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SHORT RESEARCH ARTICLE

Partial deletion of *DEPDC5* in a child with focal epilepsy

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Summary

We report on a child, aged $4^{7/12}$ 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 *DEPCD5*-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 *DEPCD5* mutations reported so far generate premature termination codons, suggesting that *DEPDC5*-related epilepsy most likely results from an haploinsufficiency mechanism.[2,3,11] Based on the observed reduced penetrance and

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variable expression of the phenotype, the participation of other modifier genes[8] or the presence of a "second hit"[8,9] 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 hemiparaesthesia 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′/5′, respectively.

Early perinatal period was uncomplicated, 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. A

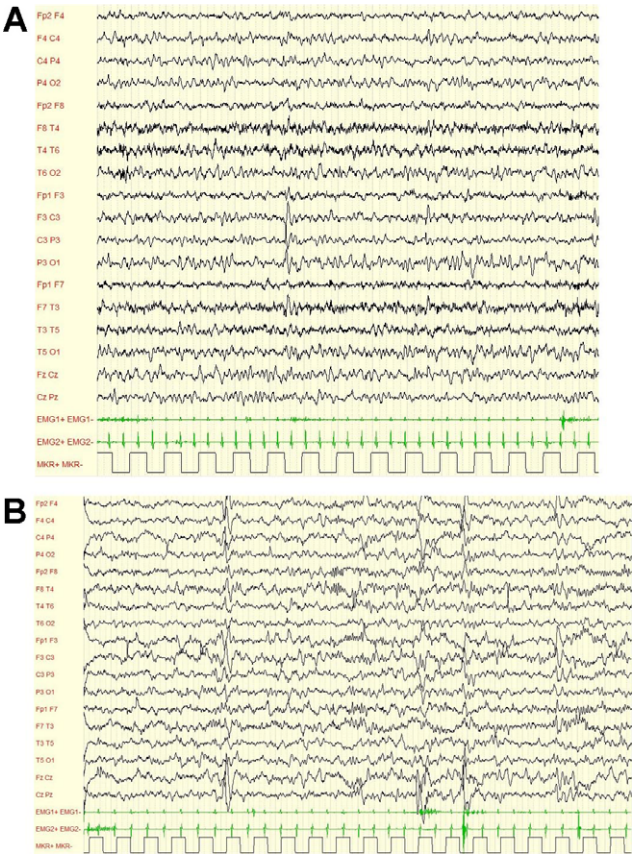

Figure 1.
(**A**) EEG polygraph recording during drowsiness at 2 years of age showed well-organized background activity and epileptiform abnormalities over the left central regions with a slight diffusion. (**B**) EEG polygraph recording during sleep at the age of $4^{7/12}$ years showed epileptiform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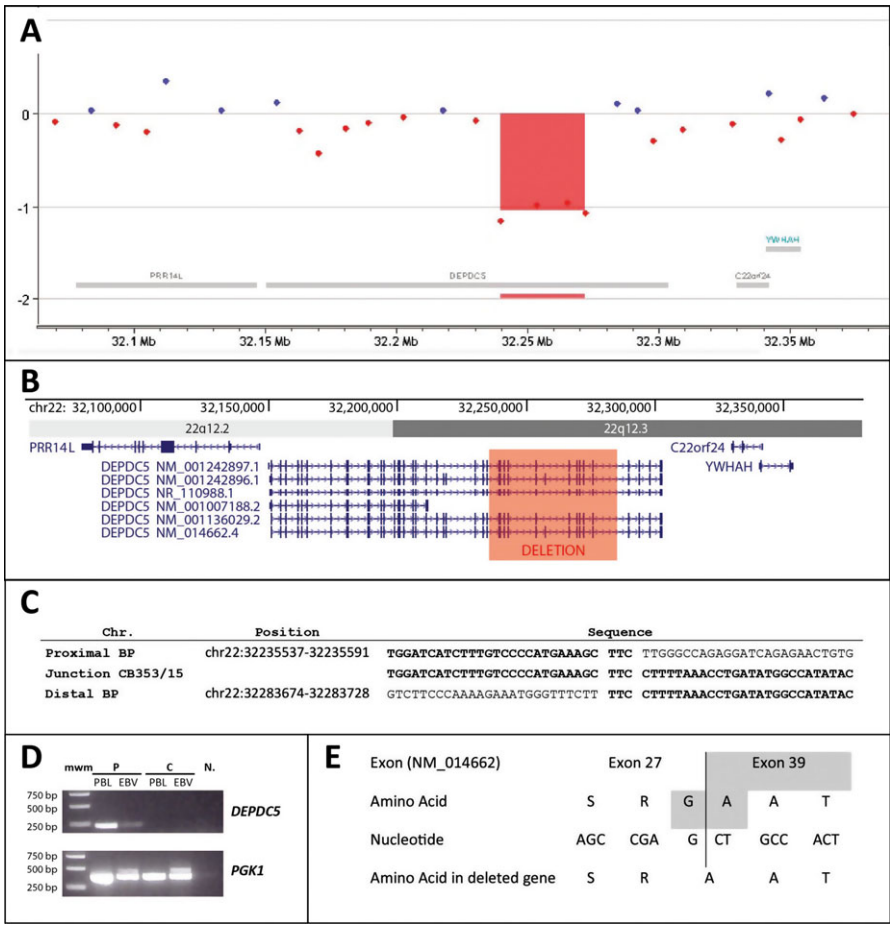

Figure 2.

(**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.

therapy with sodium valproate (VPA) was started at the age of $2^{1/12}$ 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 μg/ml.

At the present age of $4^{7/12}$ 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 μg/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

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 FFEVF, 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.[7]) or truncating mutations close to the amino terminus of the protein (Family A from Scheffer et al.;[8] Families A1 and I from Dibbens et al.[5]) 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 DUF3608 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.

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.

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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.

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