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ARTICLE

PRICKLE2 revisited—further evidence implicating *PRICKLE2* in neurodevelopmental disorders

Allan Bayat[1,2] · Sumaiya Iqbal[3,4,5] · Kim Borredy[6] · Jeanne Amiel[7,8,9] · Christiane Zweier[10,11] · Guilia Barcia[7,8,9] · Cornelia Kraus[10] · Heike Weyhreter[12] · Alexander G. Bassuk[13] · Maya Chopra[7,8,14] · Guido Rubboli[1,2] · Rikke S. Møller[1,2]

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Abstract

PRICKLE2 encodes a member of a highly conserved family of proteins that are involved in the non-canonical Wnt and planar cell polarity signaling pathway. Prickle2 localizes to the post-synaptic density, and interacts with post-synaptic density protein 95 and the NMDA receptor. Loss-of-function variants in prickle2 orthologs cause seizures in flies and mice but evidence for the role of *PRICKLE2* in human disease is conflicting. Our goal is to provide further evidence for the role of this gene in humans and define the phenotypic spectrum of *PRICKLE2*-related disorders. We report a cohort of six subjects from four unrelated families with heterozygous rare *PRICKLE2* variants (NM_198859.4). Subjects were identified through an international collaboration. Detailed phenotypic and genetic assessment of the subjects were carried out and in addition, we assessed the variant pathogenicity using bioinformatic approaches. We identified two missense variants (c.122 C > T; p.(Pro41Leu), c.680 C > G; p.(Thr227Arg)), one nonsense variant (c.214 C > T; p.(Arg72*) and one frameshift variant (c.1286_1287delGT; p.(Ser429Thrfs*56)). While the p.(Ser429Thrfs*56) variant segregated with disease in a family with three affected females, the three remaining variants occurred de novo. Subjects shared a mild phenotype characterized by global developmental delay, behavioral difficulties ± epilepsy, autistic features, and attention deficit hyperactive disorder. Computational analysis of the missense variants suggest that the altered amino acid residues are likely to be located in protein regions important for function. This paper demonstrates that *PRICKLE2* is involved in human neuronal development and that pathogenic variants in *PRICKLE2* cause neurodevelopmental delay, behavioral difficulties and epilepsy in humans.

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✉ Allan Bayat
abaya@filadelfia.dk

1 Department for genetics and personalized medicine, Danish Epilepsy Centre, Dianalund, Denmark

2 Institute for Regional Health Services Research, University of Southern Denmark, Odense, Denmark

3 Center for Development of Therapeutics, Broad Institute of MIT and Harvard, Cambridge, MA, USA

4 Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, USA

5 Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, USA

6 Department for neuropsychology, Danish Epilepsy Centre, Dianalund, Denmark

7 Department of genetics, Necker-Enfants Malades Hospital, Assistance Publique Hospital of Paris (AP-HP), Paris, France

8 UMR-1163, Imagine Institute, Paris, France

9 Paris Descartes University, Sorbonne Paris Cité, Paris, France

10 Institute of Human Genetics, Friedrich-Alexander-University, Erlangen-Nürnberg, Erlangen, Germany

11 Department of Human Genetics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

12 Department for Pediatrics, Danish Epilepsy Centre, Dianalund, Denmark

13 Department of Pediatrics and Neurology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA, USA

14 Translational Neuroscience Centre, Boston Children's Hospital, Boston, MA, USA

Introduction

The *PRICKLE2* gene [OMIM: 608501] is mapped to human chromosome 3p14 [1] and encodes the highly conserved Prickle, Espinas, Testin and Linl-1, Isl-1 and Mec-3 domain-containing protein which is involved in the non-canonical Wnt and planar cell polarity signaling pathway [2]. Prickle2 localizes to the post-synaptic density, and interacts with post-synaptic density 95 and the NMDA receptor [3]. *PRICKLE2* is highly intolerant to loss-of-function in the healthy population, demonstrated by a pLI (probability of loss-of-function intolerance) score of 1 and a loss-of-function observed/expected upper bound fraction score of 0.28 on the gnomAD database (https://gnomad.broadinstitute.org/).

Inherited biallelic variants in *PRICKLE1* have been reported in progressive myoclonus epilepsy—ataxia syndrome [4] [OMIM: 612437] while autism [5] and spina bifida [6] have been associated with heterozygous variants. *PRICKLE2* has also previously been implicated in neurodevelopmental disorders, but evidence to date is limited and conflicting. The first candidate gene study of *PRICKLE2* examined zebrafish assays, flies, mice, and human subjects [7]. In this report, zebrafish with a suspected pathogenic variant showed more convergent-extension defects than the wild-type and *PRICKLE2* knockout mice and flies were more susceptible to epileptic seizures [7]. In addition, both heterozygous and compound heterozygous variants in *PRICKLE2* were reported in several human subjects with epilepsy. One subject (subject 6) harbored a 2.22 Mb microdeletion (of unknown inheritance) encompassing *PRICKLE2*. Another subject (subject 4) belonged to a previously described pair of siblings [8] and harbored two compound heterozygous variants (c.443 G > A; p.(Arg148His) and 457 G > A, p.(Val153Ile)) in *PRICKLE2*. Whole exome sequencing has since identified compound heterozygous *POLG* variants (OMIM: 174763) in this subject and her sibling [9]. The authors argued that the reported *PRICKLE2* variants in subject 4 were unlikely to cause epilepsy and that variants in this gene should not be considered as disease causing in human [9]. Subject 5 was reported to have the variant c.1813G > T (p.Val605Phe), which today has been reported in three humans in gnomAD. In subjects 4–6, parents were unavailable for testing (personal communication).

