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## **Familial focal epilepsy with focal cortical dysplasia due to *DEPDC5* mutations**

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### **Running head: FCD and DEPDC5 mutations**

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## ABSTRACT

**Objective:** The *DEPDC5* (DEP domain-containing protein 5) gene, encoding a repressor of the mTORC1 signaling pathway, has recently emerged as a major gene mutated in familial focal epilepsies. We aimed to further extend the role of *DEPDC5* to focal cortical dysplasias (FCDs).

**Methods:** Seven patients from four families with *DEPDC5* mutations, and focal epilepsy associated with FCD were recruited and investigated at the clinical, neuroimaging and histopathological levels. The *DEPDC5* gene was sequenced from genomic blood and brain DNA.

**Results:** All patients had drug-resistant focal epilepsy, five of them underwent surgery, and one a brain biopsy. Electroclinical phenotypes were compatible with FCD II, although MRI was typical in only four cases. Histopathology confirmed FCD IIa in two patients (including one MRI-negative case) and showed FCD I in two other patients, and remained inconclusive in the last two. Three patients were seizure-free post-surgically and one had a worthwhile improvement. Sequencing of blood DNA revealed truncating *DEPDC5* mutations in all four families; one mutation was found to be mosaic in an asymptomatic father. A brain somatic *DEPDC5* mutation was identified in one patient in addition to the germline mutation.

**Interpretation:** Germline, germline mosaic and brain somatic *DEPDC5* mutations may cause epilepsy associated with FCD, reinforcing the link between mTORC1 pathway and FCDs. Similarly to other mTORopathies, a “two-hit” mutational model could be responsible for cortical lesions. Our study also indicates that epilepsy surgery is a valuable alternative in the treatment of drug-resistant *DEPDC5*-positive focal epilepsies, even if the MRI is unremarkable.

## INTRODUCTION

Focal epilepsies are the most frequent epilepsies. It is increasingly recognized that focal epilepsies are likely to have a genetic basis. During the last two decades, a number of mutations have been identified in autosomal dominant focal epilepsies, in both ion-channel (*CHRNA4*, *CHRNA2*, *CHRNA2*, *KCNT1*) and non-ion channel genes (*LGI1*, *DEPDC5*).<sup>1,2</sup> Focal cortical dysplasias (FCDs), in particular FCD II, are well-known underlying neuropathological conditions for severe therapy-refractory epilepsies.<sup>3,4</sup> Typically, FCDs II are characterized by a focal epilepsy of early onset, a drug-resistance, sleep-related seizures usually of extratemporal (often frontal) origin, and stereoelectroencephalography (SEEG) often showing subcontinuous repetitive spikes and spikes and waves alternating with bursts of fast activity.<sup>5-7</sup> In the 2011 consensus classification of the International League Against Epilepsy (ILAE), FCD I referred to architectural disturbances of cortical lamination. Dymorphic neurons are the histopathologic hallmark of FCD IIa and association with balloon cells specifies FCD IIb.<sup>8</sup> FCD II occurs mainly sporadically, but rare familial cases have been reported.<sup>9</sup> Several rare mutations in *CNTNAP2* (contactin associated protein-like 2), *PTEN* (phosphatase and tensin homolog) and *SCN1A* (alpha 1 subunit, sodium voltage-gated channel) have been associated with FCDs.<sup>10</sup>

Mutations of the *DEPDC5* (DEP domain containing protein 5) gene account for 12 to 37 % of inherited focal epilepsies, including familial focal epilepsy with variable foci (FFEVF), autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) and familial temporal lobe epilepsy (FTLE).<sup>11-13</sup> Recently, *DEPDC5* mutations were also identified in rare cases of rolandic epilepsy and unclassified focal childhood epilepsies<sup>14</sup>, as well as in three patients (in two families) with bottom-of-the-sulcus dysplasia and one with focal band heterotopia.<sup>15</sup> Recent studies have shown that *DEPDC5* is an upstream negative regulator of mammalian target of rapamycin mTOR complex 1 (mTORC1) in both mammalian cells<sup>16</sup> and yeast.<sup>17</sup> The mTORC1 signaling pathway regulates many major cellular processes including cell proliferation, growth and survival, and protein synthesis.<sup>18</sup> Upregulation of the pathway is implicated in the pathogenesis of numerous neurological conditions associated with cortical malformations and intractable seizures including FCDs IIb<sup>19</sup>, TSC (tuberous sclerosis complex)<sup>20</sup>, PMSE (polyhydramnios, megalencephaly and symptomatic epilepsy) syndrome<sup>21</sup> and HME (hemimegalencephaly).<sup>22</sup> Besides, a causal link between mTOR hyperactivation and epileptogenesis is well recognized.<sup>23</sup> Here, we confirm that mutations in mTORC1 repressor *DEPDC5* cause drug-resistant focal epilepsy associated with FCDs. We describe the

electroclinical and imaging phenotypes in seven patients from four families who were referred for epilepsy surgery, and report histological data and surgical outcomes.

