Alterations in the $\alpha_2\delta$ ligand, thrombospondin-1, in a rat model of spontaneous absence epilepsy and in patients with idiopathic/genetic generalized epilepsies

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doi: 10.1111/epi.13898

SUMMARY

Objectives: Thrombospondins, which are known to interact with the $\alpha_2\delta$ subunit of voltage-sensitive calcium channels to stimulate the formation of excitatory synapses, have recently been implicated in the process of epileptogenesis. No studies have been so far performed on thrombospondins in models of absence epilepsy. We examined whether expression of the gene encoding for thrombospondin-1 was altered in the brain of WAG/Rij rats, which model absence epilepsy in humans. In addition, we examined the frequency of genetic variants of THBS1 in a large cohort of children affected by idiopathic/genetic generalized epilepsies (IGE/GGEs).

Methods: We measured the transcripts of thrombospondin-1 and $\alpha_2\delta$ subunit, and protein levels of $\alpha_2\delta$, Rab3A, and the vesicular glutamate transporter, VGLUT1, in the somatosensory cortex and ventrobasal thalamus of presymptomatic and symptomatic WAG/Rij rats and in two control strains by real-time polymerase chain reaction (PCR) and immunoblotting. We examined the genetic variants of THBS1 and CACNA2D1 in two independent cohorts of patients affected by IGE/GGE recruited through the Genetic Commission of the Italian League Against Epilepsy (LICE) and the EuroEPINOMICS-CoGIE Consortium.

Results: Thrombospondin-1 messenger RNA (mRNA) levels were largely reduced in the ventrobasal thalamus of both presymptomatic and symptomatic WAG/Rij rats, whereas levels in the somatosensory cortex were unchanged. VGLUT1 protein levels were also reduced in the ventrobasal thalamus of WAG/Rij rats. Genetic variants of THBS1 were significantly more frequent in patients affected by IGE/GGE than in nonepileptic controls, whereas the frequency of CACNA2D1 was unchanged.

Significance: These findings suggest that thrombospondin-1 may have a role in the pathogenesis of IGE/GGEs.

KEY WORDS: Thrombospondins, Absence epilepsy, $\alpha_2\delta$ subunit, WAG/Rij rats, Genetic variants.

Ines Santolini is a pharmacologist working on the molecular mechanisms underlying absence epilepsy.

Received August 18, 2017; Early View publication September 15, 2017.

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2For details of the EuroEPINOMICS CoGIE Consortium and Genetic Commission of LICE see Appendix 1.
Absence epilepsy is a nonconvulsive type of epilepsy characterized by brief periods of unresponsiveness associated with typical spike-and-wave discharges (SWDs) on electroencephalography (EEG). SWDs are generated within a corticothalamocortical network formed by highly excitable neurons in the subgranular layer of the facial region of the somatosensory cortex interconnected with neurons of the reticular and ventrobasal thalamic nuclei.\(^1,2\) T-type voltage-sensitive Ca\(^{2+}\) channels (VSCCs) are critically involved in the generation of SWDs, as demonstrated by the ability of the T-channel blocker, ethosuximide, to normalize the activity of highly excitable cortical neurons and to reduce burst firing of reticular and ventrobasal thalamic neurons.\(^3,4\)

