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Clinical spectrum and genotype-phenotype correlations in *PRRT2* Italian patients

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ABSTRACT

Prrt2 is a neuron-specific protein expressed at axonal and pre-synaptic domains, involved in synaptic neurotransmitter release and modulation of intrinsic excitability. Mutations in *PRRT2* cause a spectrum of autosomal dominant paroxysmal neurological disorders including epilepsy, movement disorders, and hemiplegic migraine and show incomplete penetrance and variable expressivity.

We assessed the diagnostic rate of *PRRT2* in a cohort of Italian patients with epilepsy and/or paroxysmal kinesigenic dyskinesia (PKD) and evaluated genotype-phenotype correlations. Clinical data were collected using a structured questionnaire.

Twenty-seven out of 55 (49.1%) probands carried *PRRT2* heterozygous pathogenic variants, including six previously known genotypes and one novel missense mutation. A family history of epilepsy starting in the first year of life and/or PKD was strongly suggestive of a *PRRT2* pathogenic variant. Epilepsy

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patients harbouring *PRRT2* pathogenic variants showed earlier seizure onset and more frequent clusters compared with *PRRT2*-negative individuals with epilepsy. Moreover, we did also identify individuals with *PRRT2* pathogenic variants with atypical age at onset, i.e. childhood-onset epilepsy and infantile-onset PKD. However, the lack of a clear correlation between specific *PRRT2* genotypes and clinical manifestations and the high incidence of asymptomatic carriers suggest the involvement of additional factors in modulating expressivity of *PRRT2*-related disorders. Finally, our study supports the pleiotropic and multifaceted physiological role of *PRRT2* gene which is emerging from experimental neuroscience.

1. Introduction

PRRT2 is located on chromosome 16p11.2 and encodes for a 340 amino acids protein (proline-rich transmembrane protein 2) expressed in the central nervous system, consisting of a proline-rich strand in the N-terminal and two *trans*-membrane (TM) domains in the C-terminal [1]. This protein is involved in the modulation of neurotransmitter release [2] and modulates the intrinsic neuronal excitability by interacting with $Na_v1.2/1.6$ [3].

PRRT2-associated neurological disorders share autosomal dominant inheritance, incomplete penetrance, and variable expressivity [4]. The core of this spectrum is represented by infantile-onset, self-limiting epilepsy [5], paroxysmal kinesigenic (PKD) dyskinesias [6,7], and hemiplegic migraine (HM) [8]. However, *PRRT2* pathogenic variants have also been associated with other childhood-onset epilepsies, migraine/headache, and movement disorders as well as with episodic ataxia, and isolated intellectual disability [4,9].

We aimed to assess the diagnostic impact of *PRRT2* in a series of patients with epilepsy and/or PKD and explored their genotype-phenotype correlations.

2. Methods

2.1. Inclusion criteria

We collected data from patients with epilepsy and/or PKD referred for *PRRT2* testing to the Laboratory of Neurogenetics at 'Gaslini Children's Hospital' between 2014 and 2018. Individuals with additional neurological features (e.g., migraine or intellectual disability/developmental delay), were allowed to enter the study. Patients or their relatives gave written informed consent to the study, which was approved by the local EC.

Retrospective clinical information was obtained from a structured questionnaire provided by each referring physician. The following clinical information was collected: family history, age of onset, seizure types, neurological examination, EEG findings, brain MRI, and antiseizure therapy. Epileptic seizures were defined according to ILAE 2017 criteria [10] and epilepsy types were classified based on the age at onset as follows: neonatal (0–1 month), neonatal-infantile (1–5 months), infantile (6–12 months), childhood (after 1 year) onset. PKD was defined by Bruno et al. criteria [11].

2.2. Genetic analysis

EDTA blood samples were obtained from probands. DNA extraction and Sanger sequencing were performed as described [12]. Variants were denominated according to RefSeq NM_145239 and the effect of novel variants was estimated using the available tools. Segregation analysis was performed in available family members. Genetic results were blinded and disclosed to the first author (G.B.) before the analysis of the results.

3. Results

3.1. Clinical features of the probands

We collected fifty-five (29 girls) probands referred for genetic testing at a mean age of 5.7 years (range: 6 months-30 years). Median follow-up duration was 3 years (range: 4 months–20 years). A family history of epilepsy or PKD was reported in first-degree relatives of 18 probands. Most subjects (48/55) were referred for epilepsy; seven probands were tested for PKD (Supplementary Fig. 1). Two patients with PKD presented also infantile epilepsy. Follow-up data were not available for 5 patients. Additional clinical features were reported in 14 probands (25%), including mild intellectual disability/developmental delay in 11 individuals and hemiplegic migraine (HM) in one individual. Detailed clinical data are provided in Table 1.

