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## **Exome sequencing in 57 patients with Self-limited Focal Epilepsies of Childhood with typical or atypical presentations suggests novel candidate genes**

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## SUMMARY

**Objective:** Self-limited focal epilepsies of childhood (SFEC) are amongst the best defined and most frequent epilepsy syndromes affecting children with usually normal developmental milestones. They include core syndromes such as Rolandic epilepsy or “Benign” epilepsy with Centro-Temporal Spikes and the benign occipital epilepsies, the early onset Panayiotopoulos syndrome and the late-onset Gastaut type. Atypical forms exist for all of them. Atypical Rolandic epilepsies are conceptualized as belonging to a continuum reaching from the “benign” RE to the severe end of the Landau-Kleffner (LKS) and Continuous Spike-Waves during Sleep syndromes (CSWS). *GRIN2A* has been shown to cause the epilepsy-aphasia continuum that includes some patients with atypical Rolandic epilepsy with frequent speech disorders, LKS and CSWS. In the present study, we searched novel genes causing SFEC with typical or atypical presentations.

**Methods:** Exome sequencing was performed in 57 trios. Patients presented with typical or atypical SFEC, negative for *GRIN2A* pathogenic variant.

**Results:** We found rare [candidate](#) variants in 20 patients. Thirteen had occurred *de novo* and were mostly associated to atypical Rolandic Epilepsy. Two of them could be considered as disease related: a [null](#) variant in *GRIN2B* and a missense variant in *CAMK2A*. Others were considered good candidates, including a substitution affecting a splice site in *CACNG2* and missense variants in genes encoding enzymes involved in chromatin remodeling.

**Significance:** Our results further illustrate the fact that atypical SFEC are more likely to have Mendelian inheritance than typical SFEC.

**Key words:** Self-limited focal epilepsies of childhood (SFEC), Rolandic epilepsy; Panayiotopoulos syndrome; Gastaut type; atypical Rolandic epilepsy; focal idiopathic epilepsies of childhood.

**Abbreviations:**

aBFEC: atypical benign focal epilepsy of childhood

aSFEC: atypical self-limited focal epilepsies of childhood

BECTS: “benign” epilepsy with centro-temporal spikes

CECTS: childhood epilepsy with centro-temporal spikes

CEOP: childhood epilepsies with occipital paroxysms

CNS: copy-number variation

CSWS: Continuous Spike-Waves during Sleep syndromes

CTS: centro-temporal spikes

GEFS+: genetic epilepsy with febrile Seizures plus

GS: Gastaut syndrome

LKS: Landau-Kleffner

**NAFE : non acquired focal epilepsies**

PS: Panayiotopoulos syndrome

RE: Rolandic epilepsy

SFEC: self-limited focal epilepsies of childhood

## 1. INTRODUCTION

The term idiopathic focal epilepsies of childhood (IFE), which has long been used in clinical practice, is no longer formally recognized by the ILAE since its 2010 revision, nor are its members and boundaries precisely delineated.<sup>1,2</sup> The historically defined core electroclinical syndromes as “Rolandic epilepsy” (RE) or benign epilepsy with centro-temporal spikes (BECTS), or childhood epilepsy with centro-temporal spikes and the childhood epilepsies with occipital paroxysms are now arranged by age at onset, which does not reflect etiology. They are still referred as Panayiotopoulos syndrome (PS), BECTS and late onset occipital childhood epilepsy (Gastaut type). The current consensus is that the term “idiopathic” should be replaced by “genetic”, and “self-limited” concerning these entities tightly related to brain maturation. “Benign” should be avoided with respect to the now well established associated neurocognitive co-morbidities.<sup>3</sup> Consequently, in recent research studies the terminology changed and this group of focal epilepsies is increasingly termed “self-limited focal epilepsies of childhood” (SFEC).<sup>4-6</sup> The ongoing genetic dissection of epilepsy phenotypes, based on large datasets, necessarily modify our conceptual approach of epilepsies but also confirm the dichotomy: generalized versus focal; SFEC are clearly distinctive from other non-acquired focal epilepsies (NAFE) with onset in childhood, such as sleep-related hypermotor epilepsy or familial focal epilepsy with variable foci that usually do not have the same evolution with age.<sup>7</sup> Atypical SFEC exist having in common an age-specific seizure onset, a seizure resolution related to brain maturation, an absence of structural brain abnormalities, a rather characteristic EEG spike and wave element of variable expression, and a supposed genetic origin. Atypical Rolandic epilepsies cover atypical benign focal epilepsy of childhood (aBFEC), status of RE, Landau-Kleffner (LKS) and Continuous Spike-Wave during Sleep (CSWS) syndromes.<sup>8,9</sup> The prevalence and severity of atypical self-limited focal epilepsies of childhood (aSFEC) is highly variable in literature, depending on inclusion criteria, notably on