A growing body of evidence suggests a potential role for the \(\alpha_2\delta\) subunit of VSCCs in the pathophysiology of nonconvulsive epilepsy. The mutant mouse \textit{ducky}, which carries a mutation for the gene encoding for the \(\alpha_2\delta-2\) subunit, is characterized by absence seizures and ataxia. In addition, gabapentin and pregabalin, which bind to and negatively modulate \(\alpha_2\delta-1\) and \(\alpha_2\delta-2\) proteins, may precipitate absence seizures in experimental animal models and humans.\(^5,6\) The \(\alpha_2\delta\) subunit is known to regulate trafficking, current amplitude, and activation/inactivation kinetics of high-voltage activated VSCCs (e.g., L-, N-, and P/Q-type VSSCs).\(^7\) The role of \(\alpha_2\delta\) subunit in the regulation of T-type channels is less clear. Formation of a multimolecular complex between the \(\alpha_1\) subunit of T channels and auxiliary subunits (\(\alpha_2\delta, \beta, \) and \(\gamma\) subunits) has not yet been demonstrated. However, there is evidence that expression of the \(\alpha_2\delta\) subunit increases plasma membrane localization and current density of T channels\(^8-10\) (see also Lacinová et al.,\(^11\) for contrasting data). These findings are difficult to reconcile with the evidence that genetic deletion or pharmacologic blockade of the \(\alpha_2\delta\) subunit causes absence seizures (see above), unless the function of the \(\alpha_2\delta\) subunit is not restricted to the modulation of VSCCs. Of interest, \(\alpha_2\delta-1\) has been identified as a high affinity receptor for thrombospondins,\(^12\) which, in the central nervous system (CNS), are secreted from astrocytes and promote synaptic formation under physiologic and pathologic conditions.\(^13,14\) The interaction between the epidermal growth factor–like domains of thrombospondins and \(\alpha_2\delta-1\) mediates the effect of thrombospondins on synaptic formation. Of interest, the \(\alpha_2\delta\) ligand, gabapentin, inhibits the induction of excitatory synaptic formation by thrombospondins.\(^12\) This stimulates interest for the study of thrombospondins in models of absence epilepsy.

We used WAG/Rij rats, which develop spontaneous generalized bilateral symmetrical SWDs associated with absence-like behavior after 2–3 months of age.\(^15,16\) SWDs in symptomatic WAG/Rij rats are reduced by classical antiabsence drugs, such as ethosuximide and valproate, and are increased by drugs that aggravate absence seizures in humans, such as phenytoin, carbamazepine, tiagabine, and vigabatrin.\(^17-19\) This makes WAG/Rij rats a valuable model for the study of human absence epilepsy.

We now report that the transcript of thrombospondin-1 was largely reduced in the ventrobasal thalamus of WAG/Rij rats, both in the presymptomatic and symptomatic age, as compared to nonepileptic controls. We then examined the variants of \textit{THBS1} and \textit{CACNA2D1} in a cohort of patients with idiopathic/genetic generalized epilepsy (IGE/GGE) and found that variants in \textit{THBS1} but not in \textit{CACNA2D1} were enriched in patients respect to control population. These findings support a potential role for thrombospondins in epileptogenesis and raise the interesting possibility that changes in the expression and/or biologic function of thrombospondin-1 contributes to the pathogenesis of absence epilepsy.

**Methods**

**Animals** We used male WAG/Rij rats at 2 or 6 months of age raised at Radboud University, Nijmegen, The Netherlands. WAG/Rij rats of 2 months of age do not show SWDs yet, and, therefore, are considered “presymptomatic.” All 6-month-old WAG/Rij rats have about 16-20 SWDs per hour, or >200 SWDs per day,\(^15\) and are thus defined as “symptomatic.” As control rats, we used age-matched \textit{Agouti Copenhagen Irish} (ACI) rats or age-matched Wistar rats. ACI rats show no or only very few SWDs and the lowest number of SWDs of all inbred strains investigated, as assessed in a 48 h EEG evaluation study,\(^20\) and in all cases they have much less SWDs than WAG/Rij rats of the same age.\(^21\) However, ACI rats have a different genetic background with respect to WAG/Rij rats. Wistar rats have the same genetic background of WAG/Rij rats, and may occasionally develop SWDs or have no SWDs at all, depending on the substrain.\(^22,23\) Rats were housed under a 12 h-12 h light/dark cycle under standard conditions; food, water, and cage enrichment were always available. All efforts were done to cause as little discomfort as possible, and to use as few animals as was considered meaningful. Ethical approval was obtained from Ethics Committee on Animal
Research of the Radboud University Nijmegen (RU-DEC, ‘Radboud University Dier Experimenten Commissie’). For the analysis of Thbs1 and Cacna2d1 transcripts we performed two independent experiments. In a first experiment, we examined the two transcripts in the ventrobasal thalamus, somatosensory cortex, and motor cortex from WAG/Rij and age-matched ACI rats. In a second experiment, we measured the transcript of Thbs1 in the ventrobasal thalamus and somatosensory cortex in a new set of 6-month-old WAG/Rij and age-matched ACI rats. For western blot analysis of Thbs1 and Cacna2d1, we examined the two transcripts in the ventrobasal thalamus, and WAG/Rij and ACI rats for measurements in the somatosensory cortex.