## **SUBJECTS AND METHODS**

### **Patients**

Two French (families 1 and 2), one American (family 3) and one French-Canadian (family 4) families were recruited. Our criteria for inclusion in the cohort were: 1) the presence of at least two family members with focal epilepsy, 2) transmission compatible with autosomal dominant inheritance, 3) at least one family member with a diagnosis of FCD according to the electroclinical features with positive MRI, or negative MRI with histological confirmation, and 4) a pathogenic *DEPDC5* mutation in the affected family members. Nocturnal frontal lobe epilepsy (NFLE) was defined by clusters of short-duration (less than one minute) hyperkinetic, tonic or dystonic seizures, occurring predominantly during sleep. Written informed consent was obtained from all participants (or the parents of minors). The study was approved by the local ethics committee (CCPPRB of Pitié-Salpêtrière Hospital, Paris, No. 69-03, 25/9/2003).

### **Histopathology**

Human tissue specimens removed for the treatment of medically intractable epilepsy were analyzed by histology in five patients obtained from Rennes University (patients 1/IV-9 and 1/V-1), Saint-Anne Hospital in Paris (patient 2/II-2), Ann & Robert H. Lurie Children's Hospital of Chicago (patient 3/IV-2) and CHUM Notre-Dame in Montreal (patient 4/III-1). Brain specimens were fixed in 4% paraformaldehyde and embedded in paraffin. All fixed tissue blocks were sectioned at 8  $\mu$ m. Histopathological examination of the cortical specimen was carried out on hematoxylin and eosin (HE) stained paraffin-embedded tissue. FCDs were classified according to the ILAE classification.<sup>8</sup>

### **Genetic study**

Genomic DNA from blood samples was extracted using standard procedures. Genomic DNA from brain paraffin-embedded sections was available from 2 patients (1/IV-9 and 2/II-2), and was isolated and purified using the iPrep ChargeSwitch® Forensic Kit (Invitrogen™). All 42 coding exons and intron-exon junctions of *DEPDC5* (except exon 2 which was analyzed by Sanger sequencing) were analyzed by universal tailed amplicon sequencing as previously described.<sup>12</sup> *DEPDC5* cDNA refers to NCBI reference sequence # NM\_001242896.1 and

DEPDC5 protein to reference protein # NP\_001229825.1. Ultra-deep sequencing (> 1000 X) to search for mosaic mutations in family 2 was performed on MiSeq (Illumina). Paternity test was performed using the AmpFLSTR® Profiler Plus® (Life technologies) in family 2.

## RESULTS

### Clinical and neuroimaging data (see Table 1, Figure 1)

**Family 1**, diagnosed with FFEVF, was previously reported (referred to as family S)<sup>12, 24</sup> and was further investigated in this study. Patient 1/IV-9 had drug-resistant MRI-negative NFLE. Intracranial recordings by SEEG demonstrated interictal, almost continuous, rhythmic spike activity co-localized with subclinical ictal discharges arising from the left lateral premotor cortex. Low frequency stimulation in the same area elicited three clinical seizures similar to the spontaneous ones. This electrophysiological pattern was highly suggestive of FCD II, as previously described.<sup>5</sup> A focal resection guided by the results of the SEEG completely suppressed the seizures (13-year follow-up). However, due to technical problems, histopathology failed to identify the typical pattern corresponding to FCD II and only revealed a mild cytoarchitectonic perturbation compatible with FCD I (data not shown). His son, individual 1/V-4, suffered from nocturnal seizures suggestive of NFLE. A ketogenic diet in association with vigabatrin and phenytoin was effective for only 10 months. MRI showed abnormalities typical of FCD IIb in the left frontal lobe (**Fig 2A-D**). He is currently under presurgical evaluation. A second-degree female cousin of individual 1/V-4, individual 1/V-1, suffered from NFLE associated with an abnormal left precentral gyrus compatible with FCD II. A limited surgical resection was performed after SEEG at the age of 13, followed by a mild hemiparesis without reducing seizure frequency. Histopathology revealed fragmented and ischemic surgical specimens, precluding a reliable analysis (data not shown).