the amount of atypical seizure types, atypical EEG features, “accepted” preexistent neurocognitive comorbidities, and on rejection or not of definite LKS and CSWS.<sup>9-15</sup> There is still no definite consensus about which clinical and EEG features should be necessarily present for diagnosing atypical forms since the onset.<sup>16</sup>

The genetic architecture of SFEC and related disorders appears to be complex and remains largely unknown. Considering the more “benign” end of the RE-LKS-CSWS continuum, loci for the CTS EEG trait have been identified in the *ELP4-PAX6* region on 11p13 and on chromosome 15q13.<sup>17</sup> However disease relevant variants need to be recognized. Association studies have suggested other potential candidate genes, such as *RBFOX1*, *RBFOX3* encoding neuronal splice regulators, and *GABRG2*, encoding a gamma-aminobutyric acid type A receptor.<sup>18,19</sup> In our previous study of CNV analysis in 47 patients with RE and aRE, we have shown that half of them carried rare CNVs, some of them disrupting known causal genes for epilepsy.<sup>20</sup> Two patients with atypical RE carried the 600-kb recurrent duplication on chromosome 16p11.2. An independent study, on a larger group of patients, found duplication in 1.2% of the patients, confirming its role as a disease susceptibility factor for RE.<sup>21</sup> A very recent study with high-density SNP-array in 186 patients have found many candidate loci including another recurrent CNVs (deletion or duplication) at Xp22.31 in 5 patients with typical RE.<sup>22</sup> Patients with [truncating variants](#) in *KCNQ2* and *KCNQ3*, which are usually causing “benign familial neonatal epilepsy”, can also have RE later in childhood, sometimes without seizures in the neonatal period.<sup>23</sup> [Truncating](#) variants of *DEPDC5*, encoding a regulator of the mTOR pathway has been also reported in patients with RE.<sup>24</sup> For the other SFEC syndromes, the evidence for genetic basis is scarce. One recent paper suggested a role for the co-occurrence of a 22q11.2 microdeletion and of a 2q36 microduplication (inherited from the unaffected mother) in a patient with Panayiotopoulos syndrome.<sup>25</sup> *SCN1A* variants

were also occasionally reported in families with genetic epilepsy with febrile Seizures plus (GEFS+) and PS.<sup>5</sup>

An important step was achieved by identifying *GRIN2A*, which encodes a subunit of the excitatory glutamate receptor N-acetyl-o-aspartate (NMDA), as the first gene recurrently associated with the epilepsy-aphasia continuum that includes some patients with aRE, speech dyspraxia, and CSWS.<sup>26-27</sup> The aim of the present study was to search for novel genes involved in Mendelian forms of SFEC with typical or atypical presentations.

## **2. PATIENTS AND METHODS**

### **2.1 Patients**

Patients were recruited in context of the grant of the French Ministry of Health (National PHRC grant 2010 No 03-08) between 2010 and 2013, in the Department of Pediatric Neurology and the Department of Epileptology of the University Hospital of Strasbourg (n=34) and in the Department of Pediatric Clinical Epileptology, Sleep Disorders and Functional Neurology of the University Hospital of Lyon (n=23). Epilepsy syndromes were classified based on the International League Against Epilepsy website definitions (epilepsydiagnosis.org).

