ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

BRIEF COMMUNICATION

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

Thomas Scerri[1], Jessica R. Riseley[2], Greta Gillies[2], Kate Pope[2], Rosemary Burgess[3,4], Simone A. Mandelstam[3,5,6], Leanne Dibbens[7,8], Chung W. Chow[9,10], Wirginia Maixner[11,12], Anthony Simon Harvey[3,9,12,13], Graeme D. Jackson[3,4], David J. Amor[2,9], Martin B. Delatycki[2,9,14], Peter B. Crino[15], Samuel F. Berkovic[3,4], Ingrid E. Scheffer[3,4,9,13], Melanie Bahlo[1], Paul J. Lockhart[2,9] & Richard J. Leventer[9,12,13]

[1]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria, Australia
[2]Bruce Lefroy Centre for Genetic Health Research, Murdoch Childrens Research Institute, Parkville, Victoria, Australia
[3]The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia
[4]Epilepsy Research Centre, University of Melbourne, Austin Health, Melbourne, Australia
[5]Department of Radiology, Royal Children's Hospital, Melbourne, Australia
[6]Department of Radiology, University of Melbourne, Melbourne, Australia
[7]Epilepsy Research Program, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia
[8]Sansom Institute for Health Research, University of South Australia, Adelaide, Australia
[9]Department of Pediatrics, University of Melbourne, Melbourne, Australia
[10]Department of Anatomical Pathology, Royal Children's Hospital, Melbourne, Australia
[11]Department of Neurosurgery, Royal Children's Hospital, Melbourne, Australia
[12]Murdoch Childrens Research Institute, Melbourne, Australia
[13]Department of Neurology, Royal Children's Hospital, Melbourne, Australia
[14]Clinical Genetics, Austin Health, Melbourne, Australia
[15]Shriners Hospital Pediatric Research Center, Temple University, Philadelphia, Pennsylvania

Correspondence
Richard J. Leventer, Department of Neurology, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia. Tel: +61393455661; Fax: +61393455977; E-mail: richard.leventer@rch.org.au

Funding Information
This work has been supported by the Victorian Government's Operational Infrastructure Support Program and Australian Government NHMRC IRIISS. Funding was provided by the National Health and Medical Research Council of Australia, the Murdoch Childrens Research Institute and the Campbell Edwards Trust. M. B. is supported by an ARC Future Fellowship (FT100100764), and P. J. L. is supported by an NHMRC Career Development Fellowship (APP1032364).

Received: 9 February 2015; Accepted: 9 February 2015

Annals of Clinical and Translational Neurology **2015; 2(5): 575–580**

doi: 10.1002/acn3.191

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).[1] Most cases of FCD are sporadic; however, rare familial cases are described.[2,3]

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.[4–6] DEPDC5 is a component of the GATOR1 complex, a critical negative regulator of the mammalian target of rapamycin (mTOR) pathway.[7] Germline heterozygous mutations in *DEPDC5* have been associated with lesional epilepsies including BOSD type FCD.[3] Notably, there was considerable intrafamilial 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,[8,9] 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.[2] 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.[1] Resected brain tissue was assessed for mTOR activity by phospho-S6 ribosomal protein antibody staining as described previously.[10] 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[11] (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). Local re-alignment was performed with Genome Analysis Toolkit (GATK; v.5-2)[12] and variant detection and annotation utilized GATK's Unified Genotyper (v3.0-0) and ANNOVAR (version dated 2013-05-20).[13] 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[14] (v5.1.1) or Polyphen-2[15] (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.[2] 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