**Family 2** consisted of two sisters with nocturnal focal seizures. Patient 2/II-2 had drug-resistant epilepsy and underwent a presurgical assessment. MRI was negative, but FDG-PET showed focal hypometabolism in the anterior part of the left insula (**Fig 2E**). SEEG confirmed the concordance between the hypometabolic area and the typical FCD II EEG pattern, with a seizure onset zone in the insula and early spread to the frontal lobe. After a limited resection of the left anterior insula, a seizure-free period of six months was obtained, but seizure recurrence required a complementary stereotaxic thermocoagulation within the neighboring anterior insular region, with a favorable outcome (4-year follow-up). Histopathological

examination of a small fragment showed large dysmorphic neurons in favor of FCD IIa (**Fig 3A-C**). Her sister, individual 2/II-1, suffered from focal seizures suggestive of NFLE, sensitive to carbamazepine, which was discontinued two years later without seizure recurrence. Brain MRI (3 T) was normal.

**Family 3** included individual 3/IV-2 who had his first seizures at two years of age. Seizures were first sensitive to carbamazepine but a drug-resistance appeared at the age of five years. Semiology was suggestive of NFLE, MRI was normal, and presurgical evaluation implicated a precentral location. Because of the functional risks, surgery was limited to a surface biopsy from the suspect area. Histology revealed FCD I but the macroscopic anatomy (small area of pale cortex, firm to touch), suggested it may have been surrounded by FCD II (data not shown). Seizure frequency remained unchanged. Several family members on the father's side had a similar history of seizures (**Fig 1**), although all had spontaneous remission and did not participate in the study.

**Family 4** comprises a male and his nephew with high suspicion of FCD II in both. Individual 4/II-1 suffered from nocturnal sensorimotor seizures related to a dysplastic lesion located in the central region. Despite four resections, the patient was not seizure-free, but a worthwhile improvement was obtained. The excessive fragmentation of the surgical specimens precluded a reliable histologic diagnosis (data not shown). Individual 4/III-1 suffered from drug-resistant focal epilepsy from the age of four years (previously reported<sup>25</sup>). Electroclinical features, including MRI, SPECT, FDG-PET and magnetoencephalography localized the epileptogenic zone to the right anterior frontal lobe (**Fig 2F**). A right anterior frontal corticectomy led to seizure freedom off all antiepileptic drugs (seven-year follow-up). Histopathological examination revealed FCD IIa (**Fig 3B-D**).

### **Germline *DEPDC5* mutations**

We applied targeted high-coverage sequencing of *DEPDC5* exons and intron-exon junctions in the probands of the four families followed by a validation with Sanger sequencing. Pedigrees and molecular findings are illustrated in Figure 1.

*DEPDC5* c.715C>T (p.Arg239\*) mutation was previously reported to segregate among affected family members in family 1, including patients 1/IV-9 and 1/V-1 who underwent epilepsy surgery.<sup>12</sup> We showed that the *DEPDC5* transcript carrying the p.Arg239\* mutation is targeted by nonsense-mediated decay and leads to *DEPDC5* haploinsufficiency.<sup>12</sup> A novel

mutation, c.484-1G>A, located in the acceptor splice site of intron 8 and predicted to induce exon 9 skipping, was identified in the proband (2/II-2) of family 2. The c.484-1G>A mutation was also present in the affected sister (2/II-1), but was undetectable by traditional Sanger sequencing in the parents, in whom paternity was confirmed. Deep-targeted sequencing of *DEPDC5* exon 9 was subsequently performed in both parents, and allowed detection of the c.484-1G>A mutation in ~ 5% (56/1038 reads) of the alleles of the asymptomatic father (2/I-2), indicating germline mosaicism. In families 3 and 4, two novel nonsense mutations, c.1264C>T (p.Arg422\*) and c.1759C>T (p.Arg587\*) respectively, were identified. All the above mutations were considered pathogenic since they either introduced a premature stop codon or affected an essential splice site. They are not reported in the dbSNP135, 1000 Genomes Project database or the NHLBI exome variant server database.

### **Somatic *DEPDC5* mutations**

We searched for *DEPDC5* somatic mutations in brain specimens from patients 1/IV-9 and 2/II-2. DNA was extracted from paraffin-embedded tissue and *DEPDC5* sequencing performed either by targeted or traditional Sanger sequencing when amplification of targeted regions failed (exons 2, 12, 18). Sequencing confirmed the presence of the germline mutations p.Arg239\* in patient 1/IV-9 and c.484-1G>A in patient 2/II-2. In addition, we also detected a second nonsense mutation located in exon 18, c.1264C>T (p.Arg422\*) in individual 1/IV-9 (yet not detected in blood DNA with deep sequencing > 300 X) by Sanger sequencing (**Fig 4**). PCR amplification and sequencing were repeated from two independent extractions of brain tissue. The quality of brain DNA isolated from formalin-fixed, paraffin-embedded tissue precluded deep sequencing to evaluate the level of mosaicism of the p.Arg422\* mutation and to determine whether this second mutation was located in cis or trans from the p.Arg239\* germline mutation. No brain somatic mutation was identified in the second patient (individual 2/II-2).



