Partial deletion of DEPDC5 in a child with focal epilepsy

* Maria Clara Bonaglia, † Roberto Giorda, ‡ Roberta Epifanio, *Sara Bertuzzo, § Susan Marelli, ¶ Marion Gerard, ¶§ Joris Andrieux, ‡ Nicoletta Zanotta, and ‡ Claudio Zucca

Epilepsia Open, 1(3-4):140–144, 2016
doi: 10.1002/epi4.12012

**Summary**

We report on a child, aged 47/12 years, with borderline intelligence quotient, normal brain magnetic resonance imaging, and focal epilepsy. The polysomnographic electroencephalogram recording revealed asynchronous central spikes at both brain hemispheres resembling the features observed in focal idiopathic epileptic syndromes. Array comparative genomic hybridization analysis revealed a 32-kb partial deletion of the DEP domain-containing protein 5 (DEPDC5) gene, involved in a wide spectrum of inherited focal epileptic syndromes. The parental origin of the deletion could not be fully ascertained because the pregnancy had been achieved through anonymous egg donation and insemination by intracytoplasmic sperm injection. However, we demonstrate that the deletion, shared by all alternatively spliced isoforms of DEPDC5, produces a transcript presumably generating a DEPDC5 protein missing the entire DEP domain. Our findings suggest that partial deletion of DEPDC5 may be sufficient to cause the focal epilepsy in our patient, highlighting the importance of the DEP domain in DEPDC5 function. This study expands the phenotypic spectrum of DEPDC5 to sporadic forms of focal idiopathic epilepsy and underscores the fact that partial deletions, albeit probably very rare, are part of the genetic spectrum of DEPDC5 mutations.

**Key Words:** Array comparative genomic hybridization, Seizures, Copy number loss, Polysomnography EEG.

The DEP domain-containing protein 5 (DEPDC5) gene is a component of the GATOR1 complex, a critical negative regulator of the mammalian target of rapamycin (mTOR) pathway.\(^1\) DEPDC5 loss-of-function mutations have recently been identified in a broad spectrum of epileptic syndromes with different brain localization and electroclinical expression, including autosomal dominant focal epilepsies, such as autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE; MIM 600513),\(^2,3\) familial temporal lobe epilepsy (FTLE; MIM 600512),\(^4\) and familial focal epilepsy with variable foci (FFEVF; MIM 604364),\(^5\) the last of which includes individuals with both frontal and temporal epilepsy.\(^2,5\) Rolandic epilepsy (OMIM 25570), albeit rare, and other nonlesional focal childhood epilepsies are also among the DEPDC5-related epileptic syndromes.\(^6\) Patients with DEPDC5 mutations show phenotypes ranging from benign to refractory epilepsy and may present mild intellectual disability, epileptic spasms,\(^7\) and various brain malformations connecting focal epilepsy with focal cortical dysplasia.\(^8-10\)

To date, de novo DEPDC5 mutations have been reported in one sporadic case with focal epilepsy\(^5\) and, more recently, in two patients with epileptic spasms.\(^7\) The majority of the DEPDC5 mutations reported so far generate premature termination codons, suggesting that DEPDC5-related epilepsy most likely results from an haploinsufficiency...
Based on the observed reduced penetrance and variable expression of the phenotype, the participation of other modifier genes or the presence of a “second hit” influencing the clinical manifestation has been postulated.

Here, we describe and discuss the causative role of the first 32-kb partial deletion of DEPDC5, including the protein’s DEP domain, detected by whole-genome array comparative genomic hybridization (aCGH) in a 4-year-old child with nonlesional focal epilepsy, borderline intelligence quotient (IQ), mild dyspraxia, and language delay.

**METHODS**

DNA was prepared from peripheral blood, obtained after informed consent, using standard procedures. aCGH analysis was performed using the 180K Agilent kit (Agilent Technologies) according to the manufacturer’s protocol. Data analysis was performed using Agilent Cytogenomics Ed 2.5.8.1. Base positions refer to the UCSC Genome Browser Feb 2009 assembly, hg19. Quantitative polymerase chain reaction (qPCR) assays were performed on DNA from the patient and his parents using SYBR Green and were analyzed on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Long-range (LR) PCR amplification and Sanger sequencing of the deletion breakpoints’ junction were performed with standard protocols. Total RNA extraction was performed on peripheral blood lymphocytes (PBLs) and the Epstein-Barr virus (EBV) line of the patient using the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was quantified by a NanoPhotometer Pearl (Implen, München, Germany). cDNA synthesis of the transcript from the deleted DEPDC5 allele was achieved using the M-MLV Reverse Transcriptase Kit (Promega, Madison, WI) followed by PCR amplification and Sanger sequencing with standard protocols. All primer sequences are available from the authors.