In the present study, the authors chose, to conserve the wide - ranging term “atypical” as used by the referring epileptologists, fitting well with the concept of a spectrum disorder reaching “from the benign to the disabling with a genetic propensity to seizures”.<sup>8,9,28-31</sup>

Inclusion criteria were: (i) classical forms of RE, PS and GS; (ii) atypical variants of SFEC which comprised various degrees of pre-existing cognitive co-pathologies (Intellectual deficiency, Autism spectrum disorder), mixed CEOP/aCECTS forms, and children with atypical RE and EEG features as described by Massa et al., present at onset, or at evolution.<sup>10</sup>

Less well-defined entities as benign childhood seizures with affective symptoms, benign childhood epilepsy with parietal spikes, autosomal rolandic epilepsy with paroxysmal exercise-induced dyskinesia and writer's cramp, benign childhood seizures with frontal or midline spikes, and benign focal seizures of adolescence were not referred by clinicians for inclusion.<sup>32-37</sup> Sleep induced activation of paroxysmal activity was borderline or could fulfill CSWS criteria in some cases. These children could present with various transient degrees of cognitive or behavioral impairments. (iii) informed consent of parents (**Table A.1**)

Exclusion criteria were: (i) brain malformation on the MRI, to exclude acquired and symptomatic epilepsies, (ii) the identification of a *GRIN2A* pathogenic variant or of a pathogenic CNV identified by array-CGH.

Our cohort included 22 patients with typical RE, 2 patients with typical PS, 3 patients with PS/aRE features and 30 patients with aRE.

Written informed consent was obtained from the parents for their children and for themselves, according to the French Bioethics Law and to the appropriate ethical committee (n° CPP 09/40-DC-2009-1002/DC-2014-2222).

## **2.2 Exome sequencing**

Blood genomic DNA was extracted from patient's by standard protocol using the illustra DNA Extraction kit BACC3 (Ge Healthcare, Little Chalfont Buckinghamshire, UK) following the manufacturer's recommendation. Library preparation, exome enrichment, and whole exome sequencing were done at the Centre National de Recherche sur le Génome Humain (CNRGH, Evry, France). Libraries were prepared from 3µg genomic DNA using an optimized SureSelect Human Exome kit (Agilent, Santa Clara, CA, USA). Captured, purified, and clonally amplified libraries targeting the exome were the sequenced on a HiSeq 2500 (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations.

## 2.3 Bioinformatics

Sequence reads were aligned to the human genome (hg19 assembly) using the Burrows-Wheeler Aligner software. Sequence quality was assessed with FastQC 0.11.5, then the reads were mapped using BWA-MEM (version 0.7.13), sorted and indexed in a bam file (Samtools 1.4.1), duplicates were flagged (Sambamba 0.6.6), coverage was calculated (Picard-Tools 2.10.10). Variant calling was done with GATK 3.7 Haplotype Caller. Variants were then annotated with SnpEff 4.3, dbNSFP 2.9.3, gnomAD, ClinVar, HGMD, Variome Great Middle East and an internal database. Coverage for these patients was 93% at a 20x depth threshold. Criteria for selection of rare variants: i) *de novo* variants with an occurrence >3 in the gnomAD database of control individuals were excluded, ii) for compound heterozygous variants in which of the variants had an occurrence > 100, and iii) for X chromosome variants with an occurrence > 3 in males.

An additional analysis with the following criteria: a *de novo* variant in (at least) one patient and inherited variants of the same gene in another patient. For the inherited variants we studied the pathogenicity criteria (occurrence in gnomAD, SIFT, Polyphen-2 and mutation taster). The selected rare variants were classified according to the ACMG classification.