## DISCUSSION

While *DEPDC5* has been mainly linked to nonlesional familial focal epilepsies, particularly FFEVF, more recently mutations have also been detected in patients with focal epilepsy associated with cortical dysplasia and focal band heterotopia.<sup>15</sup> Thus, *DEPDC5* mutations are associated with various focal epileptic phenotypes, across the spectrum from nonlesional familial focal epilepsies to malformation-associated focal epileptic syndromes (for review<sup>1, 26</sup>). Here, we confirm the existence of *DEPDC5* mutations in patients with FCDs, and provide further information on the electroclinical, imaging and histological features, and on surgical outcome. Our findings have several original features: (1) the first report of patients with FCDs associated with *DEPDC5* mutations who underwent epilepsy surgery, and demonstration of a favorable surgical outcome; (2) the presence of histologically confirmed FCD despite a normal MRI, raising the question of potential underestimation of FCDs in genetic focal epilepsies; (3) the association between FCD type IIa and *DEPDC5* mutations suggesting that mTORC1 hyperactivation predicted in patients with mutations in *DEPDC5* may also underlie different FCD subtypes, and not only type IIb; (4) the first report of a brain somatic mutation in *DEPDC5* gene in one patient (out of two tested) in addition to an existing germline mutation supporting a two-hit hypothesis for FCD, and (5) a *DEPDC5* germline mosaic mutation transmitted by an asymptomatic father.

We previously reported that *DEPDC5* mutations may be associated with a high rate of drug-resistance.<sup>13</sup> We now report in seven patients from four families the existence of FCD in the context of inherited drug-resistant focal epilepsies caused by *DEPDC5* mutations. All patients had electroclinical phenotypes highly suggestive of FCD II. Epilepsy onset in infancy and early childhood (mean age: 18 months), high seizure frequency, drug-resistance and predominant location in fronto-central areas are characteristic of FCD II.<sup>6, 28</sup> In addition, sleep-related epilepsy previously reported as a clinical marker of FCD II<sup>27</sup> was observed in six out of seven patients. MRI showed typical features of FCD II in four patients and was considered normal in three others in accordance with the 30-40 % rate of MRI-negative FCD II cases observed in recent series.<sup>28, 29</sup> Of the three MRI negative cases, one had macroscopic abnormalities suggestive of FCD II and two had SEEG features typical of FCD II, concordant with focal hypometabolism on FDG-PET in one. This is concordant with a report showing that in patients with MRI-negative neocortical epilepsies, FDG-PET coregistered on MRI may help to detect occult FCD II.<sup>30, 31</sup> In our series, histological examination confirmed FCD II in two patients, yet failed to demonstrate FCD II in four operated/biopsied patients, which may be

due to technical reasons: fragmented specimens (individuals 1/IV-9, 1/V-1; 4/II-1), incomplete resection of the abnormal cortex (individuals 1/V-1, 4/II-1) and a biopsy (individual 3/IV-2), which was seemingly performed in the vicinity of the abnormal cortex. Finally, of the five patients who underwent resective surgery, three became seizure-free with a follow-up of 4 to 13 years and one had a worthwhile improvement, in accordance with the favorable surgical outcome usually obtained in FCD II, including MRI-negative cases.<sup>6</sup> In the two patients in whom surgery did not lead to seizure freedom, the location of the epileptogenic zone in functional areas precluded complete removal of the lesion.

Despite a drug-resistance rate of ~ 30% in patients with familial focal epilepsies<sup>24, 32</sup>, which may be higher when *DEPDC5* is causal<sup>13</sup>, epilepsy surgery is rarely performed. The reticence may derive from the absence of an MRI-detectable lesion and the presence of a mutated protein expressed ubiquitously throughout the cortex. Our study indicates that the presence of a *DEPDC5* mutation in patients with refractory focal epilepsy (whether the brain MRI is normal or not) should not preclude epilepsy surgery, since it may cure the epilepsy. Therefore we believe that drug-resistant focal epilepsies should be evaluated for surgery even in the presence of a clear genetic etiology. A key question directly emanating from our findings is whether only a subset of family members develops FCD or whether infra-radiological FCD is present in all affected family members.