Biological analysis of the principal areas of the corticothalamic network

Male WAG/Rij and age-matched control ACI or Wistar rats of 2 or 6 months of age were decapitated, and brains were rapidly dissected out and frozen. The brains from each rat were coded and codes were released after biochemical analysis. Brains were cut coronally by a cryostat, and tissue that comprises the principal area of the corticothalamic network was dissected using the coronal diagrams (between coordinates from bregma – 1.88 mm and – 3.80 mm), according to Paxinos and Watson atlas as a guide. For cortical areas, we dissected a part of the cortex that included the primary somatosensory cortex (S1) and another portion of the cortex that included the primary motor cortex (M1). A thalamic portion containing ventrobasal thalamic nuclei (VB) was also dissected.

RNA isolation, reverse transcription, and quantitative real-time PCR

Total RNA was extracted from different brain regions of the corticothalamic network in Trizol reagent according to manufacturer’s protocol. The RNA was further treated with DNase (Qiagen, Hilden, Germany) and single-stranded complementary DNA (cDNA) was synthesized from 2 μg of total RNA using superscript III (Invitrogen, Carlsbad, CA, U.S.A.) and random hexamers. Real-time polymerase chain reaction (PCR) was performed on 20 ng of cDNA by using specific primers and Power SYBR Green Master Mix (Applied Biosystem, Foster City, CA, U.S.A.) on an Applied Biosystems Step-One instrument. Thermal cycler conditions were as follows: 10 min at 95°C, 40 cycles of denaturation (15 s at 95°C), and combined annealing/extension (1 min at 60°C). Primers used were as follows: Thbs1 Forw TCGGGGCGAGGAAGCATATGA and Rev ACTGGGCAGGGTGAATGGG; Cacna2d1 Forw CAGCAATGTCTAGGATGTA and Rev ATCTGTATCCCTTTTGTGC; and β-actin Forw GTTGCATCCCGTAAAGACC and Rev TGGAAAGTGGACAGTGGAG. mRNA copy number of each gene analyzed was calculated from serially diluted standard curves simultaneously amplified with the samples and normalized against β-actin copy number. Statistical analysis was performed by two-way analysis of variance (ANOVA) and Fisher test.

Western blot analysis

Western blot analysis was carried out in the ventrobasal thalamus and in the somatosensory cortex dissected from WAG/Rij and age-matched control ACI or Wistar rats, at 2 or 6 months of age (n = 6 rats per group). Tissue samples were homogenized at 4°C in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/mL aprotinin, 1 μg/mL pepstatin, and 1 μg/mL leupeptin. Proteins were resuspended in sodium dodecyl sulfate (SDS)-bromophenol blue reducing buffer with 40 μM dithiothreitol (DTT). Western blot analyses were carried out using 8, 10, or 12% SDS polyacrylamide gels, which were electroblotted onto immunoblot polyvinylidene difluoride (PVDF) membranes (BioRad, Milano, Italy); filters were blocked overnight in TTBS (Tris-Tween-Buffer-Saline) containing 5% bovine serum albumin (BSA) or 5% non-fat milk. Specific rabbit polyclonal antibodies for Rab3A (1:1000; Cell Signaling Technology, Danvers, MA, U.S.A.), VGLUT1 (1:1,000; Cell Signaling Technology, Danvers, MA, U.S.A.), and mouse monoclonal antibody for β-actin (1:100,000; Sigma) were used. We also used four commercially available anti-thrombospondin-1 antibodies (1:300; Calbiochem, San Diego, CA, U.S.A.; 1:500; Abcam, Cambridge, MA, U.S.A.; 1:500; Thermo Scientific, Rockford, IL, U.S.A.; 1:1000, R&D Systems, Minneapolis, MN, U.S.A.). Blots were then incubated for 1 h with secondary antibodies (peroxidase-coupled anti-rabbit or anti-mouse, Amersham, Piscataway, NJ, U.S.A.) diluted 1:7,000 with TTBS. Immunostaining was revealed by enhanced chemiluminescence (ECL, Amersham).