Following this report eight additional subjects were reported to have a *PRICKLE2* related phenotype [10, 11]: six subjects from two different families in which a heterozygous *PRICKLE2* missense variant segregated with a neurodevelopmental delay and psychiatric symptoms [10], and a twin pair with autism and a de novo 6.88 Mb deletion of 3p14 (chr3: 60,472,496-67,385,119) [11]. The deleted region contained 17 genes, 5 of which are known or suspected to be related to central nervous system disorders: *FEZF2, SYNPR, ATXN7, PRICKLE2*, and *MAGI1* [11]. The authors suggested that *PRICKLE2* was the most likely cause for the autistic features exhibited by the twins [11].

These conflicting reports have resulted in uncertainty about the role of *PRICKLE2* as a human disease gene [9]. We have identified six novel subjects with presumed pathogenic variants in the *PRICKLE2* gene and describe the spectrum of symptoms, the genetic landscape and bioinformatic data associated with *PRICKLE2* related disorders. With this report, we add weight to the argument that *PRICKLE2* is implicated in a neurodevelopmental disorder.

Materials and methods

Cohort analysis

Subjects were identified through GeneMatcher [12] and recruited through their treating clinicians. Medical information including birth parameters, epilepsy, electroencephalograms (EEGs), developmental histories, brain magnetic resonance imagings (MRIs) and physical examinations were collected from the local healthcare providers. Subjects 4–6 were related while the remaining were not. The study was conducted in agreement with the Declaration of Helsinki and approved by the local ethics committees. All probands or, in case of minors, their parents or legal guardians gave informed consent.

Subject 6 was available for neuropsychological testing. She was tested with the fourth edition of the Wechsler Adult Intelligence Scale (WAIS-IV), Rey Auditory Verbal Learning Test (RAVLT), Rey Complex Figure Test (RCFT), Warrington's Recognition Memory Test for Faces (RMF), Design Fluency (5 Point Test) and Verbal Fluency Test, Sentence Repetition (NCCEA), Trail Making Test, Stroop Test and Conners Continuous Performance Test CPT3. In addition, self-report forms were administered: Behavior Rating Inventory of Executive Function (BRIEF-V) and Symptom Checklist-90-R (SCL-90-R). The references of tools employed will be shared upon request.

Genetic identification and analysis

Subjects 1–4 were investigated by whole exome sequencing ordered by healthcare providers. Due to their familiar relationship, subjects 5 and 6 were only tested for the *PRICKLE2* variant detected in subject 4. All variants are annotated using the NM_198859.4 (GRCh37/hg19) transcript of *PRICKLE2*. We predicted the functional alteration of missense variants using polymorphism phenotyping-2

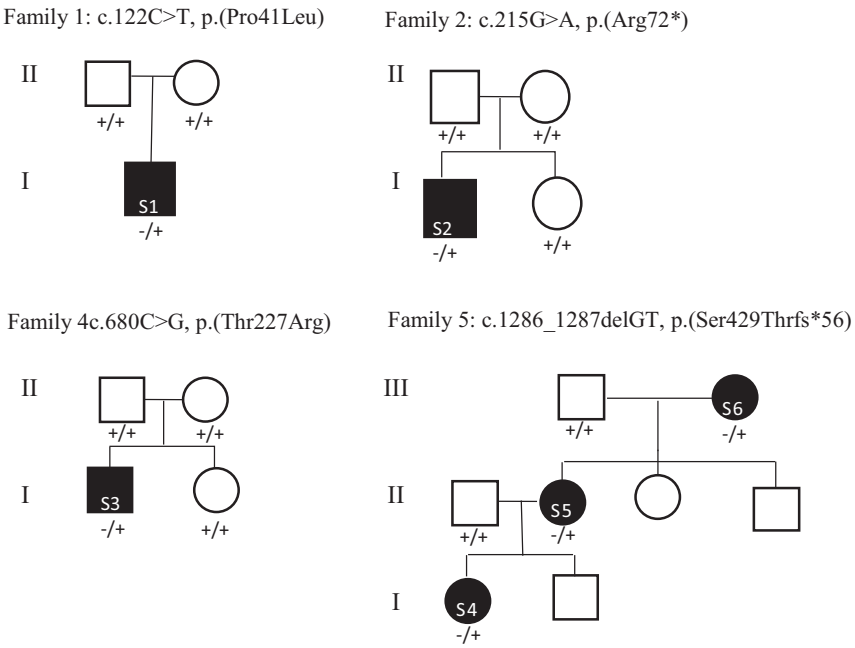

Fig. 1 Pedigree of four families with pathogenic *PRICKLE2* variants. Solid symbols (squares = males, circles = females) indicate clinically affected subjects; open symbols, unaffected subjects. Subjects with a rare and presumed disease causing variant in *PRICKLE2* are indicated by −/+; subjects tested for such rare variants and found to be negative are indicated by +/+. *S* = subject.