The finding that *DEPDC5* is associated with FCD in the context of inherited focal epilepsy agrees with evidence that *DEPDC5* acts as an mTORC1 inhibitor.<sup>16</sup> The role of *DEPDC5* in the mTORC1 cascade is particularly attractive for two main reasons: 1/other genes encoding inhibitors of mTORC1 have been shown to cause brain malformations associated with refractory epilepsy<sup>33, 34</sup>: inactivation of *TSC1* or *TSC2* causes tubers in TSC, deletion of *STRADA* causes PMSE syndrome, and loss-of-function of *PTEN* causes HME; 2/ enhanced constitutive mTORC1 activation has been shown in the balloon cells found in FCD IIb, which are thought to be the cellular marker traducing alterations of the pathway, but not in FCD IIa.<sup>35</sup> Interestingly, we did not find FCD IIb cases in our families with *DEPDC5* mutations, suggesting a continuum in both cellular and mTORC1 signaling alterations.

A long-standing, yet unproven hypothesis, is that FCD is caused by brain somatic mutations, which are increasingly identified in neurodevelopmental diseases associated with epilepsy.<sup>36</sup> Also, in families comprising a patient with Dravet syndrome, a severe epileptic encephalopathy, 7 % of *SCN1A* mutations are inherited from a parent who is somatic or germline mosaic.<sup>37</sup> Interestingly, single cell sequencing of *TSC1* and *TSC2* in tubers revealed

both germline and somatic mutations reflecting a “two-hit” mutational event.<sup>38</sup> With the finding of *DEPDC5* mutations in patients with cortical malformations, this hypothesis has become even more attractive.<sup>26</sup> We report a *de novo* somatic *DEPDC5* mutation in the brain lesional specimen of one patient with FCD. This mutation, absent from blood DNA, and superimposed on an existing, different, heterozygous germline mutation, is likely to cause a biallelic inactivation of *DEPDC5* at the site of the lesion, confirming a two-hit mutational model. However, we failed to detect a somatic mutation in a second patient, from another family. Sequencing and search for loss-of-heterozygosity of paired blood-brain DNA from additional patients will allow determination of the prevalence of *DEPDC5* somatic mutations in FCDs, and may help to resolve the puzzling question of the variability of brain areas involved in different family members within families with FFEVF. Sequencing using gene panels has been shown to be a valuable technique to detect mosaic mutations in cerebral cortical malformations.<sup>39</sup>

To conclude, our study reveals that *DEPDC5* mutations are associated with FCD in patients with familial focal epilepsy, who were originally considered as nonlesional. *DEPDC5*, like other mTORC1 inhibitor genes including *TSC1*, *TSC2*, *PTEN* or *STRADA*, may be responsible for structural alterations in the cortex with the presence of dysmorphic cells. Experimental studies on animal models are now needed to better understand the nature and extent of the consequent changes related to *DEPDC5* mutations. In particular, it remains to be proven that constitutive mTORC1 activation contributes to *DEPDC5*-related epilepsy, as seen in other so-called mTORopathies. This would, of course, have important therapeutic implications.

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## FIGURE LEGENDS

**Figure 1:** Pedigrees of the families with *DEPDC5* mutation segregation. Pedigrees of family 1 (p.Arg239\*) and family 2 (c.484-1G >A) of French origin, family 3 (p.Arg422\*) of American origin and family 4 (p.Arg587\*) of French-Canadian origin are illustrated. Individuals with FCD are boxed (in red). The respective *DEPDC5* mutations (NM\_001242896.1, NP\_001229825.1) are indicated. Individuals who carry a *DEPDC5* mutation are denoted by m/+ and those negative for mutations are denoted by +/+. Level of mosaicism of c.484-1G >A mutation is indicated in brackets.

