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Laura Licchetta, Tommaso Pippucci, Sara Baldassari, Raffaella Minardi, Federica Provini, Barbara Mostacci, Giuseppe Plazzi, Paolo Tinuper, Francesca Bisulli, On behalf of the Collaborative Group of Italian League Against Epilepsy (LICE) Genetic Study Group on SHE<ce:author-group id="aug0010">, Amedeo Bianchi, Pasquale Striano, Antonio Gambardella, Lucio Giordano, Margherita Santucci, Stefano Meletti, Giovanni Crichiutti, Carla Marini, Aglaia Vignoli, Roberto Dilena, Eleonora Briatore

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SLEEP-RELATED HYPERMOTOR EPILEPSY (SHE): CONTRIBUTION OF KNOWN GENES IN 103 PATIENTS

Laura Licchetta[1,2*], Tommaso Pippucci[3*], Sara Baldassari[4], Raffaella Minardi[1], Federica Provini[1,2], Barbara Mostacci[1], Giuseppe Plazzi[1,2], Paolo Tinuper[1,2], Francesca Bisulli[1,2]

On behalf of the Collaborative Group of Italian League Against Epilepsy (LICE) Genetic Study Group on SHE[§]

[§]Amedeo Bianchi, Pasquale Striano, Antonio Gambardella, Lucio Giordano, Margherita Santucci, Stefano Meletti, Giovanni Crichiutti, Carla Marini, Aglaia Vignoli, Roberto Dilena, Eleonora Briatore.

[1]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

[2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

[3]Medical Genetics Unit, Polyclinic Sant'Orsola-Malpighi University Hospital, Bologna, Italy

[4]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

[*]These authors contributed equally to the manuscript

[§]Collaborative Group of LICE Genetic Commission: Amedeo Bianchi (Department of Neurology and Epilepsy Centre, San Donato Hospital, Arezzo, Italy), Pasquale Striano (DINOGMI-Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, G. Gaslini Institute), Antonio Gambardella (Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy), Lucio Giordano (Neuropsychiatric Department, Spedali Civili, Brescia, Italy), Margherita Santucci (IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy), Stefano Meletti (Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, Modena,

Italy; N.O.C.S.A.E. Hospital, AUSL Modena, Italy), Giovanni Criciutti (Division of Pediatrics, Department of Medicine, University of Udine, Udine, Italy), Carla Marini (Pediatric Neurology Unit, Neurogenetics and Neurobiology Laboratories, Neuroscience Department, A. Meyer Pediatric Hospital, University of Florence), Aglaia Vignoli (Child Neuropsychiatry Unit - Epilepsy Center, San Paolo Hospital, Milan, Italy; Department of Health Sciences, University of Milan, Milan, Italy), Roberto Dilena (Servizio di Epilettologia e Neurofisiopatologia Pediatrica, UO Neurofisiopatologia, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy), Eleonora Briatore (Department of Pediatric Neurology, Santa Croce e Carle Hospital, Cuneo, Italy).

Corresponding author

Laura Licchetta

IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy

Department of Biomedical and Neuromotor Sciences, University of Bologna

Bellaria hospital, Via altura 3 Bologna, Italy 40139

Telephone: +39 0514966991; Fax: +39 0514966080

licchetta@gmail.com, laura.licchetta2@unibo.it

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Highlights

- We analyzed 103 SHE patients to estimate the mutation frequency of the known genes
- We identified mutations in *CHRNA4* (2.9%), *KCNT1* (1%), *DEPDC5* (3.9%) and *NPRL2* (1%)
- The frequency of pathogenic variants was 19% in familial and 7% in isolated cases
- *DEPDC5* shows the highest frequency, especially in cases with a structural etiology

ABSTRACT

Purpose: Genetics of Sleep-related Hypermotor Epilepsy (SHE) includes mutations in several genes that cumulative account for 30% of families. This approximate estimate comes from different case-series, each focused on the screening of a single gene. We systematically investigated a large cohort of SHE patients to estimate the frequency of pathogenic variants in the main genes thus far implicated in this epilepsy syndrome.

Methods: We selected familial and isolated cases diagnosed with clinical/confirmed SHE who underwent genetic analysis by comparable next generation sequencing (NGS) techniques (WES/ multigene epilepsy panel). The identified heterozygous variants were classified according to the American College of Medical Genetics and Genomics guidelines.