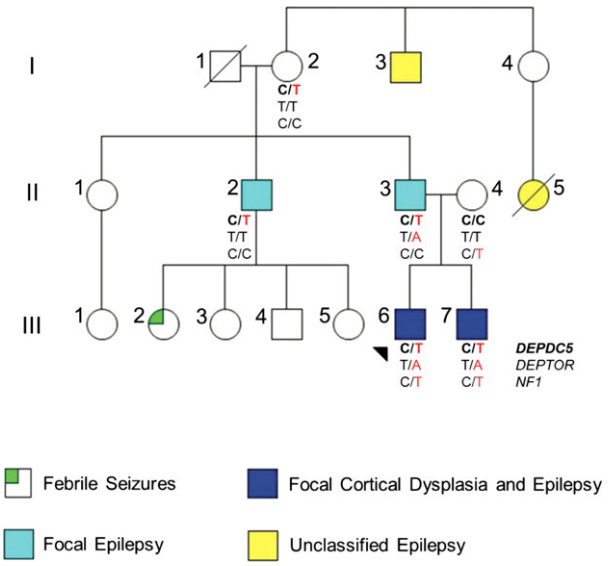

Figure 1. Pedigree structure and genotyping. Pedigree showing the epilepsy phenotypes and the genotypes for the variants identified in *DEPDC5*, *DEPTOR*, and *NF1*.

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.[4] 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.[3,16–18] Mutations in *DEPDC5*, a negative regulator of mTOR activity, cause focal epilepsy with or without a cortical malformation visible on MRI.[3,4,8,9] 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.[7] 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.[7] 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 quadrantic in the other. The Dutch family reported to have focal epilepsy and an identical mutation in *DEPDC5* all had normal brain MRI.[4] 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.[19] Similarly, while mutations in *NF1* predispose individuals to neurofibromatosis type I, NF-1 can potentially dysregulate DEPTOR activity via a cascade of interactions that