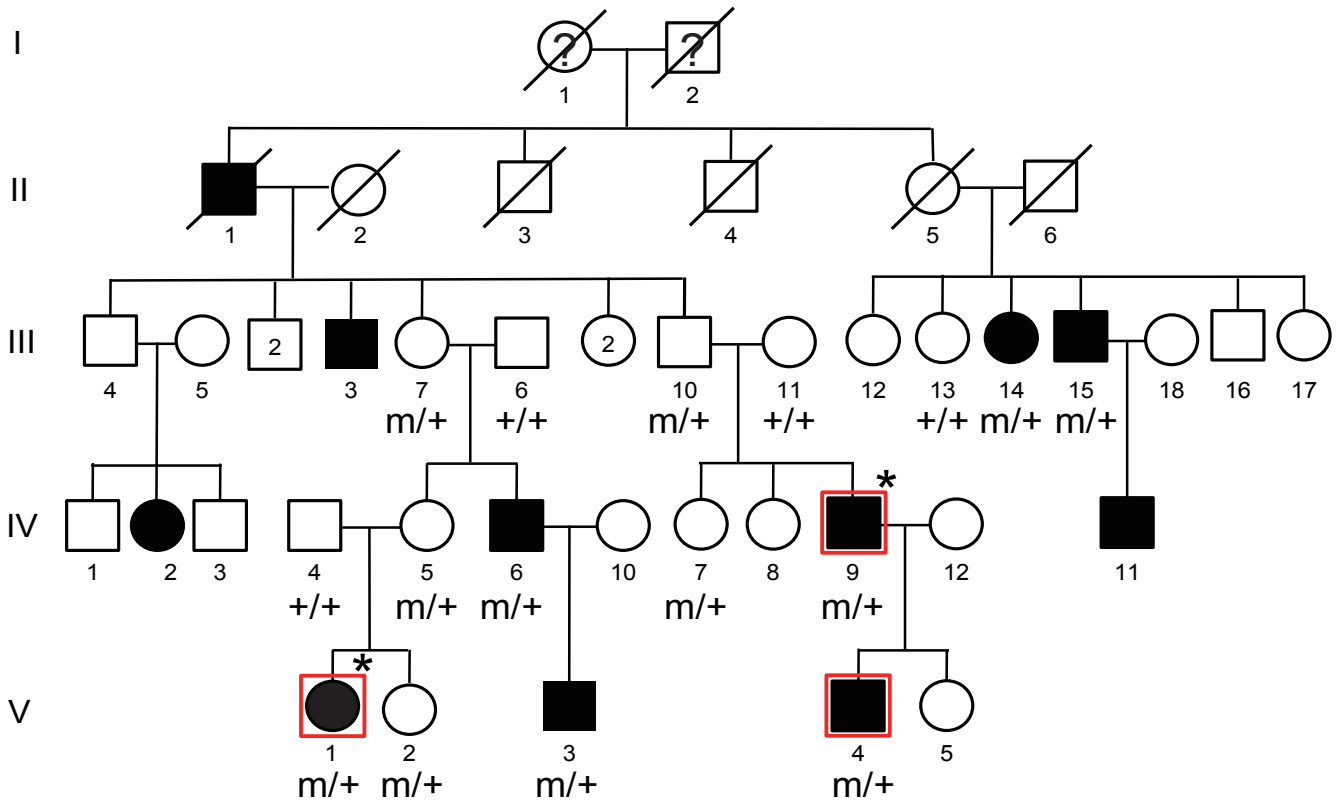
**Figure 2:** MRI of patients 1/V-4 (A-D), 2/II-2(E) and 4/III-1 (F). (A, B), coronal slices, inverted T2 weighted sequences; (C, D) axial slices; C, T1 weighted; and D, FLAIR sequences. Typical features of FCD IIb located in the left mesial superior frontal gyrus (premotor area) with a broad gyrus, blurring of the grey-white matter demarcation, subcortical hypersignal and transmantle sign (arrows). (E), FDG-PET/MRI superimposition, coronal slice; note the focal hypometabolism in the anterior part of the left insula (arrow) corresponding to the seizure onset zone and the site of FCD IIa. (F), MRI of individual 4/III-1 showing a coronal view, 3D T1 sequence. The white arrow shows the abnormal deep sulcus (FCD IIa) in the paramedian region of the right frontal pole.

**Figure 3:** Hematoxylin and eosin stained paraffin-embedded tissue from post-operative brain sections in subject 2/II-2 (A-C) and 4/III-1 (B-D), showing the presence of large dysmorphic neurons (indicated by arrows) and cortical disorganization characteristic of FCD IIa. Scale bar = 50  $\mu$ m, magnification A-B: X40, C-D: X20.

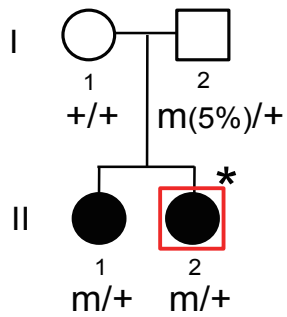
**Figure 4:** Sequence chromatograms from patient 1/IV-9 showing the detection of p.Arg239\* mutation in *DEPDC5* exon 12 in blood and brain DNA, and p.Arg422\* mutation in exon 18 in brain DNA. The mutation p.Arg422\* was not detected from blood DNA indicating that it occurred *de novo* somatically.