Results: We include 103 SHE patients (M/F:61/42) who underwent NGS. Sixteen (15.5%) were familial cases, 16.5% had focal cortical dysplasia (FCD).

We identified three pathogenic variants in *CHRNA4* (2.9%, CI: 0.6-8.3%), two of whom novel; one pathogenic variant in *KCNT1* (1%, CI: 0.02-5.29%); four loss-of-function variants in *DEPDC5* (3.9%, CI: 1.1-9.7%), one of whom never reported; finally, one missense change in *NPRL2* (1%, CI: 0.02-5.29%), already reported as pathogenic. Three out of the four patients with *DEPDC5* mutations had FCD.

Conclusions: The overall frequency of pathogenic variants in our SHE cohort was 8.7%, 19% and 7% considering familial and sporadic cases, respectively. Pathogenic variants in the GATOR1-

complex genes account for 5% of the cases. *DEPDC5* shows the highest mutations frequency, especially in patients with genetic-structural etiology. From a practical perspective, analysis of this gene is recommended even in isolated cases, because of possible implications for patient management.

Keywords: sleep-related hypermotor epilepsy• nocturnal frontal lobe epilepsy • genetics

Abbreviations: SHE: Sleep-related Hypermotor Epilepsy; ID: Intellectual disability; FCD: Focal Cortical Dysplasia

INTRODUCTION

Sleep-related hypermotor epilepsy (SHE), previously Nocturnal Frontal Lobe Epilepsy (NFLE), is characterized by hypermotor seizures arising predominantly from sleep. Recognized etiologies include acquired injuries, structural anomalies and genetic causes [1]. The first gene for SHE, *CHRNA4* (Cholinergic Receptor Nicotinic Alpha 4 Subunit MIM *118504), was identified in 1995 by linkage analysis in a large pedigree showing an autosomal dominant pattern of transmission (ADSHE). Subsequently, mutations in two homologous genes, *CHRNB2* (Cholinergic Receptor Nicotinic Beta 2 Subunit, MIM *118507) and *CHRNA2* (Cholinergic Receptor Nicotinic Alpha 2 Subunit, MIM *118502) have been highlighted [2].

For about two decades no further genetic determinants of SHE have been identified. Only since 2012, the application of next generation sequencing (NGS) technologies allowed to study nuclear pedigrees not suitable for linkage analysis, or even sporadic cases, leading to the identification of four additional main genes: *KCNT1* (Potassium Sodium-Activated Channel Subfamily T Member 1, MIM *608167) [3], *DEPDC5* (DEP Domain Containing 5, MIM *614191) [4], *NPRL2* (NPR2-like Protein, MIM *607072) [5] and *NPRL3* (Nitrogen Permease Regulator-like 3, MIM *600928) [6].

Mutations in these genes cumulative explain about 30% of families [7]. This is an approximate estimate coming from studies of different case-series (principally families), each focused on the screening for mutations in a single SHE gene. A systematic study on the contribution of each gene to the overall disorder is lacking. We studied an Italian cohort of sporadic and familial SHE patients and assessed the frequency of pathogenic variants in the main genes implicated thus far in SHE. This would provide important perspectives for clinical genetic testing, prognosis and management of the disorder.

MATERIALS AND METHODS

The study was approved by the Ethics Committee (Prot. N 945/CE; cod CE: 13084).

POPULATION AND INCLUSION CRITERIA

The study population encompasses patients referred to our Institute and diagnosed with SHE according to recommended diagnostic criteria [1]. Additional cases were referred from other Italian epilepsy Centers, thanks to the collaboration with the Italian League against Epilepsy (LICE).
All patients who, after signing appropriate consent, underwent NGS analysis were included in the present study.
We enrolled 103 individuals among sporadic and familial cases, the latter defined as having at least one relative within two degrees of relatedness affected with SHE and/or other epilepsy.
All probands underwent a comprehensive evaluation including video-polygraphic monitoring and targeted 3T-brain MRI.

GENETIC ANALYSIS

Genetic analysis was performed by two comparable NGS techniques: whole exome sequencing (WES) and a multigene NGS panel including the main SHE genes (*CHRNA4*, *CHRNB2*, *CHRNA2*, *KCNT1*, *DEPDC5*, *NPRL2*, *NPRL3*). *CRH* (corticotropin-releasing hormone, MIM *122560) and *PRIMA1* (Proline-rich membrane Anchor 1, MIM * 613851), whose variants were anecdotally implicated in inherited forms of SHE (autosomal dominant and recessive, respectively) [7, 8] but not confirmed in other cases, had been not included in the epilepsy panel. Both the NGS techniques

showed, for each gene, a coverage of 20X in more than about 90% of all targeted regions, as shown in Supplemental Table 1. Supplemental material provides further details on NGS assays.