3.2. Genetic findings and segregation analysis

PRRT2 pathogenic variants were identified in 27 (49.1%) out of the 55 probands and were inherited in 21 (78%) subjects. The inherited variants were found in a total of 37/54 (68.5%) tested relatives (21 parents, 10 siblings, 6 others) and were associated with clinical symptoms (15 epilepsy, 4 PKD) in 19 out of 37 (51%) subjects. One proband referred for infantile-onset epilepsy carried a *de novo* pathogenic variant. Segregation analysis was not available for six probands.

3.3. PRRT2-associated phenotypes

The twenty-one probands with pathogenic *PRRT2* variants and epilepsy presented with a homogeneous phenotype, characterized by focal motor seizures starting with gaze staring and head deviation, with or without secondary generalization, usually occurring in cluster(s). One patient with childhood-onset experienced focal non-motor seizures with vomit, dyscognitive features, and loss of contact. We observed a bimodal peak in seizures onset,with one peak at 6 months and another at 3 years. Most patients (18; 86%) developed epilepsy between 3 and 12 months of life. The three (14%) patients with childhood-onset symptoms experienced their first seizure between age 2.5 and 3.5 years. Mean epilepsy duration was 7.1 ± 4.8 months for all the probands, with no difference ($p=0.56$) based on the age of onset, except for one patient with childhood epilepsy who remitted after 8 years since the onset.

Ictal EEG often showed parietal-occipital epileptic activity that could eventually become bilateral and diffused (Supplementary Fig. 2). The interictal EEG during follow-up was clinically unremarkable in all patients; one female patient with childhood-onset epilepsy had photosensitivity. Available brain imaging (MRI) findings were within the normal range for the age.

Seventeen patients received anti-seizure medications. The most frequently prescribed monotherapies were phenobarbital (7), valproate (5), carbamazepine (3); two patients with childhood epilepsy

Table 1
Summary of probands' clinical features and genetic analysis results per subgroups.

Probands' phenotypes, *PRRT2* status and diagnostic yield per subgroups	*PRRT2* pathogenic variant (*n*)	*PRRT2 wild-type (n)*	*Tot* (n = 55)
Neonatal onset epilepsy (0–1 m)	*0 (0%)* – –	*1 (100%)* O: 0.2 m R: n.a.	*1* (1.8%)
Neonatal/Infantile onset epilepsy (1–5 m)	*10 (77%)* O: 4.4 ± 0.57 m R: 11.69 ± 6.42 m	*3 (23%)* O: 2,7 ± 1,4 m R: 1 patient at 4 months; 2 n.a.	*13* (23.6%)
Infantile onset epilepsy (6–12 m)	*8 (36%)* O: 6.5 ± 0.88 m R: 13.33 ± 5.29 m	*14 (63%)* O: 8.22 ± 1.86 m R: 14.22 ± 4.9 m	*22* (40%)
Childhood-onset epilepsy (>1 y)	*3 (25%)* O: 3 ± 0.5 y R: 6.5 ± 4.77 y	*9 (75%)* O: 3.14 ± 1.66 y R: 3.16 ± 0.75 y 2 n.a.	*12* (21.8%)
PKD	*6 (86%)* O: 8.3 y (range 1–25 y) R: no	*1 (14%)* O: 4 y R: no	*7* (12.7%)
Tot (n = 55)	*27* (49.1%)	*28* (51.9%)	*55*
Additional features	*7* DD/ID *1* HM	*4* DD/ID	
Family history of epilepsy and/or PKD	*11* epilepsy *1* epilepsy and PKD (67%)	*6* epilepsy (33%)	*18* (32.7%)

DD/ID, developmental delay/intellectual disability; HM, hemiplegic migraine; m, months; O, onset; PKD, paroxysmal kinesigenic dyskinesia; R, remission; y, year.
Age at onset (O) and remission (R) are presented as *mean ± standard deviation*.

received combination therapy (valproate + ethosuximide ± carbamazepine); one patient remitted without treatment; no data were available for three patients.