Patients, sequencing, and annotation of the variants

Whole-exome sequencing data from two independent cohorts of IGE/GGE patients recruited through the Genetic Commission of the Italian League Against Epilepsy (LICE) and the EuroEPINOMICS-CoGIE Consortium (Appendix 1) were analyzed. Their clinical information, including data on EEG and antiepileptic therapy, were recorded on data collection forms. Available charts and investigations were reviewed by experienced neurologists (PS, HL, PP) before making a final clinical diagnosis. Genomic DNA isolation and genetic analysis was carried out with the Nimblegen-SeqCapEZ-V244M enrichment kit on the Illumina HiSeq2000 system as described. Only samples with a minimum of 90% of all bases in the coding region of the Thbs1 and Cacna2d1 being covered by at least 11 reads were used and the Human Gene Mutation Database (HGMD) variants identified in the patients were validated.
Reduced thrombospondin-1 gene expression in the ventrobasal thalamus in spontaneously epileptic WAG/Rij rats

We used WAG/Rij rats at 2 and 6 months of age (corresponding to a presymptomatic and symptomatic age, respectively) and age-matched control ACI or Wistar rats for the analysis of thrombospondin-1, $\alpha_2\delta$-1 subunit, and two biochemical markers of glutamatergic terminals, that is, Rab3A and VGLUT1. All symptomatic WAG/Rij rats show a high frequency of SWDs recorded by EEG as exemplified in Figure 1. Thbs1 mRNA levels were largely reduced in the ventrobasal thalamus of both presymptomatic and symptomatic WAG/Rij rats, as compared to age-matched nonepileptic control rats (Fig. 2A). Levels did not differ between presymptomatic and symptomatic WAG/Rij rats (Fig. 2A), suggesting that the reduced expression of thrombospondin-1 in the ventrobasal thalamus is not an epiphenomenon of SWDs in WAG/Rij rats. No changes in thrombospondin-1 mRNA levels were found in the somatosensory and motor cortex of WAG/Rij rats at both 2 and 6 months of age (Fig. 2A). To exclude that the observed difference in thalamic Thbs1 mRNA levels between WAG/Rij and ACI rats was due to the different genetic background of the two strains of rats, we performed an additional experiment in which the transcript of Thbs1 was measured in the ventrobasal thalamus of WAG/Rij rats and Wistar rats at 6 months of age. Levels were significantly lower in the ventrobasal thalamus of WAG/Rij rats, whereas no changes were found in the somatosensory cortex (Fig. 2B). We were unable to measure thrombospondin-1 protein levels in brain regions of WAG/Rij and control rats because we could not detect a clean band, corresponding to the deduced molecular size of thrombospondin-1 by western blot analysis, using four commercially available anti-thrombospondin-1 antibodies.