(PolyPhen-2) [13], SIFT (Sorting intolerant from tolerant) [14], MutationTaster (http://www.mutationtaster.org), and CADD (Combined annotation dependent depletion) [15] (https://cadd.gs.washington.edu). The Genome Aggregation Database (gnomAD v.2.1.1; https://gnomad.broadinstitute.org/) was employed to check the presence of the variants in control populations. All variants were described according to HGVS-nomenclature recommendations using Mutalyzer software (https://mutalyzer.nl/).

In subject 4, a genetic variant of unknown clinical significance was observed in heterozygous state in the HESX homeobox 1 (*HESX1*) gene, described as c.309 G > T, leading to the missense variant p.(Leu103Phe). The variant was not registered in dbSNP, but it was seen in 1/246146 healthy controls in gnomAD. It was not reported in HGMD. The prediction software SIFT and MutationTaster indicated a damaging effect of the variant, while PolyPhen-2 predicted it to cause a tolerant effect on protein function.

The *HESX1* gene encodes a conserved homeobox protein that is a transcriptional repressor in the developing forebrain and pituitary gland. Pathogenic variants have been associated with growth hormone deficiency with pituitary anomalies, combined pituitary hormone deficiency and septooptic dysplasia and both autosomal dominant and autosomal recessive modes of inheritance have been observed. We believe that the clinical features of the subject 4 is not compatible with a *HESXI*-deficiency.

Bioinformatic analysis of subject variant properties

The BLAST (Basic Local Alignment Search Tool) search was run with default parameters from UniProt webpage (https://www.uniprot.org/) to identify the close homologs of PRICKLE2. The program Clustal [16] was used for aligning the sequences. Mapping of posttranslational modification (PTM) sites on PRICKLE2 sequence was obtained directly from the PhosphoSitePlus web portal (https://www.phosphosite.org/uniprotAccAction?id=Q7Z3G6).

Results

We recruited six subjects from four unrelated families harboring rare heterozygous variants in *PRICKLE2*. Age spanned from 7 years to 68 years of life and two subjects were males. We identified two missense variants (c.122 C > T; p.(Pro41Leu), c.680 C > G; p.(Thr227Arg)) and two truncating variants (c.214 C > T; p.(Arg72*), c.1286_1287delGT; p.(Ser429Thrfs*56)). While the p.(Ser429Thrfs*56) variant segregated with the phenotype in a family with three affected females, the three remaining variants occurred de novo. The pedigrees are shown in Fig. 1, and a brief overview of the clinical, MRI, electrophysiological and genetic features of the six novel *PRICKLE2* cases is provided in Table 1.

Perinatal period

Data on pregnancy and birth was not available for subject 6. For the remaining subjects pregnancies were uneventful and 20-week gestation scans were normal. All babies were born at term. Birthweights were all within normal parameters (2nd–98th centiles).

Table 1 Clinical and genetic findings in six subjects with presumed pathogenic *PRICKLE2* variants.

Subject	Subject 1	Subject 2	Subject 3	Subject 4 (III-1, daughter of subject 5)	Subject 5 (II-2, mother of subject 4)	Subject 6 (I-2, grandmother of subject 4)
gDNA change	Chr3:64184482 G > A	Chr3:64148736 G > A	Chr3:64138965 G > C	Chr3:64132879delAC	Chr3:64132879delAC	Chr3:64132879delAC
DNA change (NM_198859.4)	c.122 C > T	c.214 C > T	c.680 C > G	c.1286_1287delGT	c.1286_1287delGT	c.1286_1287delGT
Protein change	p.(Pro41Leu)	p.(Arg72*)	p.(Thr227Arg)	p.(Ser429Thrfs*56)	p.(Ser429Thrfs*56)	p.(Ser429Thrfs*56)
Inheritance	De novo	De novo	De novo	Inherited	Inherited	Unknown[a]
CADD	Deleterious	–	Deleterious	–	–	–
SIFT	Damaging	–	Damaging	–	–	–
Polyphen2	Probably damaging	–	Benign	–	–	–
MutationTaster	Disease causing	–	Disease causing	–	–	–
Ethnicity	Caucasian	African	Caucasian	Caucasian	Caucasian	Caucasian
Age at inclusion	7 years	10 years	12 years	17 years	41 years	68 years
Gender	Male	Male	Female	Female	Female	Female
Epilepsy	Questionable	No	Yes	Yes	Questionable	No
Age at seizure onset	2 years: questionable seizures	Not relevant	9 months	Onset of eyelid myoclonic from the age of 3 years but onset of bilateral tonic-clonic seizures at 10 years of age	14 years	Not relevant
Seizure type at onset	Unclear: seizure-like episodes, but several normal EEGs. A focal epilepsy was suspected	Not relevant	Myoclonic and bilateral TCS	Bilateral TCS	Unclear: seizure-like episodes, but several normal EEGs	Not relevant
Seizure types during disease course	NA	Not relevant	1) myoclonic jerks, 2) GTCS	1) GTCS 2) Eyelid myoclonic with absences	NA	Not relevant
Febrile seizures	No	No	No	No	No	No
Status epilepticus	No	NO	No	No	No	NO
Overall antiepileptic drug response	Not relevant	Not relevant	Seizure-free	Disappearance of absences og GTCS; persistence of eyelid myoclonia	Not relevant	Not relevant
Development prior to epilepsy onset	Mildly delayed	Not relevant	Mildly delayed	Mildly delayed	NA	Not relevant
EEG findings	Normal background activity. Interictal focal occipital slowing but no epileptorm discharges	Not relevant	Mildly slowed background activity with irregular theta activities intermixed. In P3, inter-ictal bursts of irregular generalized spike-wave discharges	Mildly slowed background activity with irregular theta activities intermixed. Photoparoxysmal response by IPS; episodes of eyelids myoclonia triggered by eye closure or IPS associated with diffuse rhythmic sharp waves	Normal EEG	Not relevant