The probands with PKD and pathogenic *PRRT2* variants suffered from attacks starting between age 8 and 10 years in four cases; the other two subjects experienced the first symptom at age 12 months and 25 years, each. All the attacks occurred in concomitance with sudden movements, with retained consciousness, often preceded by a sensory aura in the lower limbs or head. Five probands were successfully treated with carbamazepine. During the first decade of life, one patient developed hemiplegic migraine (HM). The attacks were characterized by intense pulsating headache with paresthesia, left upper limb atonia, aphasia, lasting up to 2 h, and then followed by headache and confusion. Mild intellectual disability/developmental delay occurred in 7 probands harbouring pathogenic *PRRT2* variants.

The phenotype showed by family members harbouring *PRRT2* pathogenic variants did reflect that showed by the affected proband. Self-limiting epilepsy with predominant focal motor seizures and neonatal/infantile-onset was reported in 9 relatives (3 parents, 4 siblings, 2 others) whereas infantile-onset occurred in three individuals (2 parents, 1 sibling); absence epilepsy was reported in two parents and febrile seizures in one sibling. PKD occurred in 3 individuals from the same family (father, paternal grandmother, paternal aunt); PKD and infantile epilepsy co-segregated in another kindred, affecting the maternal grandmother but not proband's asymptomatic mother.

Among family members carrying *PRRT2* pathogenic variants, 11 individuals were unaffected (8 parents, 3 siblings); insufficient clinical information was available in 5 subjects. Overall, the penetrance of PRRT2 pathogenic variants was 89% (76% for epilepsy, 50% for PKD) in our cohort.

3.4. Genotype-phenotype correlations

The diagnostic yields for *PRRT2* genetic sequencing in different probands subgroups are summarized in Fig. 1. Genotype-phenotype correlations are summarized in Fig. 2.

Seven different heterozygous genotypes were identified in our probands: c.649dupC (21, of whom 1 *de novo*; 77.8%), c.649del (1; 3.7%), c.649C>T (1; 3.7%), c.679C>T (1; 3.7%), c.809T>A (1; 3.7%), c.872C>T (1; 3.7%), and whole gene deletion (1; 3.7%).

In line with Literature, the most common pathogenic variant was c.649dupC (p.R217Pfs*8), which was identified in 21 probands and 30 family members (8 healthy subjects) featuring both epilepsy and PKD; mild ID/DD co-occurred in 5 probands with epilepsy. No clinical information was available for six relatives.

The c.649C>T (p.R217X) variant did partially segregate in a small family with PKD and infantile epilepsy. The frameshift/truncating variant c.649del (p. Arg217Glufs*12) was identified in a man with mild ID and PKD from age 25 years. The truncating variant c.679C>T (p.R227X) was associated with infantile epilepsy and inherited from the healthy father. The missense variant c.872C>T (p.Ala291Val) was found in one family, associated with neonatal/infantile epilepsy, febrile seizures and one unaffected member. A whole gene deletion (p.0) was identified in a girl with focal non-motor seizures from age 3–8 years and mild intellectual disability. The deletion did not include other genes and was inherited from the healthy father. The missense variant c.809T>A (p. Ile270Asn) was identified in a girl with dyskinetic attacks triggered by sudden initiation of a movement and preceded by a sensory aura on the retro-popliteal region from age 9 years. In the following years, the patient did also suffer from migraine. This variant, inherited from the healthy father, was novel and absent in the clinical (HGMD and ClinVar) and the general population (gnomAD) databases. It was considered "likely pathogenetic" according to ACMG criteria as it affects a conserved nucleotide (GERP+ 4.32) and is predicted to have a deleterious effect. Detailed genotype-phenotype associations in the families can be found in Supplementary Table 1.

We also looked whether probands with *PRRT2* pathogenic variants manifest distinctive features compared to *wild-type (wt)* probands. The diagnostic rate for *PRRT2* variants was 67% (12/18) in probands with a history of epilepsy and/or PKD in first-degree family