Figure 1

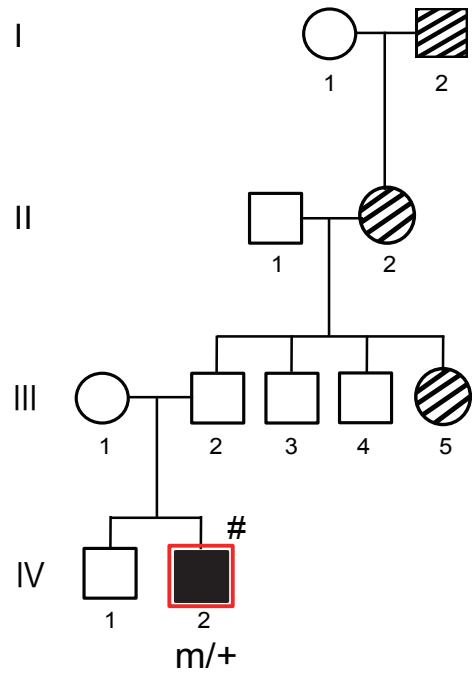
Family 1 : c.715C>T / p.Arg239\*



Family 2 : c.484-1G>A



Family 3 : c.1264C>T / p.Arg422\*



Family 4 : c.1759C>T / p.Arg587\*

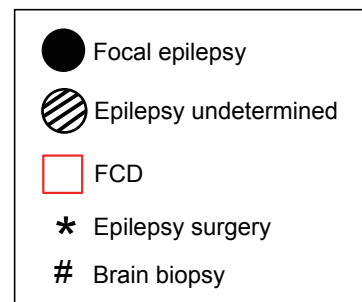
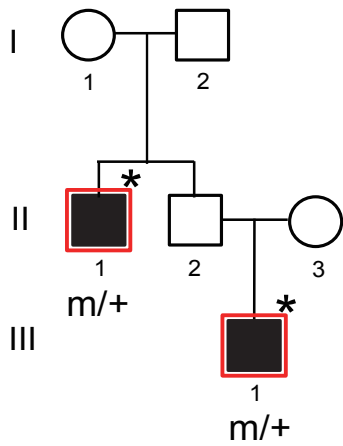