The identified variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [9] and segregation verified by Sanger sequencing. For pathogenicity predictions we used M-CAP (Mendelian Clinically applicable Pathogenicity, http://bejerano.stanford.edu/mcap/) for missense variants and HSF (Human Splicing Finder v3.0, http://www.umd.be/HSF/) for splice-region variants.

STATISTICS

Continuous variables were presented as mean±standard deviation, categorical variables as absolute and relative frequency (%). We used binomial Exact test to calculate 95% confidence intervals (CI).

RESULTS

STUDY POPULATION

We included 103 patients (M/F:61/42) diagnosed with SHE who underwent NGS. The mean age at epilepsy onset was 11.7±3.65 years. Sixteen patients (15.5%) had a family history for SHE (6.8%) and/or other epilepsy (8.7%). Twenty-six (25.2%) patients had brain abnormalities on MRI or detected only after histopathologic analysis of surgical specimen. In 17 (16.5%) the abnormalities were consistent with focal cortical dysplasia (FCD), confirmed by histopathology in four (3.9%).

GENETIC ANALYSIS

Fifteen patients underwent the multigene epilepsy panel and 88 WES (Supplemental material). Table 1 summarizes the genetic findings. Figure 1 provides the mutation frequency of SHE genes in the whole series and among familial and sporadic cases (1A), with the pedigrees of cases not reported (1B).

We identified three pathogenic variants in *CHRNA4* (2.9%, CI: 0.6-8.3%). The p.Ser284Leu (rs28931591) occurred *de novo* in a patient with early-onset refractory seizures and intellectual disability (ID) (Figure 1B, pedigree 1). The variant is a hotspot mutation associated with a CpG hypermutable site in the TM2 domain, the major pore-forming part of each nicotinic acetylcholine

receptor (nAChR) subunit, and corresponds to the p.Ser252Leu mutation reported in four families and one isolated case [2]. A novel missense change p.Ser284Trp, affecting the same amino acid residue of the previously mutation (p.Ser284Leu), was detected in a sporadic case with refractory SHE and borderline IQ (Figure 1B, pedigree 2). The variant is predicted to be damaging and it is absent in the healthy father and brother (healthy mother deceased). Finally, one novel heterozygous missense change p.Gly307Val segregates in two affected sisters of the ADSHE pedigree 3 (Figure 1B), inherited from the asymptomatic father. It is predicted as being damaging and affects a conserved amino acid residue located in the first extracellular loop between the transmembrane domains TM2 and TM3. Moreover, since missense variants with incomplete penetrance are a common mechanism of disease in *CHRNA4*-related ADSHE, we considered this change as causative (see ACMG scores, Table 1).

In an isolated patient with ID (Figure 1B, pedigree 4) we identified a *de novo* pathogenic variant in *KCNT1* (1%, CI: 0.02-5.29%): the missense change p.Ala934Thr (rs397515403) has been already reported as pathogenic in a patient with Malignant Migrating Focal Seizures of Infancy (MMFSI) [10]. Instead, our patient had a typical SHE. She presented at age 9 years with asymmetric tonic seizures showing a spontaneous remitting-relapsing evolution, without a clear-cut drug-resistance.

We found four loss-of-function variants in *DEPDC5* (3.9%, CI: 1.1-9.7%): one novel frameshift (p.Thr381Hisfs*15) was detected in a sporadic case (Figure 1B, pedigree 5), while the remaining (p.Arg389Profs*2, p.Arg422*, c.193+1G>A) have been already published (Table 1) [11, 12]. Interestingly, three of these patients have FCD.

Finally, the p.Leu105Pro in *NPRL2* (1%, CI: 0.02-5.29%) was detected in one familial case already reported [5].

We also identified novel/ultra-rare missense changes in *CHRNA4*, *CHRNB2*, *KCNT1* and *DEPDC5*, classified as variants of unknown significance (VoUS) [9] (Supplementary table 2).