Table 1 (continued)

Subject	Subject 1	Subject 2	Subject 3	Subject 4 (III-1, daughter of subject 5)	Subject 5 (II-2, mother of subject 4)	Subject 6 (I-2, grandmother of subject 4)
Current intellectual ability	Mildly delayed		Mildly delayed	Mildly-moderately delayed	Mildly delayed	Is able to read and write but has spelling problems and dyslexia
Current developmental delay	Mildly delayed	Mildly-moderately delayed	Mildly delayed	Mildly delayed	Mildly delayed	NA (is able to walk and use both hands)
Age of sitting	9 months		12 months	NA	NA	NA
Age of walking	12 months	17 months	18 months	15 mdr	NA	NA
Present motor ability	Grossmotor and finemotor delay	Finemotor delay	Grossmotor and finemotor delay (difficulties holding a pen or a zipper)	Finemotor delay (difficulties holding a pen or a zipper)	Able to walk but has grossmotor and finemotor difficulties. Tremor of the hands	NA (is able to walk and use both hands)
Age of first words	24 months	Unknown	12 months	12 months	14 months	NA
Age of first sentences	30 single words at 3 years of age	Unknown	Unknown	Unknown	Unknown	NA
Present verbal ability	Short sentences, mild articulation problems	Talks in sentences, adapted answers	Short sentences, mild articulation problems	Able to speak but slightly delayed in articulation	Able to speak but slightly delayed in articulation	Normal
Problems with social interaction	Yes	Yes	Yes	Yes	Yes	Yes (in addition a severe depression since adulthood)
Stereotypies	No	No	No	No	No	NA
Austism or autistic features	Yes	No	No	No	No	No
ADHD	Yes	Yes	Yes	Yes, hyperactive, impulsive	Yes, hyperactive, impulsive	NA
Bipolar disorder	No	No	No	No	No	Yes
Stubborn	NA	Yes	Yes	No	No	NA
Rigid and unflexible behavior	Yes	Yes	Yes	Yes	Questionable	NA

ADHD Attention deficit hyperactivity disorder, *CADD* Combined annotation dependent depletion, *Chr* chromosome, *EEG* Electroencephalograms, *TCS* tonic-clonic seizures, *IPS* Intermittent photic stimulation, *SIFT* Sorting Intolerant From Tolerant.

[a]Parents unavailable for testing.

Neurodevelopment

The motor milestones were delayed in subjects 1–5. All could walk independently before 18 months of life (ranging from 12 to 18 months of life) and acquired the ability to run and climb stairs. However, their gait remained unsteady and all had difficulties with finemotor tasks such as holding a pen, closing a zipper or buttoning their clothes. Subjects 2–5 used their first single words around 12–18 months of life, subject 1 at 2 years of life and all subjects eventually learned to communicate using short sentences. No subjects lost skills. Subjects 1–5 had learning difficulties, but typically within the mild to moderate spectrum. Behavioral issues were reported in all six subjects. There were recurring themes, such as anxiety and shyness associated with difficulty in understanding social situations and making friendships. Subjects 1–5 were diagnosed with an attention deficit hyperactive disorder manifesting with a lack of attention span and restless movements. Obsessions and fixation on a favorite object or routines or disliking changes with behavior deteriorating at times of stress were also reported (subject 1–4). Subjects 1–4 were described as having a low anger threshold and had challenging behavior such as inconsolable upset, anger tantrums, and occasional aggressive outbursts; however, no self-injurious behavior was described. During infancy, subject 3 had severe sleep disturbance which resolved with age. Subject 2 had sleep apnea which resolved after adenotonsillectomy. Subject 1 had a formal diagnosis of autism spectrum disorder (ASD).