## 2.4 Sanger sequencing for confirmation of the selected variants from exome sequencing

After exome analysis, selected variants were confirmed by PCR and direct sequencing using parents and patient's DNA. PCR amplification was done with 50 ng of genomic DNA Fast Taq DNA polymerase (Roche, Boulogne Billancourt, France). Primers used to amplify the coding and flanking non-coding regions were designed using Primer 3. Fifty-microliter PCR products were purified and both strands were sequenced at Eurofins Biotech (<https://www.eurofinsgenomics.eu/>). Sequence chromatograms were analyzed with Seqscre version 2.5 software (Applied Biosystems, Courtaboeuf, France).

### 3. RESULTS

Our study, based on 57 trios, was first aimed at identifying *de novo* variants in known and candidate genes for SFEC with typical and atypical presentations with prediction of pathogenicity. *De novo* rare variants were found in 13 patients (22.8%) (**Table 1**). Nine of them were mainly found in patients with aRE, one in a patient with PS/aRE features with transient CSWS, one in a patient with typical RE, and the last one in a patient with PS. The rare variants corresponding to one of the tested modes of inheritance and with *in-silico* predictions of pathogenicity were selected (**Table 1**). One **null** variant was found in a gene associated with epileptic disorders, *GRIN2B*. A missense variant was found in *CAMK2A* who was shown to cause a phenotype with intellectual deficiency and autism spectrum disorders when this study was ongoing. These two variants could be classified as pathogenic. Six other *de novo* variants occurred in genes that have been associated with intellectual disability, autism spectrum, dystonia, or seasonal depression (*HDAC9*, *KMT2B*, *CACNG2*, *TSC2*, *ASXL2*, and *NPAS2*). The c.295+1G>C variant of *CACNG2* was predicted to affect splicing. The others are missense variants with variable predictions of pathogenicity (**Table 1**).

In addition to these *de novo* variants, we also found several missense rare variants in disease-causing genes (**Table A.1**), but with a different neurological phenotype, following different modes of inheritance, including *MAGED1* and *HDAC8* (inherited from the mother), *GRIN2B* (inherited from unaffected parent), as well as *ADGRV1*, *BRAT1* and *KIF1C* (bi-allelic variants).

At the moment, all *de novo* variants and all inherited variants, detected in this study other than the *GRIN2B* and *CAMK2A* variants, should be considered of unknown significance.

#### 4. DISCUSSION

In this trio-based exome sequencing study, we searched for rare candidate variants in patients diagnosed with self-limited focal epilepsies in childhood with typical and atypical presentations. Due to the recruitment of a large majority of sporadic cases, our first aim was to search for *de novo* variants but we also studied other Mendelian modes of inheritance.

Whereas typical SFEC are well defined, the spectrum of aSFEC is lesser delimited. An atypical form of benign focal epilepsy of childhood (aBFEC) was first recognized by Aicardi and Chevrie in 1982 and confirmed by numerous subsequent studies, further expanding the range of atypical clinical and electroencephalographic features of atypical Rolandic epilepsy (aRE) also referred as aCECTS. The electroclinical criteria to diagnose aRE or more largely aSFEC are far to be consistent, despite efforts for classification and elaboration of predictive EEG factors for menacing LKS/CSWS evolutions.<sup>8-10,15,38-39</sup> Notably, preexisting atypical features are reported in literature, as variable degrees of cognitive impairment, autistic features or abnormal neurological status, overlapping with a subset of developmental epileptic encephalopathies (DEEs), including frequently severe language disorders. Some of the aRE descriptions could bear a resemblance to the so called ‘pseudo-Lennox type’ described by Hahn et al. or could be included in the subgroup presenting with early developmental dysphasia, as reported by Echenne et al.<sup>40,41</sup> Thus, the still imprecise term ‘aRE’ reflect to some extent obvious difficulties in clinical assessment and as a result, these heterogeneous atypical forms of SFEC are not included as specific syndromic entities in the updated ILEA revision of seizure/epilepsy classification and terminology, although their prevalence, (essentially assessed for aRE), is variable and can reach up to 50% depending on inclusion criteria.<sup>1,3,9,11,12-15</sup> In addition mixed phenotypes CEOP/CECTS and atypical forms of CEOP overlapping with CSWS are increasingly recognized.<sup>42</sup>