**RESULTS**

**Clinical case**

The male child, aged 4 7/12 years, was born to healthy nonconsanguineous parents aged 44 (father) and 47 (mother) years. Family history was negative for febrile seizures and epilepsy but significant for migraine in the paternal lineage. Since the age of 8 years, the father suffered from migraine with visual aura associated with hemiparesis on the right side.

The couple underwent in vitro fertilization, achieved via egg donation and insemination by intracytoplasmic sperm injection (ICSI), leading to the conception of the child reported here.

Delivery, following an unremarkable 36-week gestation, was normal with a birth weight of 2.550 kg (25th), length of 48 cm (25–30th), and head circumference of 33 cm (25–50th). Apgar scores were 10/10 at 1 min, respectively.

Early perinatal period was unremarkable, apart from mild hyperbilirubinemia treated with 24 h of phototherapy. Mild psychomotor development delay was noted: the infant walked at the age of 18 months and did not utter single words until he was 2 years old. At 19 months, he experienced his first short-lasting (60 s) epileptic seizures, characterized by staring with drooling and paroxysmal hypotonia on the right side of the body, sometimes with falls. These episodes had a frequency from one to three times weekly. At this time, his electroencephalogram (EEG) recording showed epileptiform abnormalities on the left central regions with a slight diffusion (Fig. 1A). Neurologic examination revealed mild dyspraxia and language delay. Psychometric tests (Griffiths) documented borderline intellectual disability (overall IQ: 83). Metabolic screening tests and brain magnetic resonance imaging (MRI) were normal.

Figure 1. (A) EEG polygraph recording during drowsiness at 2 years of age showed well-organized background activity and epileptiform abnormalities over the left central regions with a slight diffusion. (B) EEG polygraph recording during sleep at the age of 4 7/12 years showed epileptiform abnormalities over bilateral central areas, asynchronous, with a prevalence over the right hemisphere. Background activity is well organized during both wakefulness and sleep.

Epilepsia Open © ILAE
therapy with sodium valproate (VPA) was started at the age of 21\(^{1/2}\) years, achieving a reduction of seizure frequency and duration, but not a completely seizure-free period. The VPA dosage was progressively increased to 800 mg/day (42 mg/kg). VPA plasma level was 108 \(\mu\)g/ml.

At the present age of 47\(^{1/2}\) years, a polysomnographic EEG recording showed for the first time epileptiform abnormalities during sleep over bilateral central areas, asynchronous, with a prevalence over the right hemisphere (Fig. 1B). Background activity was well organized both during wakefulness and sleep. The patient receives a dose of 32 mg/kg of VPA. VPA plasma level is 94.5 \(\mu\)g/ml.

Stature and weight are respectively at the 90th and >97th percentile; head circumference is at the 25–50th percentile. The patient shows minor facial dimorphisms, including flat malar region, epicanthic folds, narrow palpebral fissures, long eyelashes, flat nasal bridge, asymmetric small ears with prominent helices and abnormal antihelices, short philtrum, and widely spaced deciduous teeth. His level of developmental functioning (cognitive, motor, and language) does not show any decline, and no seizures have been observed during the last 3 months.

DNA molecular analysis

Array CGH analysis, requested as a first-tier diagnostic protocol, revealed a copy number loss of ~32 kb at 22q12.3 with breakpoints at 32,229,940–32,239,190 Mb and 32,271,611–32,283,790 Mb. The deletion involved a portion of DEPDC5 (Figs. 2A, B). Quantitative PCR

Figure 2.
(A) detail of the aCGH profile of chromosome 22 showing an interstitial deletion at 22q12.3 (orange box); genes included in the deleted region are listed on the bottom. (B) Schematic representation of our patient’s deletion (orange box); RefSeq genes, including known DEPDC5 isoforms, are shown (GRCh37/hg19). (C) Genomic location and sequence of the 22q12.3 deletion breakpoint junction (GRCh37/hg19). (D) Agarose gel analysis of the portion of the DEPDC5 transcript spanning exons 27–39 in PBL and EBV samples from the proband (P), control PBL and EBV samples (C), and water control (N); the fragment amplified only in the proband because the full-length transcript is too large to be amplified with our protocol. A portion of the human PGK1 transcript including exons 1–3 was amplified as a control. GeneRuler 1 Kb DNA ladder (Thermo Fisher) was used as a molecular weight marker (mwm). (E) Detail of the in-frame junction between exons 27 and 39 of DEPDC5 showing the nucleotide sequence of the transcript as well as the amino acid sequence of the deleted protein. 