Subjects 1–4 attended a school for children with special needs while subjects 5–6 attended a public school with additional support. Subjects 4–6 reached adolescence but none completed an education. Subject 5 completed 9th grade and made several attempts to continue education but was unable to complete any of them. She failed to hold a job as she easily fatigued and had difficulties with concentration. She therefore went on early retirement at the age of 40 years.

Subject 6 had normal motor and verbal development. Despite spelling difficulties, she was an avid reader. She completed 7th grade of primary school, had no further education. When testing subject 6 we found a FSIQ and a GAI within average. No evidence of an attention deficit or working memory deficit were found. Her performance in RAVLT was normal, while her performance in the two nonverbal memory tests (RCFT and RMF) was in the low normal range. The Stroop Test showed inhibition difficulties and the BRIEF-V self-report form indicated general difficulties with executive functioning in daily life. No other signs of executive dysfunction were detected. When interviewed, her primary cognitive complaints were poor facial recognition and a poor sense of direction as well as trouble with retrieval of autobiographical memory. She was in an emotionally stable period at the time of the testing, which was supported by her neutral scores in the SCL-90-R. Subject 6 reported episodes of anxiety in her childhood. In adulthood she was diagnosed with a bipolar disorder and borderline personality disorder. She had recurrent periods of severe depression and received psychiatric treatment, including electroconvulsive therapy (ECT), throughout most of her adulthood.

Subjects 3 and 4 were diagnosed with epilepsy, while subjects 1 and 5 were suspected as having epilepsy but never reached a formal diagnosis (Table 1). Onset of seizures in patients 3 and 4 was 9 months and 3 years of life respectively. Both experienced a generalized epilepsy with bilateral tonic-clonic seizures in addition to myoclonic jerks in the upper limbs (subjects 3) and eyelid myoclonia with absences (subject 4). In this latter subject, evidence of photosensitivity to intermittent photic stimulation (IPS) during EEG is compatible with a syndromic diagnosis of eyelids myoclonia with absences, i.e., Jeavons syndrome [17]. Seizures were not provoked by fever. EEGs were available in both and showed a mildly slowed background activity intermixed with irregular theta activities (Fig. 2). In subject 3, inter-ictal bursts of irregular generalized spike-wave discharges (sometimes with a posterior lead) were recorded. In subject 4, IPS elicited a photoparoxysmal response; in addition, episodes of eyelids myoclonia triggered by eye closure or IPS were recorded (Fig. 2B). The overall drug response was favorable. Subject 3 became seizure free following monotherapy with levetiracetam and subject 4 is treated with levetiracetam and topiramate and is partially seizure free (Table 1).

Subjects 1 and 5 had respectively staring episodes and episodes with presumed involuntary movements of the extremities, but both repeatedly had normal EEGs. The two remaining subjects never experienced epileptic seizures.

Brain MRI scans were available in 5/6 subjects and they were reported as normal in all subjects.

Other clinical features

There was no involvement of the cardiac, pulmonary, dental or ear-nose-throat systems. Dysmorphic features were not reported. Vision and hearing were normal in all subjects and no structural abnormalities were reported. Subject 3 had dermatitis. Besides a high anterior hairline reported in subject 3, no abnormal hair or nails were reported. Gastrointestinal problems involved chronic constipation in one case (subject 2), and feeding difficulties in the neonatal period (subject 1) that resolved spontaneously.

Computational analysis of variants

We identified four different heterozygous variants (two missense (c.122 C > T; p.(Pro41Leu) and c.680 C > G,