We included 22 patients presenting with typical RE, 2 patients with typical PS and three patients with overlapping PS/aRE features and 30 patients with aRE. We identified 13 *de novo* rare variants in genes, expressed in the brain or with a function potentially related to the phenotype of our patients, and with some *in silico* prediction in favor of a deleterious effect. Two *de novo* variants could be definitely be classified as disease causing in our patients: *GRIN2B* and *CAMK2A*. Two rare variants for *GRIN2B* were found in the present study. *GRIN2B* encodes the GluN2B subunit of the NMDA receptor. Pathogenic variants of this gene have been shown to be associated with a wide range of neurodevelopmental disorders. Moderate to profound intellectual deficiency and autistic behavior are the main clinical features. Half of the patients also suffer from epilepsy with highly variable presentations, including focal or generalized seizures, or infantile spasms, with age of onset ranging from birth to late childhood.<sup>43</sup> In the present study, the *de novo* null variant p.(Glu55\*), predicted to lead to a truncated protein, was considered as disease causing, in patient EPI30 who had aRE with bilateral spikes and intellectual deficiency. In addition, we also found the *GRIN2B* p.(Tyr738His) missense variant, in patient EPI25 who had aRE with moderate intellectual deficiency. It was absent from the gnomAD database. It is located in the S2 ligand-binding domain. *In silico* predictions are in favor of a deleterious effect. However, this variant was inherited from the unaffected mother. Due to the fact that incomplete penetrance is uncommon for pathogenic *GRIN2B* variations, we classified it as a variant of unknown significance.

We also found the *de novo* missense variant p.(Pro212Leu) of *CAMK2A*, a gene functionally related to *GRIN2B* and *GRIN2A*, in a patient with aRE with intellectual deficiency and autistic features (patient EPI62). *CAMK2A* is a regulator of synaptic plasticity, whose activation is necessary and sufficient for hippocampal long-term potentiation induction and synaptic plasticity.<sup>44</sup> *CAMKII $\alpha$*  is functionally related to NR2A and NR2B. *CAMKII $\alpha$*  binding to the

NMDAR and its ability to regulate the membrane insertion and activity of AMPAR are crucial for regulating synaptic strength. The *de novo* missense variant p.(Pro212Leu) was found in a patient with intellectual deficiency and autistic features. This patient had typical oro-pharyngeal or hemifacial motor and rare secondary generalized tonic clonic seizures with salivation. Background EEG was normal, showing the typical CTS-EEG trait at wake, becoming transiently continuous over Rolandic regions in sleep. He was classified as aRE with respect to the comorbid cognitive features and the focal CSWS aspects. Seizures resolved spontaneously at age of ten years. EEG normalized at wake with only sporadic asynchronous CTS seen in sleep. The variant was predicted to be deleterious and was absent from gnomAD database. *CAMK2A* is highly intolerant to loss of function and missense variants (pLI=1, Z-score=5.27) and was considered as a good candidate. We then contacted other researchers through the Matchmaker Exchange network to find other patients with pathogenic variants in this gene.<sup>45</sup> We gathered 14 patients with an ultra-rare variant, 13 of them being confirmed to be *de novo*.<sup>46</sup> Two other patients also carried the p.(Pro212Leu) variant, which is another strong argument in favor of its pathogenicity. Epilepsy and especially aRE did not appear to be a common manifestation in *CAMK2A* patients since only one other patient had complex partial seizures. However, the precise epileptic phenotype was not reported in this publication which mainly deals with genetics and basic science.