In contrast to thrombospondin-1, no changes in the transcript of Cacna2d1 were found in the ventrobasal thalamus, somatosensory, and motor cortex of WAG/Rij rats as compared to ACI rats (Fig. 2C). In the somatosensory cortex, the transcript of $\alpha_2\delta$-1 showed a trend to a reduction with age in both WAG/Rij and ACI rats, whereas a significant increase with age was seen in the motor cortex (Fig. 2C). Immunoblot analysis showed no changes in $\alpha_2\delta$-1 protein levels in the ventrobasal thalamus of WAG/Rij rats at 2 or 6 months of age, as compared to age-matched ACI or Wistar rats (Fig. 3A). Knowing that thrombospondins enhance the formation of glutamatergic synapses by interacting with $\alpha_2\delta$-1, we extended the analysis to Rab3A and VGLUT1, which are established presynaptic markers of glutamatergic terminals. Rab3A levels did not change in the ventrobasal thalamus of presymptomatic and symptomatic WAG/Rij rats, as compared to ACI or Wistar rats (Fig. 3B). In contrast, there was a significant strain- and age-related reduction (but not strain × age difference) of VGLUT1 in the ventrobasal thalamus of WAG/Rij rats as compared to ACI or Wistar rats (Fig. 3C). No changes in $\alpha_2\delta$-1, Rab3A, or VGLUT1 protein levels were found in the somatosensory cortex of WAG/Rij rats as compared to age-matched ACI rats (Fig. 3D–F).

Genetic variants in the THBS1 gene encoding for thrombospondin-1 are more frequent in IGE/GGE patients

Overall, 238 patients affected by IGE/GGE were included in the analysis (Table S1). One hundred forty-three patients were affected by childhood absence epilepsy (CAE); 21 patients by juvenile absence epilepsy (JAE); 47 patients by juvenile myoclonic epilepsy (JME); and 27 patients by generalized tonic–clonic seizures alone (EGTC). All subjects were of European descent (Italian 128, German 54, Finnish 22, Dutch 11, British 9, Danish 8, Turkish 6). The cohort included 138 female subjects (58%). Age at epilepsy onset ranged from 5 years to 38 years, with a median of 8 years. Most of cases (n = 183) derived from multiplex families with at least two affected family members. Most of the patients (n = 189; 79.4%) were treated with one antiseizure drug, usually valproate or levetiracetam.

In this cohort, we identified a total of 11 variants (8 distinct HGMD missense mutations in THBS1 and 3 in CACNA2D1) in the whole IGE/GGE cohort (total frequency = 4.62%). Details of the identified variants and their frequency are reported in Table 1. Variants in THBS1 were enriched in the IGE/GGE cohort (8/238; 3.6%) compared with control population (1829/121,230; 1.5%).
In contrast, variants in $CACNA2D1$ were not more frequent in patients (3/238; 1.26%) than in control populations (733/120,817; 0.6%) ($p = 0.37$) (Table 2). Syndrome subdivision analysis failed to demonstrate that variants in both genes were specifically enriched in CAE syndrome (Table S2).

Figure 2.
Reduced $Thbs1$ transcript in the ventrobasal thalamus of WAG/Rij rats. mRNA levels of $Thbs1$ in the ventrobasal thalamus, somatosensory cortex, and motor cortex of WAG/Rij and ACI rats at 2 and 6 months of age are shown in (A). mRNA levels of $Thbs1$ in the ventrobasal thalamus and somatosensory cortex of 6-month-old WAG/Rij rats and age-matched Wistar rats are shown in (B). mRNA levels of $Cacna2d1$ in the ventrobasal thalamus, somatosensory cortex, and motor cortex of WAG/Rij and ACI rats at 2 and 6 months of age are shown in (C). In (A) and (C), and (B), values are means ± standard error of the mean (SEM) of 4-5, and 6 animals per group, respectively. In (A), a strain effect for the thalamus was found (two-way ANOVA: $F_{1,15} = 45.65$, WAG/Rij < ACI); post hoc test (Fisher’s Least Significant Difference, LSD) confirmed that this strain effect was present for both 2 and 6 months groups *$p < 0.05$. In (B), (Student’s t test, $t_{10} = 6.81$, *$p < 0.05$) strain difference in thalamus: 6-month-old WAG/Rij < age-matched Wistar rats. In (C), age effect in motor cortex *$p < 0.05$ (two-way ANOVA, $F_{1,13} = 6.56$, older < younger rats); post hoc test (Fisher’s LSD) confirmed that this age effect was present in both strains *$p < 0.05$.