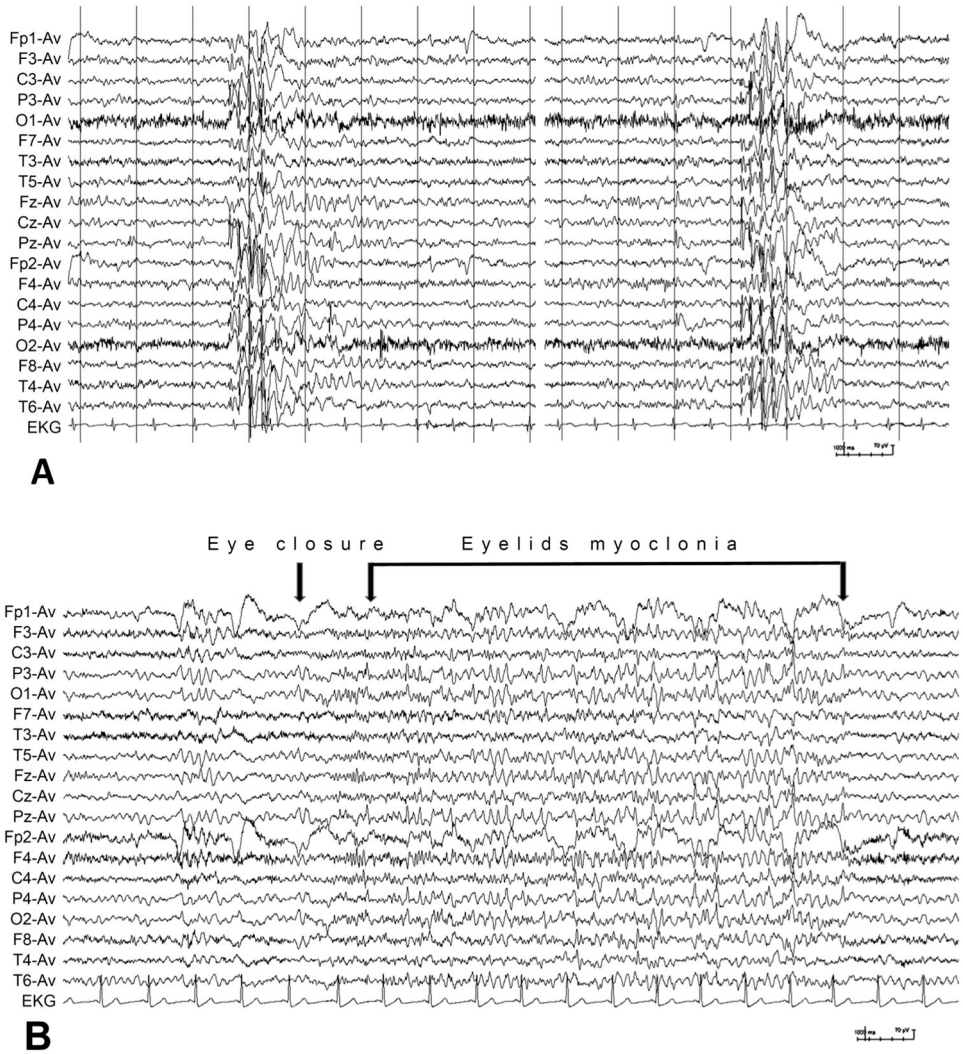

Fig. 2 Electroencephalograms (EEG) of subjects with epilepsy and a disease causing variant in *PRICKLE2*. A Electroencephalogram (EEG) in subject 3 showing a mildly slowed background activity intermixed with irregular theta activity; frequent inter-ictal bursts of irregular generalized spike-wave discharges (sometimes with a posterior lead) were recorded. **B** EEG in subjects 4 with an episode of eyelids myoclonia triggered by eye-closure. Eyelids myoclonia appear about 1 second after eye-closure associated in the EEG with diffuse rhythmic sharp waves at about 5–6 Hz. This EEG activity and the eyelids myoclonia disappear upon eye opening.

p.(Thr227Arg)) and two truncating variants (c.1286_1287delGT; p.(Ser429Thrfs*56) and c.214 C > T; p.(Arg72*)), all of which were absent from GnomAD. All variants were predicted to be damaging by different prediction tools. Variants were spread across the gene and there was no clustering in a specific domain, making it difficult to establish a genotype–phenotype correlation based on the landscape of pathogenic variants.

We utilized multiple orthogonal bioinformatic approaches and gained insights into the functional importance of the amino acid residues mutated in the two de novo missense variants, Pro41Leu and Thr227Arg. We hypothesized that residues that are evolutionarily conserved across species may be functionally important to protein activity or stability. We used a BLAST search with the human PRICKLE2 canonical isoform protein sequence as input and identified homolog protein sequences from six other mammalian species (MACMU, PANTHER, SHEEP, BOVINE, RAT and MOUSE) with a minimum sequence identity of 89.431%. We aligned the seven cross-species homolog sequences and observed that the mutated amino acids Pro41 and Thr227 are fully conserved across the species ('*' in supplementary Fig. 1A). This indicates that these residues may be required for protein function. It has been also proposed that functionally essential residues in protein sequence tend to be conserved across the gene family members (i.e., paralogs) [10] within the same species. We aligned human *PRICKLE1* and *PRICKLE2* canonical isoform protein sequences and observed that the mutated residues in the missense variants reported in this study are conserved across the paralog sequences as well ('*' in supplementary Fig. 1B).

We further investigated if the variant positions (Pro41 and Thr227) fall in the regions that undergo purifying selection and thus can be presumed to be essential. To this end, we used the residue-wise missense tolerance ratio (MTR) [11] score that measures the observed degree of selection of missense variants over the expected rate based