Epilepsia Open © ILAE
performed on the patient and his father demonstrated that the 22q12.3 deletion was not inherited from the father.

The breakpoint location characterized by aCGH was further refined by qPCR, amplified by long-range PCR (LR-PCR), and sequenced. The position and sequence of the breakpoint junction, shown in Fig. 2C, are consistent with a microhomology-mediated end joining (MMEJH) mechanism of repair and lead to the deletion of exons 28–38 (transcript variant 4, uc011alu.2) of DEPDC5.

The analysis of the DEPDC5 gene structure and the location of the deletion suggested the possibility of a splicing between exons 27 and 39 leading to an in frame transcript (Figs. 2D, E). Therefore, we amplified cDNA synthesized from the patient’s PBLs and EBV line with primers on exons 26/27 and 39 of the DEPDC5 transcript and obtained a patient-specific fragment of 227 bp containing a perfect junction between exons 27 and 39 (Figs. 2D, E).

**DISCUSSION**

Herein, we have identified by aCGH analysis a 32-kb deletion encompassing exons 28–38 of DEPDC5 in a patient with cryptogenic focal epilepsy associated with borderline IQ, mild apraxia, and speech delay. DEPDC5 mutations are a common genetic cause of several autosomal dominant focal epileptic syndromes among which FFEEVF, characterized by seizures originating in different brain areas among different members of the same family and an onset range from infancy to adulthood, is the most common.2,5

Because of his borderline IQ, good response to antiepileptic drug therapy, absence of brain abnormalities, and sporadic occurrence of critical focal motor phenomena, the child was classified as affected by cryptogenic focal epilepsy with central left spikes. Only at the last evaluation did the patient’s polysomnographic EEG recording reveal asynchronous epileptiform abnormalities with a prevalence over the central regions of both hemispheres (Fig. 1B) resembling the features observed in focal idiopathic epileptic syndromes.6 In our patient, a decrease in the frequency of critical focal phenomena has been successfully obtained with antiepileptic drug monotherapy. At present, the child has been fully seizure-free for two and half months, and no regression of his cognitive functions has been observed.

Whole-genome array was requested as the first genetic test in the presence of cryptogenic focal epilepsy, as suggested by previous studies.12,13 The copy number loss we detected by aCGH within DEPDC5 can be classified as pathogenic owing to the major role of this gene in various epileptic syndromes.3,11 We could not establish inheritance and parental origin of the deletion because the pregnancy was achieved through egg donation from an anonymous donor inseminated by ICSI. Therefore, we cannot exclude that the rearrangement might be a consequence of the in vitro fertilization (IVF) procedure itself. Indeed, embryos from IVF patients inseminated by ICSI exhibit high rates of aneuploidies and de novo structural chromosome aberrations, which are not restricted to arrested or poorly developing embryos but are also common in good-quality IVF embryos.14,15

We were able to determine the deletion’s boundaries and demonstrate that a transcript missing exons 28–38, presumably generating a DEPDC5 protein missing aa 840–1344, and therefore the entire DEP domain, is present in the patient’s PBLs and EBV-transformed lymphoblastoid line. The majority of DEPDC5 mutations discovered so far generate premature termination codons, suggesting that haploinsufficiency could be the main mechanism underlying the pathogenesis of this epileptic syndrome with or without developmental brain cortical malformations.11 Very recently, a Depdc5 knockout rat model was created to test the loss-of-function hypothesis.16 Lack of one copy of Depdc5 in the rat results in several neuropathological abnormalities, including neuronal migration defects and alteration of electrophysiological properties, resembling the rodent models of mTORopathies, as well as sporadic human focal epilepsies and focal cortical dysplasia (FCD) type II.16

On the other hand, the Database of Genomic Variation and Phenotype in Humans Using Ensembl Resources (DECIPHER) case 269955, with a deletion encompassing DEPDC5 and 14 other genes, has nonsyndromic mental retardation, but no epilepsy, whereas his carrier father is totally asymptomatic.

