Mutations in mTOR regulator DEPDC5 cause focal epilepsy with brain malformations

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AUTHOR CONTRIBUTIONS
I.E.S. wrote the first draft of the manuscript. I.E.S and S.F.B. oversaw collection and phenotypic analysis of families, coordinated the study and co-wrote the manuscript. S.E.H. designed and performed molecular studies and analyzed molecular data including WES data. D.E.C., B.M.R., P.T., L.L., F.B., F.P., S.F.B and I.E.S performed clinical and neuroradiological phenotyping. S.M. and A.C. carried out brain imaging analyses and interpretation. B.L.H performed molecular studies and interpreted that data. J.G. interpreted molecular data. M.G.R. carried out molecular data interpretation and co-wrote the manuscript. L.M.D. designed and oversaw the molecular genetic aspects of the study, coordinated the study and co-wrote the manuscript. All authors contributed to the editing of the manuscript.
Abstract

We recently identified DEPDC5 as the gene for familial focal epilepsy with variable foci and found mutations in >10% of small families with non-lesional focal epilepsy. Here we show that DEPDC5 mutations are associated with both lesional and non-lesional epilepsies, even within the same family. DEPDC5-associated malformations include bottom-of-the-sulcus dysplasia (3 members from 2 families), and focal band heterotopia (1 individual). DEPDC5 negatively regulates the mTOR pathway which plays a key role in cell growth. The clinicoradiological phenotypes associated with DEPDC5 mutations share features with the archetypal mTORopathy, tuberous sclerosis, raising the possibility of therapies targeted to this pathway.
Introduction

Mutations in *DEPDC5* (Dishevelled, Egl-10 and Pleckstrin Domain Containing protein 5 (DEPDC5)) were recently identified as a major cause of focal epilepsy, including the autosomal dominant disorder Familial Focal Epilepsy with Variable Foci (FFEVF)\(^1,2\). FFEVF is characterized by focal epilepsies arising in different cortical regions in different family members without apparent lesions; temporal and frontal lobe foci predominate. Mutations were found in 7/8 published large families with FFEVF and in 10/82 (12%) smaller families comprising two or more individuals with focal epilepsy\(^1\). The majority of mutations encoded premature termination codons of *DEPDC5* consistent with haploinsufficiency. Penetrance was incomplete, varying from 50-82%.

Here we report Australian family A with six affected males with focal epilepsy and a *DEPDC5* protein truncation mutation in which two individuals have frontal focal cortical dysplasia. We performed further imaging of our available patients with *DEPDC5* mutations and identified malformations in two more cases. The concept that a single gene mutation may produce non-lesional focal epilepsy in one family member and focal cortical dysplasia in another challenges previous notions of the separation of genetic malformation syndromes from non-lesional epilepsy syndromes. Whilst rare individuals with genetic malformation syndromes have no visible lesion on imaging (eg mildly affected subjects with *DCX* mutations\(^3\)), the inverse, where a proportion of subjects with a genetic epilepsy syndrome have a subtle malformation has not previously been described.
Methods

Phenotyping

The proband of family A was referred to the epilepsy clinic at Austin Health. Family B was the original Australian family in which FFEVF was described (family A1\(^1\),\(^2\)). Family C is Italian family I\(^1\).

Family members underwent electroclinical phenotyping using a validated seizure questionnaire and review of medical records and investigations\(^3\),\(^4\). Informed consent was obtained from all family members and a parent or legal guardian in the case of minors or individuals with intellectual disability. The study was approved by the Human Research Ethics Committees of Austin Health (H2007/02961) and the University of South Australia.

MRI analysis

MRI was performed for 5/6 affected males in family A, including 1.5 Tesla (T) in four and 3T imaging in one. Five members of family B had 3T MRI studies. A newly affected member of Italian family C (C:IV:1) had a 1.5T MRI study. MRI scans were reviewed systematically with evaluation of the deep grey matter and cortex including sulcal and gyral patterns, white matter signal and morphology, hippocampi, midline structures, cerebellum, brainstem and ventricles.

Whole Exome Sequencing and High Resolution Melting (HRM) Analysis

Whole exome sequencing (WES) was performed for two members of Family A. Coding sequences were enriched using the SureSelect Human All Exon 50Mb kit (Agilent Technologies, Santa Clara, CA). Following sequence capture and amplification, fragments were sequenced using the SOLiD v4 instrument (Applied Biosystems, Carlsbad, CA). Sequence reads were aligned to the human reference sequence (hg19) using BWA\(^5\). Sequence variants were reported with SAMtools and annotated using SeattleSeq (http://snp.gs.washington.edu/SeattleSeqAnnotation/). The data were filtered using SeattleSeq to remove synonymous and non-coding variants.
All available family members and controls (anonymous Australian blood donors) were tested for the \textit{DEPDC5} variant by High-Resolution Melting (HRM) analysis using the LightScanner\textsuperscript{®} (Idaho Technology, Salt Lake City, UT, USA). Sequence variants were validated by Sanger sequencing. The \textit{DEPDC5} mutations in families B and C were reported previously\textsuperscript{1}.