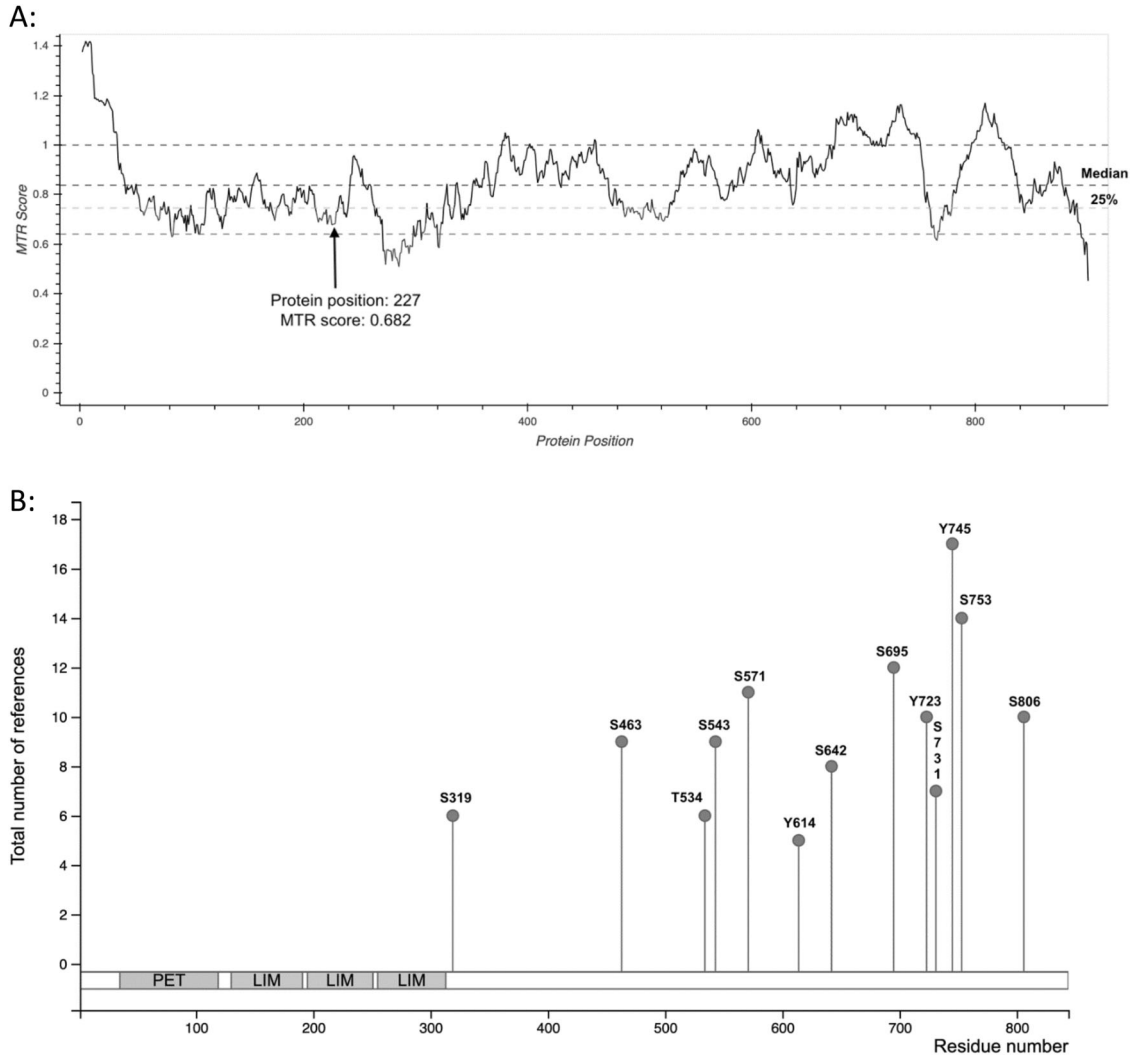

Fig. 3 Computational analysis of variants. Mapping of (**A**) missense tolerance ratio (MTR) and (**B**) map of post-translational modification (PTM) sites in *PRICKLE2* protein sequence. Figure 3A is obtained from MTR viewer webpage (http://biosig.unimelb.edu.au/mtr-viewer/geneviewer/PRICKLE2). The missense constrained amino acid residue affected by one of the variants in our cohort, Thr227Arg, is labeled. Figure 3B is taken from PhosphositePlus webpage.

on one of the largest cohorts of population genetics data, the gnomAD. Although Pro41 position is not missense constrained per MTR score, it is worth noting that there is no Pro41 substituting variant present in the gnomAD database the position. The position Thr227, on the other hand, is found to be highly intolerant to missense variation (MTR score value = 0.682), Fig. 3A. Overall, these observations suggest that the missense variants in our cohort alter highly conserved amino acid residues.

Finally, we examined the PRICKLE2 protein sequence for the presence of PTM sites, which are particularly important for modulating the protein function. We leveraged the PhosphoSitePlus database [12] for this analysis. We observed that starting from the end of the 3rd LIM zinc-binding domain to the end of the PRICKLE2 protein, especially the C-terminal part, is enriched with 13 phosphorylation sites having a minimum of five references for high-throughput or low-throughput identification of the sites (Fig. 3B). Note that, the studied frameshift variant Ser426Thrfs will supposedly eliminate or alter these sites, which may perturb the signaling and/or regulatory functions modulated by these PTM sites.

Discussion

This study shows that *PRICKLE2* related disorder has some of the hallmarks of developmental encephalopathy ± epilepsy and that subjects share a similar but relatively mild phenotype. This study is highly warranted for several reasons. *PRICKLE2* related disorders are likely to be rare. Children presenting with mild developmental delay and

behavioral difficulties such as that associated with *PRICKLE2* related disorder may not be referred for gene panels or whole exome sequencing. Thus a *PRICKLE2* related disorder is likely to be underdiagnosed.

