the germline mutation in *DEPDC5* is sufficient in isolation to cause cortical dysplasia or whether additional germline or somatic variants of mTOR pathway genes may also contribute to the severe cortical

dysplasia seen in these siblings. Additional studies of mTOR pathway genes in germline DNA and DNA from resected brain tissue from sporadic FCD cases will be required to explore this hypothesis.

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## Author Contributions

Dr. Scerri performed bioinformatic analysis, interpreted the data, and wrote the manuscript. Ms. Riseley performed molecular analysis and read/contributed to the manuscript. Ms. Gillies performed sample acquisition, molecular analysis, and read/contributed to the manuscript. Ms. Pope performed patient recruitment, sample acquisition, and read/contributed to the manuscript. Dr. Burgess performed patient recruitment, sample acquisition, and read/contributed to the manuscript. Dr. Mandelstam interpreted brain imaging and read/contributed to the manuscript. Dr. Dibbens performed molecular analysis and read/contributed to the manuscript. Dr. Chow interpreted pathological data, provided histopathological images, and read/contributed to the manuscript. Dr. Maixner provided tissue samples and read/contributed to the manuscript. Dr. Harvey provided clinical data and tissue samples and read/contributed to the manuscript. Dr. Jackson interpreted brain imaging and read/contributed to the manuscript. Dr. Delatycki contributed to the design of the study and read/contributed to the manuscript. Dr. Amor contributed to the design of the study and read/contributed to the manuscript. Dr. Crino performed immunohistochemical analysis and read/contributed to the manuscript. Dr. Berkovic contributed to the conceptualization/design of the study, provided and interpreted the clinical data, and read/contributed to the manuscript. Dr. Scheffer contributed to the conceptualization/design of the study, provided and interpreted the clinical data, and read/contributed to the manuscript. Dr. Bahlo performed bioinformatic analysis, interpreted the data, and read/contributed to the manuscript. Dr. Lockhart contributed to the conceptualization/design of the study, performed molecular and bioinformatic analysis, interpreted the data, and co-wrote the manuscript. Dr. Leventer conceptualized and designed the study, provided and interpreted clinical and imaging data, and co-wrote the manuscript.

## Conflict of Interest

Dr. Bahlo reports grants from National Health and Medical Research Council of Australia Program Grant, other from Australian Research Council Fellowship, during the conduct of the study. Dr. Berkovic reports grants from

National Health and Medical Research Council, grants from NINDS, during the conduct of the study; grants from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis Jansen Cilag, outside the submitted work. In addition, Dr. Berkovic has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies. No financial return. Dr. Berkovic was a consultant to Bionomics and Athena diagnostics over 4 years ago issued, and a patent submitted by University of Melbourne for DEPDC5 testing pending. Dr. Lockhart reports grants from National Health and Medical Research Council, during the conduct of the study. Dr. Scheffer reports grants from NHMRC, grants from NIH, during the conduct of the study; other from Annals of Neurology, other from Epileptic Disorders, other from Neurology, personal fees from UCB, personal fees from Athena Diagnostics, personal fees from Transgenomics, personal fees from GlaxoSmithKline, personal fees from Biocodex, outside the submitted work. In addition, Dr. Scheffer has a patent Diagnostic and Therapeutic Methods for EFMR (Epilepsy and Mental Retardation Limited to Females) with royalties paid. All other authors declare no conflicts of interest.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** List of genes causative or potentially associated with brain malformations derived from extensive literature searches, including key search terms such as “brain malformation,” “cortical dysplasia”, and “cortical malformations.” It includes all currently known genes associated with brain malformations with potential interacting partners and associated pathway genes identified by STRING analysis.