\textbf{Results}

\textit{Family A (Table)}

Family A comprised six affected males related through their three clinically unaffected mothers who were sisters (Figure 1A). Five had frontal lobe epilepsy (FLE) and one had unclassified focal seizures. Two were well controlled on carbamazepine and four required multiple anti-epileptic medications. Both males with malformations had refractory epilepsies. One male had intellectual disability and psychiatric problems; his brother had recurrent psychosis from 21 years.

Whole exome sequencing and mutation analysis of A:III:2 and A:III:8 identified a heterozygous nonsense mutation, c.418C>T; p.Gln140* in \textit{DEPDC5}. This mutation was present in all six affected individuals studied, their clinically unaffected mothers and grandmother. The mutation was absent in EVS and dbSNP databases. This mutation is predicted to truncate the protein early and lead to haploinsufficiency, either due to nonsense-mediated decay of the mutant transcript or to the translation of a truncated protein lacking critical functional domains.

\textit{Imaging abnormalities (Table)}

Three individuals had a bottom-of-the-sulcus-dysplasia (BOSD)\textsuperscript{5,6}. Cortical thickening was associated with loss of grey-white differentiation at the bottom of the sulcus in the right middle frontal lobe in A:III:2, the right medial superior frontal lobe in A:III:8 and in the depths of two adjacent sulci in the left superior frontal lobe in the proband B:III:3. B:III:3’s abnormality had not
been identified on 1.5T imaging despite comprehensive evaluation for possible epilepsy surgery. The remaining 4 family members of family B had normal 3T MRI and 3 affected individuals in family A had normal 1.5T MRI studies.

C:IV:1 had unilateral subtle band heterotopia within the white matter adjacent to dysplastic cortex in the left frontal lobe. Blurring of the grey-white matter junction involving part of the cingulate cortex and left frontal cortex was seen. This 6-year-old boy developed frontal lobe seizures at 4 years. Seizures were controlled on carbamazepine. He had the familial c.279+1G>A *DEPDC5* mutation\(^1\). His affected mother had a normal MRI\(^1\).

**Discussion**

We show that *DEPDC5* mutations are associated with focal cortical dysplasia in some individuals (Figure 1), while their affected relatives, who carry the same mutation, do not have a detectable structural change. This observation of lesional and non-lesional cases was found in three families with different *DEPDC5* mutations.

The malformations varied from focal cortical dysplasia to subtle band heterotopia with the predominant pattern being BOSD. BOSD is a type II focal cortical dysplasia in which the dysplastic features are maximal at the depth of a sulcus tapering to a normal gyral crown\(^6,7\). The characteristic features on MRI are thickening of cortex, blurring of grey-white junction and subcortical signal abnormality often extending to the ventricle as a transmantle sign\(^8,9,10\). The predominant pattern of BOSD is likely to represent focal cortical dysplasia type IIB although pathological confirmation is lacking\(^6\).
DEPDC5 protein is part of the GATOR1 complex, a negative regulator of the mammalian target of rapamycin complex 1 (mTORC1), in the mTOR pathway\textsuperscript{11}. mTOR is a key signalling pathway that regulates processes involved in cell growth and homeostasis in response to a range of metabolic cues\textsuperscript{12}. Rag guanosine triphosphatases (GTPases) enable mTORC1 translocation to the lysosomal surface for activation. However, under conditions of amino acid deprivation, GATOR1 inhibits mTORC1 activation by acting as a GTPase Activating Protein for Rag-GTPases\textsuperscript{11}. Activation of the mTORC1 pathway leads to increased synthesis of proteins and lipids to generate new cell membranes, increased cellular metabolism and energy production. GATOR1-mediated inactivation blocks these downstream effects.