One of the most interesting candidate gene, in our results, was *CACNG2* (patient *EPI22*), which encodes stargazin (also known as TARP- $\gamma$ 2), a synaptic adaptor protein that binds to AMPAR and regulates its synaptic trafficking, as well as its channel properties.<sup>47</sup> *In silico* predictions for splicing suggest that the c.295+1G>C single nucleotide lead to the abolition of the donor splice site, without other splice site in the 100 next bp. A missense variant in *CACNG2*, p.(Val143Leu), has been reported previously in a patient with moderate non-syndromic intellectual disability.<sup>48</sup> He was 8-year-old at the time of the study and was not

reported as having epilepsy, whereas patient EPI22 presented with focal Rolandic seizures and concomitantly significant activation of spike waves in sleep without regression. Functional studies, performed in HEK293 cells and in transfected hippocampal neurons, suggested that this variant cause a reduction in glutamatergic transmission. We hypothesize that the c.295+1G>C, predicted to lead to production of an abnormal isoform, may have similar consequences.

The other 10 *de novo* variants found in our patients were considered as potential candidates, although, additional data are required to classify them as disease-causing or rare polymorphisms. These variants are discussed in **Text A.1**.

The present study suggests that genetic variants with strong effects are almost exclusively found in patients with aRE and preexistent significant neurodevelopmental comorbidities.

This is consistent with results in our previous array-CGH study.<sup>20</sup> In addition, we did not find a rare variant by exome sequencing in genes for which we found a CNV in our former array-CGH studies, neither in patients with typical RE nor in those with CSWS or LKS, nor in candidate genes reported from other studies. Of course, a great number of them, including those presented here, will probably not be definitely confirmed to be involved in the pathophysiology of SFEC/aSFEC. Present results together with previous data from the literature illustrate the wide genetic heterogeneity of this spectrum disorder and highlight the fact that, despite the supposed “genetic propensity to seizures” to date only a small amount of the overall genetic factors have been identified. Importantly, pathogenic variants were not found in patients with typical RE, nor with typical PS, even if their number is small to draw definite conclusions. This is in accordance with prior studies, suggesting that Mendelian inheritance may not play a major role at the benign end of the SFEC-spectrum, as suggested by previous authors.<sup>49,50</sup> Our results strengthen the hypothesis of a probably multifactorial pattern of inheritance. The identification of risk factors to develop typical SFEC and related

disorders, due to the rather limited number of patients and to the study-design is beyond the scope of this study. An exome-wide association study, investigating rare variants in 194 patients with typical and atypical RE, showed a significant association for pathogenic variants (CADD15 and [truncating or null variants](#)) only for *GRIN2A*.<sup>51</sup> No gene or gene-set was significantly enriched for variants after correction for multiple-testing. This negative result may probably be related to the still limited size of that cohort.

[Recent data from the international Epi25k consortium on 17,606 individuals with different types of epilepsies showed enrichment in individuals with NAFE of are variants of several genes, including \*LGII\*, \*TRIM3\* and \*GABRG2\*.<sup>52</sup> However, due to the clinical heterogeneity of the NAFE group involved in that study, we cannot evaluated whether some of these gene factors could be shared by SFEC/aSFEC disorders.](#)

## 5. CONCLUSION

In conclusion, the present study intended to identify novel candidate genes by exome sequencing in patients with self-limited focal epilepsies of childhood with typical and atypical presentations. Potential candidate variants were detected in 20% of the patients, nearly exclusively in the group of aRE displaying significant neurodevelopmental comorbidities. Two variants, in *GRIN2B* and *CAMK2A*, could definitely be considered as disease-causing. We conclude that these results do not support a Mendelian mode of inheritance for typical RE. Data about the genetic architecture of PS and GS with typical or atypical features/evolutions are still scarce. Unraveling the genetic basis of the entities composing this wide-ranged SFEC/aSFEC continuum will require future study designs, comprising very large cohorts, providing sufficient power, to identify causative gene variants and their polygenic interactions.