DISCUSSION

We performed a genetic study on 103 SHE patients and provided the frequency of mutations in the main genes so far implicated in SHE. The main innovation of the study is the size of the cohort investigated by a systematic approach. Overall, we identified pathogenic variants in 8.7% of the whole. The detection rate in familial and sporadic cases was 19% and 7%, respectively.

Among the nAChR subunits genes, we found pathogenic variants only in *CHRNA4*, which account for about the 3% among familial and isolated SHE cases.

Mutations in *KCNT1* account for 1.15% of our sporadic cases. Although this gene has been implicated in early-onset refractory SHE with ID/psychiatric disorders [3] our patient did not show features of disease severity except for ID, even carrying the same *de novo* missense mutation as a reported patient with MMFSI [10]. Other variants in *KCNT1* give rise to either SHE or MMFSI, suggesting that the genotype-phenotype correlations is not straightforward [13].

Altogether, we found pathogenic variants in *DEPDC5* and *NPRL2,* encoding for components of the mTOR GATOR1-complex, in about 5% of our patients. This percentage is slight less than other cases-series implicating GATOR1-complex genes in 6.93% of heterogeneous autosomal dominant focal epilepsies [5]. However our result is justified by the fact that isolated cases are predominant in our cohort (84.5% versus 15.5% familial cases). *DEPDC5* showed the highest mutation rate, especially in patients with malformation of cortical development, confirming its relevance in genetic–structural etiology of SHE. In this view, detection of mutations in this gene may represent a red flag for FCDs, the most common potentially treatable architectural disorder underlying refractory epilepsies. In apparent non-lesional cases carrying pathogenic variants of GATOR1-complex genes, repeated and careful review of targeted, high-resolution neuroimaging is needed to highlight subtle structural abnormalities susceptible of surgery. Although mutated cases who underwent epilepsy surgery are anecdotal, this has proved to be curative in most of them [11], suggesting that epileptogenesis is underpinned by a genetically-determined cerebral structural lesion, even in the presence of germline mutations.

We did not detect pathogenic variants in the gene encoding the third component of GATOR1-complex, *NPRL3*, so far implicated in five among familial and sporadic cases with SHE [11].

CONCLUSIONS

This study of a representative case-series of SHE confirms the genetic heterogeneity of the syndrome and the prominent role of GATOR-1 complex genes, in particular *DEPDC5*. From a clinical perspective, the sequencing of these genes is worth even in isolated cases for whom a genetic etiology is not primarily considered, because of possible implications for the diagnostic work-up and clinical management.

Table 1: Pathogenic and likely pathogenic variants in SHE-associated genes identified in our cohort.

GENE	**FAM/ SPO**	**IDENTIFIED PATHOGENIC VARIANTS**				**Inheritance**	
		Chromosomal position (GrCH37)	**c.DNA nucleotidic change**	**Protein aminoacidic change**	**Mutation type**		
CHRNA4	Spo	g.61981912G>A$^{(W)}$	c.851C>T	p.Ser284Leu	*Missense*	*De novo*	
	Spo	g.61981912G>C$^{(W)}$	c.851C>G	**p.Ser284Trp**	*Missense*	Incomplete segregation study	
	Fam	g. 61981843C>A$^{(W)}$	c.920G>T	**p.Gly307Val**	*Missense*	Paternal	
KCNT1	Spo	g.138671275G>A$^{(W)}$	c.2800G>A	p.Ala934Thr	*Missense*	*De novo*	
DEPDC5	Spo	g.32200849dupC$^{(P)}$	c.1165dupC	p.Arg389Profs*2 [11]	Frameshift	Unknown	
	Fam	g.32202154C>T$^{(P)}$	c.1264C>T	p.Arg422*[11]	Nonsense	Paternal	
	Spo	g.32156689G>A$^{(W)}$	c.193+1G>A	p.(?)[12]	Canonical splice-site variant	Maternal	
	Spo	g.32202115delA$^{(W)}$	c.1225delA	**p.Thr409Hisfs*15**	Frameshift	Unknown	
NPRL2	Fam	g.50387121A>G$^{(W)}$	c.314T>C	p.Leu105Pro[5]	*Missense*	Maternal	

Abbreviations: FAM: familial; SPO: sporadic; (P) panel; (W) WES; M-CAP: Mendelian Clinically Applicable Pathogenicity; D: possibly pathogenic variant; N/A: not available.
ACGM scores to assess the variant pathogenicity according the Americans College of Medical Genetics guideline [9]:
PVS1: Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease;
PM1: Located in a mutational hotspot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation;
PM2: Absent from controls (or at extremely low frequency if recessive)
PM6: Assumed de novo, but without confirmation of paternity and maternity;