Since DEPDC5 acts as a repressor of mTOR activity, DEPDC5 mutations are predicted to result in excessive mTOR signaling. Consistent with this, individuals with DEPDC5 mutations share similar features with patients with other mTORopathies such as tuberous sclerosis with dysplastic lesions, focal epilepsy, autism spectrum disorders and intellectual disability (Figure 2). No clinical evidence of multisystem involvement was found in individuals with DEPDC5 mutations. Although DEPDC5 mutations do not lead to tuber development per se, the concept of a two-hit hypothesis, well established in tuberous sclerosis, may also apply here\textsuperscript{13, 14}. While the DEPDC5 mutations are dominantly inherited in the patients presented here, it is likely that de novo mutations occur in other individuals. Thus a DEPDC5 mutation together with a second genetic hit might confer misregulated growth in a somatic lineage, producing a cortical malformation. This ‘second somatic hit’ may be a mutation in the other DEPDC5 allele, or in another mTOR pathway gene. De novo mutations in DEPDC5 do occur: de novo mutations were detected in family M\textsuperscript{1} and in affected individual C:III:1 (Figure 1C). The affected grandparent C:II:2 (Figure 1C) is possibly mosaic for the de novo mutation or may be a phenocopy.
DEPDC5 mutations are associated with co-morbid features including intellectual disability, autism spectrum disorders and psychiatric features. In family A, A:III:8 is the most severely affected individual with refractory NFLE, psychosis and severe intellectual disability. His affected brother had well-controlled epilepsy and recurrent psychosis necessitating hospitalisation in adult life.

Similar co-morbidities have been reported in tuberous sclerosis and other familial focal cortical dysplasia disorders such as autosomal recessive CNTNAP2 mutations in Amish families and in polyhydramnios, megalencephaly and symptomatic epilepsy (PMSE). PMSE is caused by a homozygous deletion disrupting the gene encoding the pseudokinase STRADA, an upstream inhibitor of mTORC1. Murine malformations due to loss of Strada are rescued by rapamycin inhibiting mTOR. Moreover, a rapamycin (sirolimus) trial in children with this disorder reduced seizure frequency and improved receptive language. Evaluation of rapamycin and related molecules for alleviation of seizures and neuropsychiatric comorbidities in patients with DEPDC5 mutations is now an exciting prospect in translational neuroscience.

The mechanisms by which focal epilepsies arise in DEPDC5 mutation carriers without brain lesions remain to be elucidated. The recognition of subtle dysgenesis is limited by the performance capabilities of current imaging technology. Perhaps these individuals will ultimately be shown to have similar but more subtle malformations of cortical development that correlate with the localization of their focal epilepsy. These observations emphasize the need for repeated review of imaging for subtle abnormalities that may not be immediately appreciated and may render a patient suitable for epilepsy surgery. Our findings provide further evidence for the placement of DEPDC5 focal epilepsies in the group of mTORopathies and reinforce that this pathway may be more crucial to the common group of focal epilepsies than previously appreciated.
<table>
<thead>
<tr>
<th>Ped Ref</th>
<th>Age</th>
<th>Gender</th>
<th>Syndrome</th>
<th>Seizure onset</th>
<th>Seizure offset</th>
<th>TCS (Y/N)</th>
<th>EEG findings</th>
<th>MRI findings</th>
<th>AED response</th>
<th>Co-morbidities</th>
<th>Pub</th>
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<tr>
<td>A:III:2</td>
<td>32, M</td>
<td>M</td>
<td>FLE</td>
<td>7</td>
<td>Ongoing</td>
<td>Y</td>
<td>NK</td>
<td>Cortical thickening and loss of grey-white differentiation at the bottom of the sulcus in the right middle frontal lobe</td>
<td>Multiple, CBZ good</td>
<td>No</td>
<td>-</td>
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<tr>
<td>A:III:3</td>
<td>26, M</td>
<td>M</td>
<td>Unclassified</td>
<td>12</td>
<td>14</td>
<td>Y</td>
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<td>ND</td>
<td>Controlled with Migraine</td>
<td>CBZ</td>
<td>-</td>
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<td>A:III:5</td>
<td>24, M</td>
<td>M</td>
<td>FLE</td>
<td>7</td>
<td>22</td>
<td>N</td>
<td>Right frontal epileptiform</td>
<td>Normal</td>
<td>Controlled with No</td>
<td>VPA, LTG, VGB</td>
<td>-</td>
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<td>A:III:6</td>
<td>20, M</td>
<td>M</td>
<td>FLE</td>
<td>15</td>
<td>18</td>
<td>N</td>
<td>Left frontal epileptiform</td>
<td>Normal</td>
<td>Controlled with No</td>
<td>LTG, VPA</td>
<td>-</td>
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<tr>
<td>A:III:7</td>
<td>27, M</td>
<td>M</td>
<td>FLE</td>
<td>10</td>
<td>Ongoing</td>
<td>Y</td>
<td>Bilateral anterior quadrant epileptiform discharges, R&gt;L</td>
<td>Normal</td>
<td>Controlled with No</td>
<td>Psychosis</td>
<td>-</td>
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<td>A:III:8</td>
<td>23, M</td>
<td>M</td>
<td>FLE</td>
<td>1</td>
<td>Ongoing</td>
<td>Y</td>
<td>Right frontal epileptiform</td>
<td>Cortical thickening and loss of grey-white differentiation at the bottom of an abnormal sulcus in the right frontal lobe</td>
<td>Refractory to CBZ, GBP, LTG, VPA</td>
<td>Severe ID, ASD, psychosis</td>
<td>-</td>
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<td>Ped Ref</td>
<td>Age</td>
<td>Sex</td>
<td>Condition</td>
<td>Duration</td>
<td>Status</td>
<td>Finding</td>
<td>Controlled with</td>
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<tr>
<td>B:III:3</td>
<td>25, F</td>
<td>FLE</td>
<td>Left frontal epileptiform</td>
<td>19</td>
<td>N</td>
<td>Cortical thickening and loss of grey-white differentiation involving the depths of two adjacent abnormal sulci in the left superior frontal lobe.</td>
<td>CBZ, CLB, LTG, PHT</td>
<td>No</td>
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<tr>
<td>C:IV:1</td>
<td>6, M</td>
<td>FLE</td>
<td>Bilateral posterior slowing L&gt;R in drowsiness, not epileptiform</td>
<td>Ongoing</td>
<td>Y</td>
<td>Blurring of the grey-white differentiation involving part of the cingulate cortex and left frontal cortex. Subtle band heterotopia within the white matter adjacent to the dysplastic cortex in the left frontal lobe.</td>
<td>CBZ</td>
<td>No</td>
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</table>