Figure 2

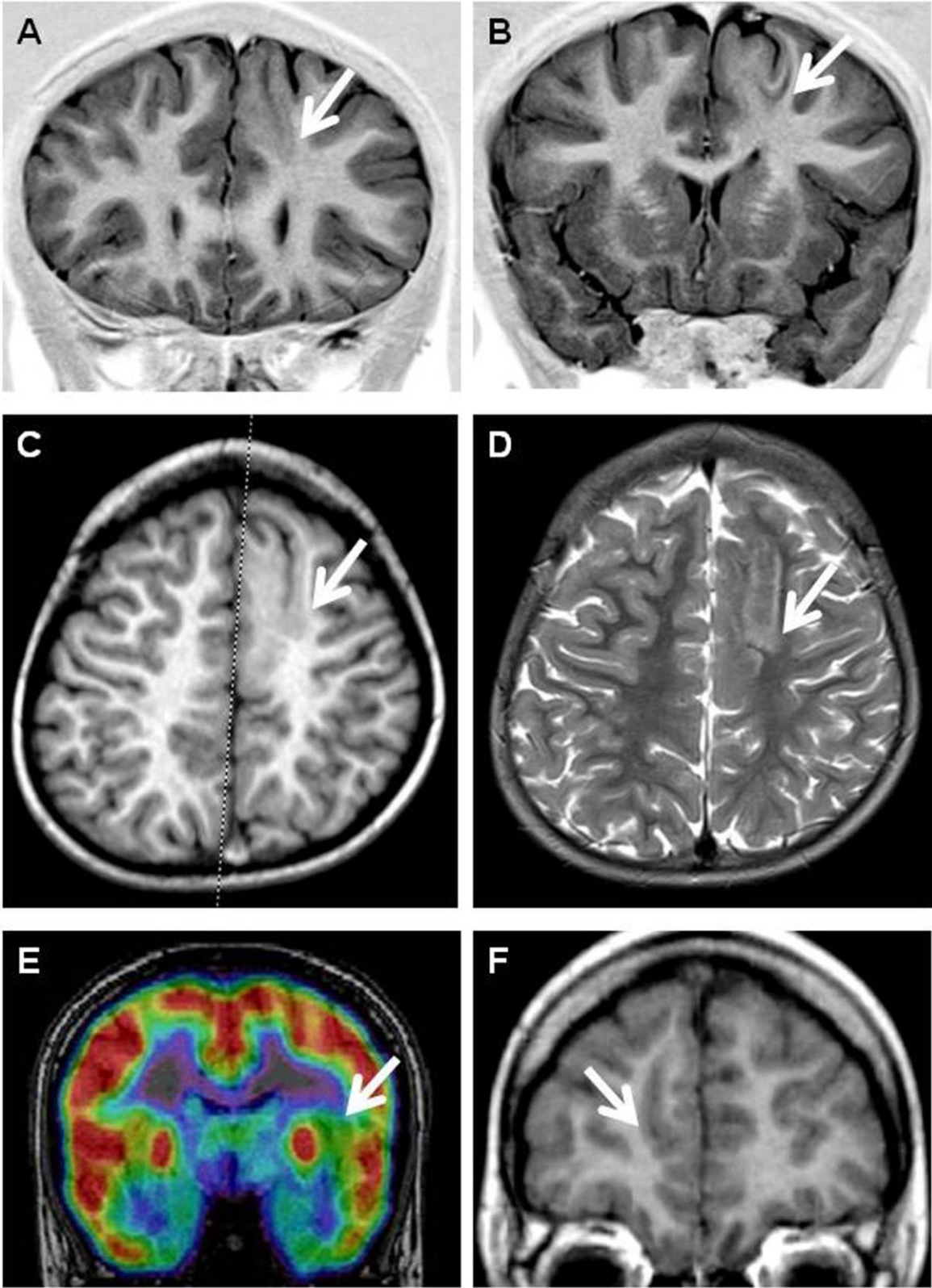


Figure 3

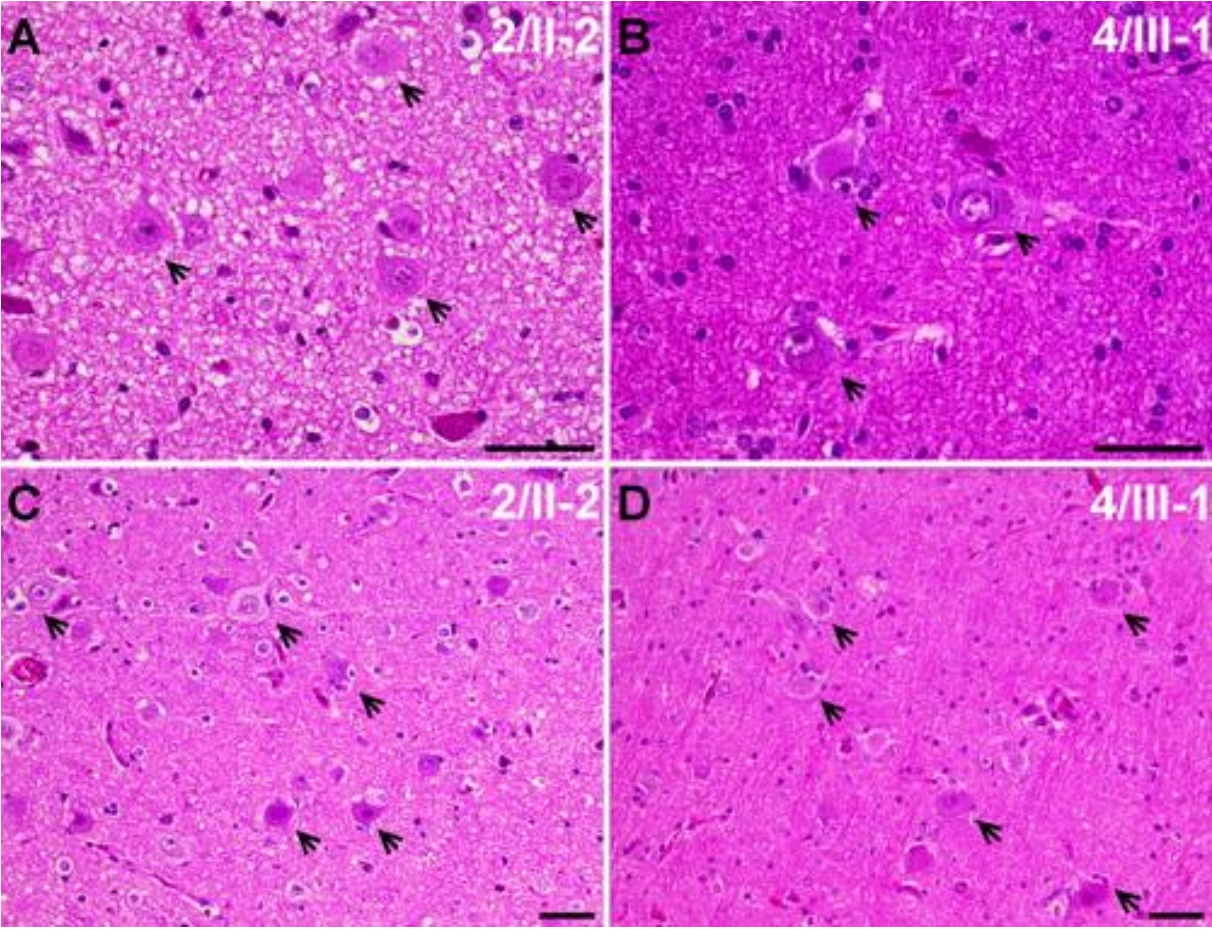


Figure 4