Secondly, our study suggests that *PRICKLE2* is involved in human neuronal development and that pathogenic variants in *PRICKLE2* cause a neurodevelopmental disability with core symptoms that include learning difficulties, altered social interaction, autistic features, ADHD and, in some cases, epilepsy. In comparison, mice with disruption of *PRICKLE2* display behavioral abnormalities including altered social interaction, learning abnormalities, and behavioral inflexibility [10]. The *PRICKLE2* deficient mice are less flexible and less interested in social interaction compared to their wild-type counterparts [10]. Taken together, these mouse behavioral data parallels the behavioral characteristic of subjects with *PRICKLE2*-deficiency.

Both heterozygous and homozygous *PRICKLE2* mutant mice display a decreased seizure threshold compared to that of wildtype littermates [7]. Also, compared to controls, the seizure-inducing GABA receptor antagonist pentylenetetrazole caused more epileptiform discharges in *PRICKLE2* deficient mice [7]. A similar phenotype conferred by homozygous *PRICKLE2* variants was found in a drosophila model: In a model for seizure sensitivity, a glass vial containing flies was vortexed, inducing seizures in flies genetically predisposed to this disorder [7]. The flies demonstrated dramatic twitching behavior similar to seizures observed in mice and humans and electrographic recordings have substantiated these findings [7]. The seizures were easily treatable with valproate acid but prevented or delayed flies from climbing the vial wall, which is the normal behavior of wild-type flies [7]. In comparison, seizures in our human subjects were not a recurrent feature but when present they were also easily treatable. Seizures occurred in both subjects with missense and nonsense variants. Onset of seizures was in infancy and no subjects experienced a developmental regression. Hopefully these results, together with additional cases, will lead to a better understanding of *PRICKLE2* related disorder, phenotype–genotype correlations, exploration of the natural history, a community for affected subjects and perhaps even tailored treatment options.

While more than 20 subjects with homozygous missense variants in *PRICKLE1* are reported to have PME [18], epileptic seizures were only present in two out of the six cases we report. As in *PRICKLE1*, our subjects presented with generalized epilepsy with myoclonic seizures (one of them featuring a phenotype compatible with the syndrome of eyelids myoclonia, i.e., Jeavons syndrome) [17]. Yet a in our cohort the epilepsy course is mild, non-progressive and drug-responsive. Finally, subjects generally do not have any congenital structural anomalies. If we disregard the previously published case 4 [7], whose phenotype is fully explained by the detected disease causing *POLG* variants [9], and the three published cases with deletions involving *PRICKLE2* [7, 11], we find that there is a correlation between the degree of neurodevelopmental delay and the severity of psychiatric features amongst subjects with a single-nucleotide variant in *PRICKLE2*.

Interestingly, subject 6 was 68 years of age at time of diagnosis and neuropsychological testing. Only limited data were available about her childhood. Her poor autobiographical memory may be attributed to her bipolar disorder and a chronic severe depression with ECT treatments [19, 20], rather than her borderline personality disorder [21]. Her executive dysfunction is less likely to be related to the ECT treatments [22, 23] as such treatments may not negatively affect cognition [24]. In addition, a study comparing the cognitive function of subjects diagnosed with a bipolar disorder with depressed subjects and healthy controls found little evidence of a frontal- or temporal-lobe dysfunction in subjects with bipolar disorders [25]. We suggest that the executive dysfunction found in subject 6 is neither exclusively caused to the bipolar disorder nor the ECT. We cannot exclude that the dysfunction results from the combined effect of her rare *PRICKLE2* variant, her bipolar disorder and the ECT treatment. Natural history studies are needed to explore if bipolar disorders are a feature of *PRICKLE2*-deficiency with onset in adolescence and adulthood. At present time we are unable to explain the milder phenotype in subject 6 but speculate that it might be caused by mosaicism, reduced penetrance or be secondary to a different polygenic risk score than her affected family members. Future functional tests are needed to better understand these clinical findings.

Computational analysis of variants show both missense and truncating variants are likely to contribute to the disease phenotype. With a pLI score of 1, *PRICKLE2* gene is highly intolerant to a loss-of-function in the general population. Although overall *PRICKLE2* gene has not been reported as missense constrained by gnomAD database (miss z-score = 1.72), the de novo variants analyzed in this paper were found to alter functionally important amino acid residues (Fig. 3 and supplementary Fig. 1), hence likely to be damaging. Since our subjects share an overlapping phenotype this could support the claim that the missense variants also result in a loss-of-function. Functional analysis in subject variants are needed to provide further evidence for this.

Conclusion

This paper provides additional evidence supporting the hypothesis that *PRICKLE2* is involved in human neuronal

development and that heterozygous pathogenic variants in *PRICKLE2* cause neurodevelopmental delay, psychiatric symptoms and epilepsy in humans. However, additional studies are needed to further support this claim, to delineate the phenotypic spectrum, to inform genotype–phenotype correlation and to pave the way toward precision medicine for *PRICKLE2* related illness.

Data availability

Anonymized data including data not published in this article will be made available by request from any qualified investigator. The variants described in this manuscript have been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) access number SUB9364907.

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Compliance with ethical standards

Conflict of interest None of the other authors have any competing interests to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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