AED= anti-epileptic drug, ASD= Autism spectrum disorder, CBZ= Carbamazepine, CLB= Clobazam, EEG= electroencephalograph, F= female, FCD= focal cortical dysplasia, FLE= Frontal Lobe Epilepsy, GBP= Gabapentin, ID= intellectual disability, LTG= Lamotrigine, M= male, MRI= magnetic resonance imaging, ND= not done, NK= not known, Ped Ref = pedigree reference, PHT= Phenytoin, Pub = previous publication, TCS= Tonic-clonic seizure, VGB= Vigabatrin, VPA= Sodium Valproate.
Legends

**Figure 1** - Pedigrees of families and MRI showing malformations

Individuals who have a *DEPDC5* mutation are denoted by m/+ and those negative for mutations are denoted by +/+. Images are from the individuals with a black circle. **(A) Pedigree of Australian Family A, *DEPDC5* c.418C>T (p.Gln140*).** Individual A:III:2 (bottom left): Coronal T1 image showing cortical thickening and loss of grey-white differentiation at the bottom of a sulcus in the right middle frontal lobe. Individual A:III:8 (bottom right): Coronal T1 image showing cortical thickening and loss of grey-white differentiation at the bottom of an abnormal sulcus in the right medial superior frontal lobe. **(B) Pedigree of Australian Family B, *DEPDC5* c.21C>G (p.Tyr7*).** Individual B:III:3 sagittal (left) and axial T1 (right) images show cortical thickening and loss of grey-white differentiation involving the depths of two adjacent abnormal sulci in the left superior frontal lobe. **(C) Pedigree of Italian Family C, *DEPDC5* c.279+1G>A.** Individual C:IV:1 axial T1 image (upper) shows blurring of grey-white differentiation involving part of the cingulate cortex and left frontal cortex. Coronal T1 image (lower) shows subtle band heterotopia in the white matter adjacent to dysplastic cortex in the left frontal lobe.

**Figure 2** - *DEPDC5* mutations and tuberous sclerosis show overlapping phenotypes and imaging abnormalities associated with activation of the mTOR pathway (modified from 11).
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References


Family A DEPDC5 c.418C>T (p.Gln140*)

Family B DEPDC5 c.210C>G (p.Tyr70*)

Family C DEPDC5 c.279+1 G>A

171x233mm (300 x 300 DPI)
**DEPDC5 phenotypes**
- Focal epilepsy
- Onset usually childhood - adolescence
- Epilepsy usually mild
- Intellectual disability rare
- Autistic spectrum disorders rare
- Familial or de novo mutations
- Variable penetrance
- Dysplastic lesions in some

**Tuberous Sclerosis**
- Focal epilepsy
- Onset usually infancy - childhood
- Epilepsy often severe
- Intellectual disability common
- Autistic spectrum disorders common
- Familial or de novo mutations
- Variable penetrance
- Multiple tubers with type IIb dysplasia

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250x170mm (300 x 300 DPI)
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