doi: 10.1111/epi.13898
Thrombospondin-1, one of the five members of the thrombospondin family, is widely expressed in the organism and is known to regulate fundamental cell biologic processes, such as cell attachment to extracellular matrix, cytoskeletal dynamics, and cell migration. In the developing CNS, astrocyte-secreted thrombospondin-1 promotes synaptogenesis, neuronal migration, and axonal growth.13,26–29 The demonstration that thrombospondin-1 interacts with the α2δ-1 subunit to stimulate the formation of excitatory synapses12 raised interest on the potential role of thrombospondin-1 in disorders that are targeted by α2δ ligands, such as neuropathic pain and epilepsy. Several lines of evidence suggest that an abnormal synaptogenesis contributes to epileptogenesis, that is, to the process by which the brain develops epilepsy. Accordingly, temporal lobe epilepsy in patients and pilocarpine-induced epilepsy in rats are associated with an increased hippocampal and cortical expression of ephrinB3 and its receptor EphB3, which play a key role in synaptogenesis and mechanisms of synaptic reorganization and plasticity.30 Other proteins that regulate synaptogenesis, such as synapsin II and synaptophysin, have been also implicated in the pathophysiology of epilepsy.31,32 Of interest, matrix metalloproteinases (MMPs), which cleave extracellular matrix (EM) proteins and regulate synaptogenesis, have an active role in epileptogenesis in different experimental animal models,33 and the EM protein, SC1, translocates from neuronal cell bodies to excitatory nerve terminals following status epilepticus in the rat lithium-pilocarpine model.34

Recent evidence suggests that thrombospondin-1, by promoting the formation of new excitatory synapses, contributes to the development of a hyperexcitable neuronal network, which is a critical event in epilepsy. Mendus et al.35 have found that mice lacking thrombospondin-1 or...
Thrombospondins 1 and 2 were more sensitive to pentylenetetrazole kindling. These mice also showed a reduced expression of α2δ-1/2 protein levels in the cortex, suggesting that the thrombospondin/α2δ axis is a key regulator of susceptibility to seizures.35 The importance of the thrombospondin/α2δ axis in the pathophysiology of epilepsy is supported by the evidence that mice overexpressing α2δ-1 show epileptiform activity and behavioral arrests associated with an increased number of excitatory synapses in the cortex.36

Here, we showed for the first time that expression of the Thbs1 gene encoding for thrombospondin-1 was substantially reduced in the ventrobasal thalamus of WAG/Rij rats, which represent an established genetic animal model of spontaneous absence epilepsy.15,16 Remarkably, a large reduction in Thbs1 mRNA levels was observed both in presymptomatic and symptomatic WAG/Rij rats, suggesting that a reduced production of thrombospondin-1 in the thalamus was not secondary to the occurrence of SWDs in the corticothalamocortical network underlying absence seizures. As opposed to mice lacking thrombospondin-1,35 we did not detect changes in α2δ-1 mRNA and protein levels associated with the reduction of thrombospondin-1 in WAG/Rij rats. In contrast, WAG/Rij rats showed lowered VGLUT1 levels in the thalamus with respect to nonepileptic control rats, suggesting that the reduced expression of Thbs1 gene might have caused an impaired formation of excitatory synapses in the thalamus of WAG/Rij rats.