The heterozygous microdeletion of DEPDC5, encompassing all of the gene’s alternatively spliced isoforms and generating protein products missing the DEP domain (Figs. 2B, D, E), may be sufficient to activate mTORC1 signaling and consequently cause the focal epilepsy observed in our patient, highlighting the importance of the DEP domain in DEPDC5 function. Some members of families with focal epilepsy and missense mutations within the DUF3 domain (Family D from Carvill et al.) or truncating mutations close to the amino terminus of the protein (Family A from Scheffer et al.,5 Families A1 and I from Dibbens et al.) showed a variety of cortical lesions, whereas most patients with mutations downstream of the DUF domain did not. We can speculate that the DEP domain may have a role in epileptogenesis, and impairment of the DUF360 domain may contribute to the additional brain malformations. Additional patients with partial loss of DEPDC5 and animal models of Depdc5 partial loss will help to elucidate the role of this gene in focal epilepsy, both sporadic and familial. Our study also suggests that patients with familial focal epilepsy negative at DNA sequencing analysis should be screened by aCGH or a specific DEPDC5 multiplex ligation-dependent probe amplification (MLPA) test to discover possible partial microdeletions, which may be overlooked by whole-exome or targeted massive parallel DNA sequencing.

Epilepsia Open, 1(3-4):140–144, 2016
doi: 10.1002/epi4.12012
In conclusion, our findings:

1. Suggest that partial deletion of DEPDC5 may be sufficient to activate mTORC1 signaling and consequently cause the focal epilepsy in our patient, highlighting the importance of the DEP domain in DEPDC5 function.

2. Confirm and expand the role of DEPDC5 mutations in a wide spectrum of focal epileptic syndromes, including sporadic forms of focal idiopathic epilepsy, and highlight the fact that partial deletions, albeit probably very rare, belong to the genetic spectrum of DEPDC5 mutations.

ACKNOWLEDGMENTS

We are grateful to the family for participating in this study.

This study was supported by a grant of the Italian Ministry of Health (RC 2015) to M.C.B. and to C.Z. (RC 2012). This study makes use of data generated by the DECIPHER community. A full list of centers that contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via e-mail from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust.

DISCLOSURE

We declare that none of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

1. Bar-Peled L, Chantarupong 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.

2. Ishida S, Picard F, Rudolf G, et al. Mutations of DEPDC5 cause autosomal dominant focal epilepsies. Nat Genet 2013;45:552–555.

3. Picard F, Makrythanasis P, Navarro V, et al. DEPDC5 mutations in families presenting as autosomal dominant nocturnal frontal lobe epilepsy. Neurology 2014;82:2101–2106.

4. Striano P, Serioli E, Santulli L, et al. DEPDC5 mutations are not a frequent cause of familial temporal lobe epilepsy. Epilepsia 2015;56: e168–e171.

5. 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.

6. Lal D, Reithlater EM, Schubert J, et al. DEPDC5 mutations in genetic focal epilepsies of childhood. Ann Neurol 2014;75:788–792.

7. Carvill GL, Crompton DE, Regan BM, et al. Epileptic spasms are a feature of DEPDC5 mTORopathy. Neurol Genet 2015;1:e17.

8. 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.

9. Baulac S, Ishida S, Marsan E, et al. Familial focal epilepsy with focal cortical dysplasia due to DEPDC5 mutations. Ann Neurol 2015;77:675–683.

10. Scerri T, Riseley JR, Gillies G, et al. Familial cortical dysplasia type IIA caused by a germline mutation in DEPDC5. Ann Clin Transl Neurol 2015;2:575–580.

11. Baulac S. Genetics advances in autosomal dominant focal epilepsies: focus on DEPDC5. Prog Brain Res 2014;213:123–139.

12. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. PLoS Genet 2010;6:e1000962.

13. Olson H, Shen Y, Avallone J, et al. Copy number variation plays an important role in clinical epilepsy. Ann Neurol 2014;75:943–958.

14. Voet T, Vanneste E, Vermeesch JR. The human cleavage stage embryo is a cradle of chromosomal rearrangements. Cytogenet Genome Res 2011;133:160–168.

15. Mertzaniidou A, Wilton L, Cheng J, et al. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. Hum Reprod 2013;28:256–264.

16. Marsan E, Ishida S, Schramm A, et al. Depdc5 knockout rat: a novel model of mTORopathy. Neurobiol Dis 2016;89:180–189.