PP1:Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease
PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease;
PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.);
PP4: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology;
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.
Novel pathogenic variants are indicated in bold

Declarations of interest: none

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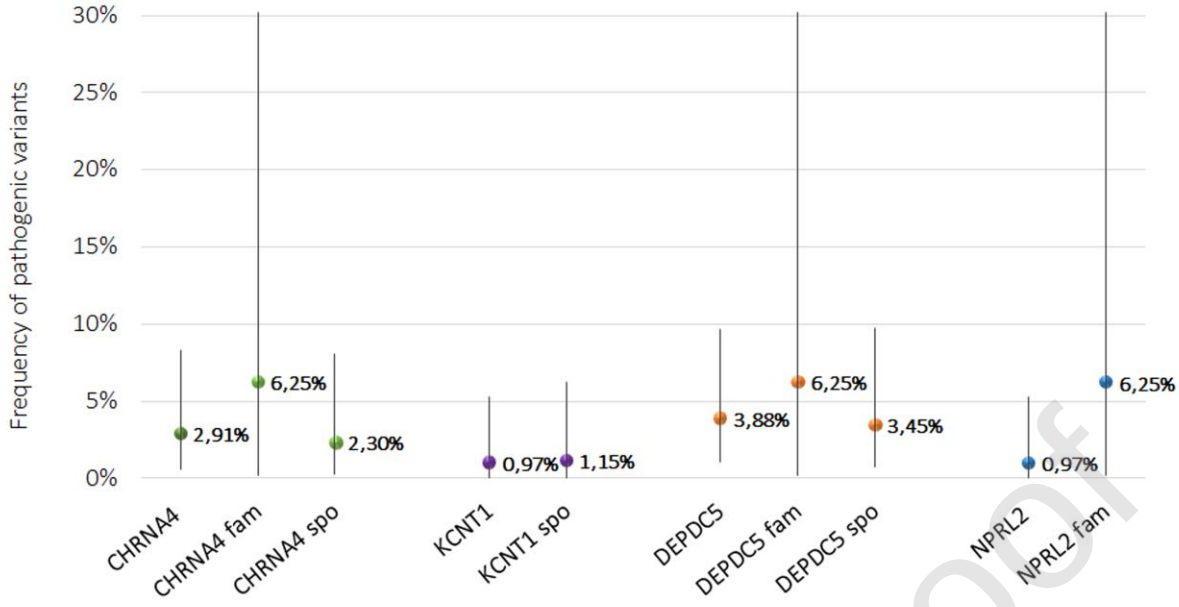
Figure legends

Figure 1A: Frequency of pathogenic variants in SHE genes in our case-series. For each mutated gene the overall frequency of mutations and their distribution among familial (fam)/sporadic (spo) cases are reported.

Figure 1B: pedigrees of familial/sporadic cases carrying pathogenic variants in SHE genes (unpublished cases).

A



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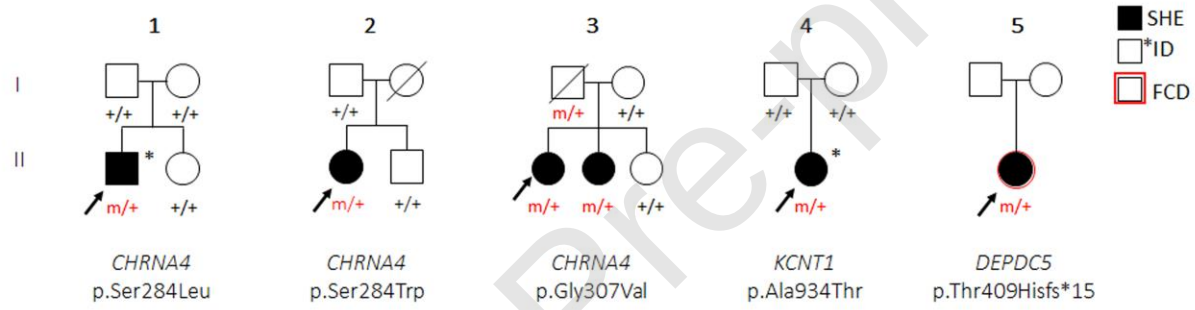

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