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**Table 1. *De novo* rare genetic variants found in the present study.**

Case	Phenotype	Gene	Chr12:g.14018980C>A (NM_000834.3)	c.163G>T p.(Glu55*)	Occurrence gnomAD (htz)	Poly phen2	SIFT	MT	Protein function	pLI	Z score	Relation to neurological diseases	Significance
EPI30	aRE	<i>GRIN2B</i>	Chr12:g.14018980C>A	c.163G>T p.(Glu55*)	0	-	-	-	NMDA receptor	1	6,74	Epilpetic encephalopathy, intellectual disability	Pathogenic
EPI62	aRE	<i>CAMK2A</i>	Chr5:g.149631371G>A (NM_015981.3)	c.635C>T p.(Pro212Leu)	0	1	0	1	calcium/calmodulin- dependent protein kinase II	1	5,27	Intellectual disability and autism	Pathogenic
EPI22	aRE	<i>CACNG2</i>	Chr22:g.36983511C>G (NM_006078.4)	c.295+1G>C p.?	0	-	-	-	AMPA receptor regulatory protein	0,96	3,44	Mental retardation, AD 10	Likely pathogenic
EPI9	RE	<i>AMZ1</i>	Chr7:g.2749305G>A (NM_133463.3)	c.803G>A p.(Gly268Asp)	1	1	0	NA	Basic aminopeptidase	0	-4,12	Critical interval of the 7p22.3p22.2 microdeletion	VUS
EPI19	aRE-OE	<i>EPHB6</i>	Chr7:g.142564758G>A (NM_004445.5)	c.1682G>A p.Arg561Gln	0	0,97	0	0,99	Ephrin receptor	0,25	1,32		VUS
EPI23	aRE	<i>PPP1R12C</i>	Chr19:g.55614832T>G (NM_017607.3)	c.676A>C p.(Thr226Pro)	0	0,99	0	1	Regulation of actin cytoskeleton	0,46	1,42		VUS
EPI28	aRE	<i>TSC2</i>	Chr16:g.2104414C>T (NM_000548.3)	c.454C>T p.(His152Tyr)	0	0	0,37	0,512	Down regulation of the mTORC1 pathway	1	0,89	Tuberous sclerosis	VUS
EPI33	aRE	<i>HDAC9</i>	Chr7:g.18687598G>A (NM_058176.2)	c.1226G>A p.(Arg409Gln)	2	1	0,42	1	Histone deacetylase	1	0,88	Candidate gene for autism	VUS

EPI40	RE	<i>PTPRD</i>	Chr9:g.8331710C>G (NM_002839.3)	c.5406G>C p.(Gln1802His)	0	1	0	1	Protein tyrosine phosphatase, receptor	1	0		VUS
EPI49	aRE	<i>HDAC3</i>	Chr5:g.141004916C>T (NM_001355039.1)	c.1076G>A p.(Arg359His)	0	0,87	0,13	NA	Histone deacetylase	0,96	4,28		VUS
EPI50	aRE	<i>KMT2B</i>	Chr19:g.36229072G>A (NM_014727.2)	c.7852G>A p.(Glu2618Lys)	0	0,99	0	1	Lysine methyltransferase	0,99		Early-onset generalized dystonia	VUS
EPI55	aRE	<i>ASXL2</i>	Chr2:g.25965437C>T (NM_018263.4)	c.3769G>A p.(Asp1257Asn)	0	0,99	0,53	NA	Chromatin protein	0,99	-0,01	Shashi-Pena syndrome	VUS
EPI64	PS	<i>NPAS2</i>	Chr2:g.101611863del (NM_002518.3)	c.2294del p.(Val765fs)	0	-	-	-	Transcription factor	0,99	2,47	Seasonal depression	VUS

RE = Rolandic epilepsy; aRE = atypical Rolandic epilepsy; OE = occipital epilepsy; PS = Panayiotopoulos syndrome; MT = mutation taster; NA = not available; VUS = variant of unknown significance

## ANNEXES

**Table A.1.** Clinical data of the patients included in the present study.

**Table A.2.** Rare inherited genetic variants, of unknown significance, found in the present study