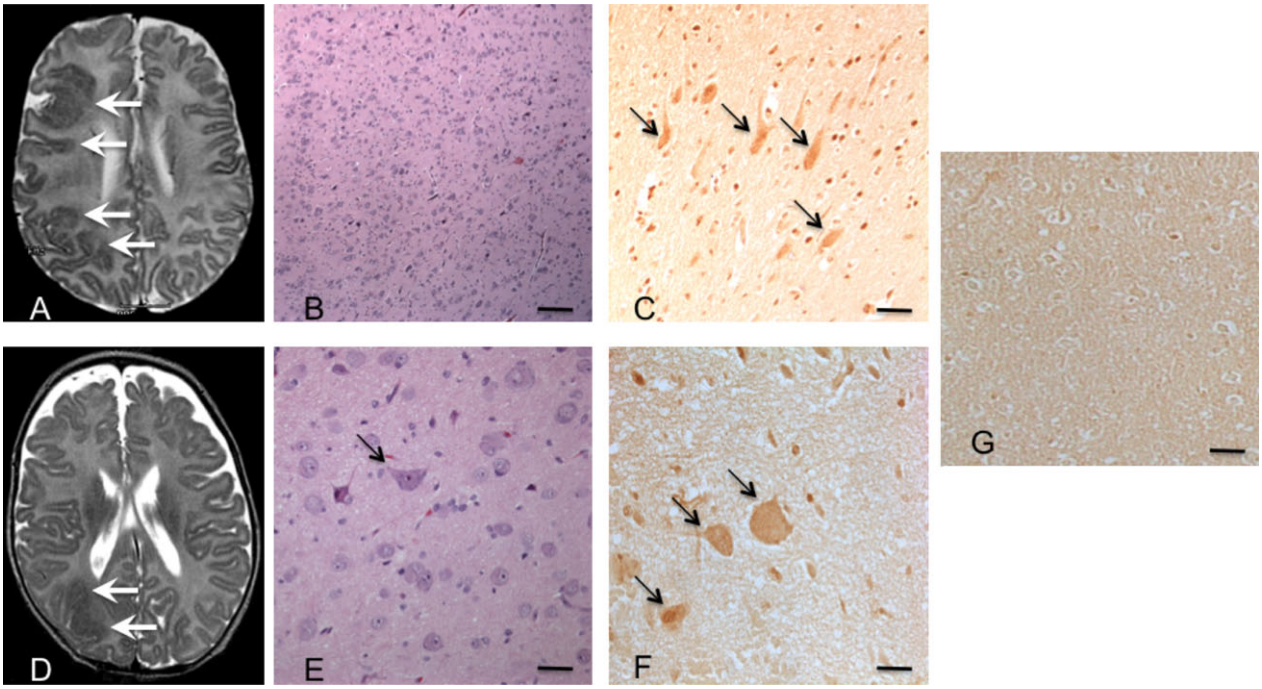

Figure 2. Brain MRI, histopathology, and phospho-S6 immunostaining. Top row is patient III:6 and bottom row is patient III:7. (A and D) are axial T2-weighted MRIs at age 6 and 14 weeks, respectively, showing multiple areas of cortical thickening and blurring of the gray white junction often maximal at 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 μm; F, 80 μm; C, E, and G, 200 μm).

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

Chr	Position	Ref allele	Alt allele	Gene	Transcript	Exon	Coding change	Protein change	Damaging?[1] 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.
[1]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).[20] 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.

Acknowledgments

We thank the family for participating in this study. We are grateful for the generous support of the Lefroy and Handbury families. This work has been supported by the Victorian Government's Operational Infrastructure Support Program and Australian Government NHMRC

IRIISS. Funding was provided by the National Health and Medical Research Council of Australia, the Murdoch Childrens Research Institute and the Campbell Edwards Trust. M. B. is supported by an ARC Future Fellowship (FT100100764), and P. J. L. is supported by an NHMRC Career Development Fellowship (APP1032364).

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.

References

1. Blumcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. Epilepsia 2011;52:158–174.
2. Leventer RJ, Jansen FE, Mandelstam SA, et al. Is focal cortical dysplasia sporadic? Family evidence for genetic susceptibility Epilepsia 2014;55:e22–e26.
3. Scheffer IE, Heron SE, Regan BM, et al. Mutations in mammalian target of rapamycin regulator DEPDC5 cause focal epilepsy with brain malformations. Ann Neurol 2014;75:782–787.
4. Dibbens LM, de Vries B, Donatello S, et al. Mutations in DEPDC5 cause familial focal epilepsy with variable foci. Nat Genet 2013;45:546–551.
5. Ishida S, Picard F, Rudolf G, et al. Mutations of DEPDC5 cause autosomal dominant focal epilepsies. Nat Genet 2013;45:552–555.
6. Martin C, Meloche C, Rioux MF, et al. A recurrent mutation in DEPDC5 predisposes to focal epilepsies in the French-Canadian population. Clin Genet 2014;86:570–574.
7. Bar-Peled L, Chantranupong L, Cherniack AD, et al. A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 2013;340:1100–1106.
8. D'Gama AM, Geng Y, Couto JA, et al. mTOR pathway mutations cause hemimegalencephaly and focal cortical

dysplasia. Ann Neurol 2015; In Press. doi: 10.1002/ana.24357
9. Baulac S, Ishida S, Marsan E, et al. Familial focal epilepsy with focal cortical dysplasia due to DEPDC5 mutations. Ann Neurol 2015; In Press. doi: 10.1002/ana.24368
10. Baybis M, Yu J, Lee A, et al. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. Ann Neurol 2004;56:478–487.
11. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002;30:97–101.
12. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–1303.
13. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
14. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4:1073–1081.
15. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–249.
16. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. Nat Genet 2012;44:941–945.
17. Poduri A, Evrony GD, Cai X, et al. Somatic activation of AKT3 causes hemispheric developmental brain malformations. Neuron 2012;74:41–48.
18. Riviere JB, Mirzaa GM, O'Roak BJ, et al. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 2012;44:934–940.
19. Peterson TR, Laplante M, Thoreen CC, et al. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell 2009;137:873–886.
20. Brundage ME, Tandon P, Eaves DW, et al. MAF mediates crosstalk between Ras-MAPK and mTOR signaling in NF1. Oncogene 2014;33:5626–5636.

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.