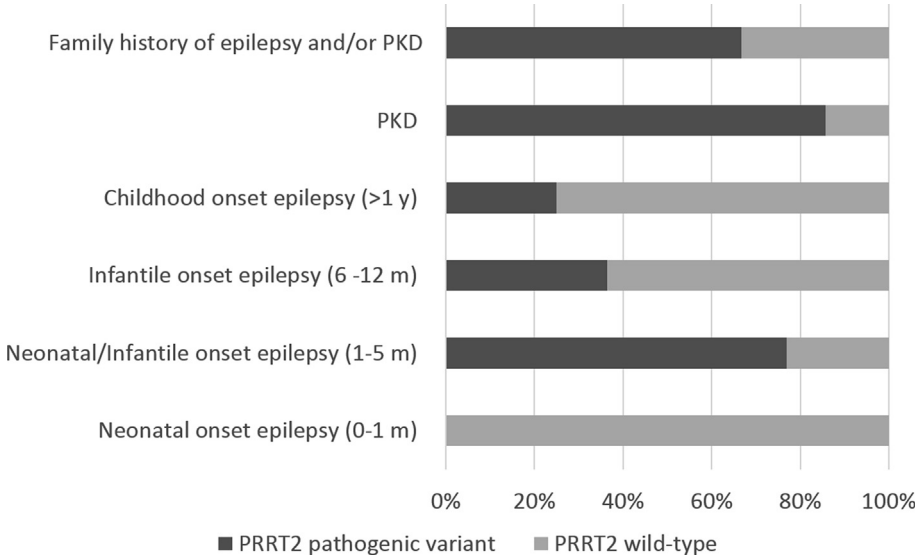

Fig. 1. Diagnostic yield of *PRRT2* sequencing in different subgroups of probands.

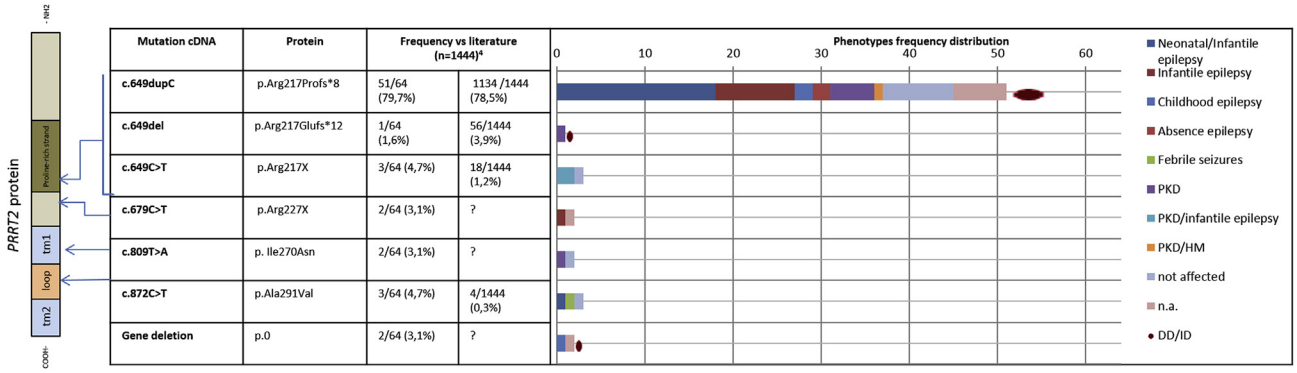

Fig. 2. List of the pathogenic variants, the effect on protein, the frequency in our cohort and the associated phenotypes.
DD/ID: developmental delay/intellectual disability; HM: hemiplegic migraine; PKD: paroxysmal kinesigenic dyskinesia.

members. Seizures clusters were more frequent in *PRRT2*-mutated (19; 90%) compared to *wild-type* (15; 55%) ($p = 0.012$, *Test Fisher*) patients. Individuals harbouring a pathogenic *PRRT2* variant showed earlier onset epilepsy (5.4 ± 1.3 months, mean and SD) within the 1st year of life compared to *wt* patients (7.9 ± 2.7 months) ($p = 0.003$, unpaired *t*-test). This is also reflected by the diagnostic yields for *PRRT2* variants based on the age at onset of epilepsy: 77% in neonatal/infantile epilepsy and 36% in infantile epilepsy.

4. Discussion

PRRT2 was the most common single-gene epilepsy (1 per 9970) in a large prospective National epidemiological cohort study showing that presentation before the age of 6 months with afebrile focal seizures were significantly associated with a genetic diagnosis [13].

In this study, we adopted wide clinical inclusion criteria to introduce an element of the originality. A *PRRT2* pathogenic variant was found in the 44% of probands with epilepsy and in the 86% of probands with PKD. The 67% of probands who referred a family history of epilepsy and/or PKD tested positive for *PRRT2* pathogenic variants; thus, positive family history is strongly suggestive for *PRRT2* mutations. However, no family history of *PRRT2* spectrum disorders was reported in 40% of the probands with pathogenic *PRRT2* variants (50% of cases with PKD and 52% of seizures cases), which is way higher than reported in the Literature (1–12%) [4]. This can be explained by the incomplete penetrance, and by a recall bias of the family members concerning early life events.

Our analysis confirms the major causative role of *PRRT2* pathogenic variants in self-limited epilepsy of the first months of life, especially when family history is present. We did also show that the occurrence of cluster(s) of focal seizures in the first year of life is strongly indicative for *PRRT2* disorder, supporting the recent findings by van Roest et al. [14], in which about half of the patients carried a missense variant or a deletion involving *PRRT2*.

PRRT2 is also a major gene accounting for PKD [15,16]. Indeed, the mutation rate for PKD in our cohort is 86%. We cannot exclude nor predict the future occurrence of PKD in our patients and, therefore, the family should be advised about this possible evolution during genetic counselling. A prospective study may be beneficial to assess the impact of *PRRT2* pathogenic variants to predict the development of a paroxysmal disorder later in life. In our series, one proband with PKD and c.649dupC developed hemiplegic migraine (HM) in the first decade. HM is the third common diagnosis in *PRRT2* pathogenic variants (2.35%) [4], suggesting a causal association. Other forms of migraine are also reported, with or without aura, but whether *PRRT2*

variants predispose to migraine cannot be established due to its high prevalence in the general population.

Recently, childhood-onset epilepsy has been reported as part of the *PRRT2* phenotypic spectrum [17]. In this study, we did also identify patients with unusual clinical features (i.e. 3 with childhood-onset epilepsy, 1 with early-onset PKD) who would have been neglected if adopting narrow inclusion criteria (i.e. infantile-onset epilepsy and childhood-onset PKD).

In this study, the most common pathogenic variant c.649dupC (p.Arg217Profs*8) causes frameshift with a premature stop codon and is located in the nine-cytosine stretch, the mutational hotspot in the *PRRT2* gene. The c.649dupC variant was found in all the different phenotypes of our cohort. Other common variants, c.649C>T (p.Arg217X) and c.649del (p.Arg217Glufs*12) are located in the same stretch. In the Literature, these variants have been identified in different populations and across different phenotypes, including *de novo* occurrence in sporadic cases, highlighting the mutational hotspot of the region [16]. The other identified pathogenic variants c.679C>T (p.Arg227X), c. 809T>A (p. Ile270Asn), c.872C>T (p.A291V), and the gene deletion led to the same paroxysmal phenotypes, with no significant differences in presentation with those underlined by the most common C duplication in 649. These findings confirm the variability and pleiotropy linked to *PRRT2* variants. We did also identify a previously unreported missense variant, i.e., c.809T>A (p.Ile270Asn), located in a highly preserved residue towards the C-terminus, in the first transmembrane domain of the protein (Fig. 2). According to Zhao SY et al., changes clustered in this region affect the protein levels or the localization of the protein at the membrane, thus leading to a loss-of-function [18].

Segregation analysis in our families with pathogenetic *PRRT2* variants confirms the incomplete penetrance of the *PRRT2*-related diseases, suggesting the presence of unknown factors acting at different levels, such as modifier genes or methylation patterns. The incomplete penetrance may also represent a challenge when interpreting genetic results; in fact, the identification of a *PRRT2* variant does not exclude the presence of possible causative variants in other genes. Therefore, the electro-clinical evaluation must remain a major diagnostic tool and guide the interpretation of genetic data.

5. Conclusions

PRRT2 is a gene characterized by a remarkable pleiotropy, leading to a spectrum of disorders ranging from seizures to movement disorders, and still evolving through the progressive association with new clinical entities. Indeed, we report patients with unusual clinical presentation (childhood-onset epilepsy, early-onset PKD). There are no clear genotype-phenotype correlations established that can predict the phenotype in a carrier, especially the manifestation of a movement disorder later in life. Future studies may lead to describe more precise correlations by the screening of wider cohorts of patients and individuals from the general population. Finally, the importance of good clinical records and case reports needs to be underlined, as the clinics represents the main tool for the interpretation of the increasing amount of genetic data.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2020.06.005.

References

[1] P. Rossi, B. Sterlini, E. Castroflorio, et al., A novel topology of proline-rich transmembrane protein 2 (*PRRT2*): hints for an intracellular function at the synapse, J. Biol. Chem. 293 (12) (2018 Mar 23) 4581, https://doi.org/10.1074/jbc.AAC118.002651.

[2] F. Valtorta, F. Benfenati, F. Zara, J. Meldolesi, *PRRT2*: from paroxysmal disorders to regulation of synaptic function, Trends Neurosci. 39 (10) (2016 Oct) 668–679, https://doi.org/10.1016/j.tins.2016.08.005.

[3] F. Fruscione, P. Valente, B. Sterlini, et al., *PRRT2* controls neuronal excitability by negatively modulating Na+ channel 1.2/1.6 activity, Brain 141 (4) (2018) 1000–1016.

[4] D. Ebrahimi-Fakhari, A. Saffari, A. Westenberger, C. Klein, The evolving spectrum of *PRRT2*-associated paroxysmal diseases, Brain 138 (Issue 12) (December 2015) 3476–3495, https://doi.org/10.1093/brain/awv317. Schubert J, Paravidino R, Becker F, et al., PRRT2 mutations are the major cause of benign familial infantile seizures. Hum Mutat. 2012 Oct;33(10):1439-43. doi: 10.1002/humu.22126.

[5] W.J. Chen, Y. Lin, Z.Q. Xiong, et al., Exome sequencing identifies truncating mutations in *PRRT2* that cause paroxysmal kinesigenic dyskinesia, Nat. Genet. 43 (12) (2011) 1252–1255.

[6] X.R. Liu, D. Huang, J. Wang, et al., Paroxysmal hypnogenic dyskinesia is associated with mutations in the *PRRT2* gene, Neurol. Genet. 2 (2) (2016 Mar 22) e66, https://doi.org/10.1212/NXG.0000000000000066.

[7] F. Riant, E. Roze, C. Barbance, et al., *PRRT2* mutations cause hemiplegic migraine, Neurology 79 (21) (2012 Nov 20) 2122–2124, https://doi.org/10.1212/WNL.0b013e3182752cb8.

[8] I. Maini, A. Iodice, C. Spagnoli, et al., Expanding phenotype of *PRRT2* gene mutations: a new case with epilepsy and benign myoclonus of early infancy, Eur. J. Paediatr. Neurol. 20 (3) (2016 May) 454–456.

[9] I.E. Scheffer, S. Berkovic, G. Capovilla, et al., ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology, Epilepsia 58 (4) (2017) 512–521, https://doi.org/10.1111/epi.13709.

[10] M.K. Bruno, M. Hallett, K. Gwinn-Hardy, et al., Clinical evaluation of idiopathic paroxysmal kinesigenic dyskinesia, Neurology 63 (12) (Dec 2004) 2280–2287, https://doi.org/10.1212/01.WNL.0000147298.05983.50.

[11] F. Zara, N. Specchio, P. Striano, et al., Genetic testing in benign familial epilepsies of the first year of life: clinical and diagnostic significance, Epilepsia 54 (3) (2013 Mar) 425–436, https://doi.org/10.1111/epi.12089. Epub 2013 Jan 29.

[12] J.D. Symonds, S.M. Zuberi, K. Stewart, et al., Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort, Brain 142 (8) (2019 Aug 1) 2303–2318, https://doi.org/10.1093/brain/awz195.

[13] A. van Roest, A. Van de Vel, D. Lederer, B. Ceulemans, The clinical and genetic spectrum in infants with (an) unprovoked cluster(s) of focal seizures, Eur. J. Paediatr. Neurol. (2019 Dec 13), https://doi.org/10.1016/j.ejpn.2019.12.003 pii: S1090-3798(19)30423-30424.

[14] A. Méneret, C. Gaudebout, F. Riant, M. Vidailhet, C. Depienne, E. Roze, *PRRT2* mutations and paroxysmal disorders, Eur. J. Neurol. 20 (6) (2013 Jun) 872–878, https://doi.org/10.1111/ene.12104. Epub 2013 Feb 9. Review. PubMed PMID: 23398397.

[15] F. Becker, J. Schubert, P. Striano, et al., *PRRT2*-related disorders: further PKD and ICCA cases and review of the literature, J. Neurol. 260 (2013) 1234–1244.

[16] L. Yang, C. You, S. Qiu, et al., Novel and de novo point and large microdeletion mutation in PRRT2-related epilepsy, Brain Behav. (2020), e01597, https://doi.org/10.1002/brb3.1597, 00.

[17] S.Y. Zhao, L.X. Li, Y.L. Chen, et al., Functional study and pathogenicity classification of *PRRT2* missense variants in *PRRT2*-related disorders, CNS Neurosci. Ther. (2019 May 23), https://doi.org/10.1111/cns.13147.