No changes in THBS-1 gene expression or VGLUT1 protein levels were found in the site of origin of the SWDs, the somatosensory cortex, suggesting that a possible defect in thalamic synaptogenesis combines with a cortical generator in the pathophysiology of absence seizures. The relevance of a defective synaptogenesis in the pathophysiology of absence seizures is supported by the evidence that stargazer mice, which show absence seizures, are characterized by an impairment of synaptic formation in the cerebellum,37 which exerts a modulatory role on generalized SWDs through the firing of deep cerebellar nuclei.38,39 Double mutant zi/zi, tm/tm rats, which represent another rat model of spontaneous absence epilepsy, show a reduced expression of the synaptic vesicle proteins, SV2A and synaptotagmin-1.40 A thalamic defect of excitatory synapses may reinforce the hyperpolarizing milieu generated by γ-aminobutyric acid (GABA) released from neurons of the reticular thalamus, thereby facilitating the recovery of T channels from inactivation in ventrobasal thalamic neurons, which ultimately results into pathologic oscillations of the corticothalamocortical network.3 This hypothesis warrants further investigation. Nevertheless, the suggestion that a defect of excitatory neurotransmission is involved in the pathophysiology of absence seizures is supported by the evidence that two types of glutamate receptors, the mGlu1 and mGlu5 metabotropic glutamate receptors, are downregulated in the

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**Table 1. Summary of THBS1 and CACNA2D1 variants identified in the IGE/GGE cohort and their frequency from the ExAC collection**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic position</th>
<th>Protein change</th>
<th>MAF</th>
<th>No. minor allele/No. total alleles</th>
<th>MAF in Europeans</th>
<th>No. minor allele/No. total alleles in Europeans</th>
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</thead>
<tbody>
<tr>
<td>THBS1</td>
<td>15:39874505 G/A</td>
<td>p.R60H</td>
<td>0.0004949</td>
<td>6/121,230</td>
<td>0.0000152</td>
<td>1/66,600</td>
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<tr>
<td>THBS1</td>
<td>15:39874642 G/T</td>
<td>p.A106S</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>THBS1</td>
<td>15:39874742 A/G</td>
<td>p.H139R</td>
<td>0.0001324</td>
<td>16/120,830</td>
<td>0.0001355</td>
<td>9/66,438</td>
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<tr>
<td>THBS1</td>
<td>15:39874918 C/T</td>
<td>p.R198C</td>
<td>0.0003462</td>
<td>4/115,536</td>
<td>0.0000313</td>
<td>2/63,914</td>
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<tr>
<td>THBS1</td>
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<td>p.R773H</td>
<td>0.0001483</td>
<td>18/121,366</td>
<td>0</td>
<td>0/66,698</td>
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<tr>
<td>THBS1</td>
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<td>p.R810Q</td>
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<td>p.G891R</td>
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<td>p.D1045A</td>
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<td>CACNA2D1</td>
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<td>CACNA2D1</td>
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<td>0.007649</td>
<td>324/120,468</td>
<td>0.004103</td>
<td>272/66,300</td>
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MAF, Minor allele frequency. For ExAC collection, see http://exac.broadinstitute.org/

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**Table 2. Statistical analysis of THBS1 and CACNA2D1 variants observed in patients affected by IGE/GGE and in subjects from the ExAC collection**

<table>
<thead>
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<th>Gene</th>
<th>Patients</th>
<th>ExAC collection</th>
<th>Total</th>
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<td>THBS1</td>
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<td>1829</td>
</tr>
<tr>
<td></td>
<td>Wild-type</td>
<td>230</td>
<td>121,183</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>238</td>
<td>121,230</td>
</tr>
<tr>
<td></td>
<td>% of mutated</td>
<td>3.36</td>
<td>1.5</td>
</tr>
<tr>
<td>CACNA2D1</td>
<td>Mutated</td>
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<td>733</td>
</tr>
<tr>
<td></td>
<td>Wild-type</td>
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<td>120,084</td>
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<tr>
<td></td>
<td>Total</td>
<td>238</td>
<td>120,817</td>
</tr>
<tr>
<td></td>
<td>% of mutated</td>
<td>1.26</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Two-tailed chi-square with Yates correction. For ExAC collection, see http://exac.broadinstitute.org/
ventrobasal thalamus of symptomatic WAG/Rij rats, and pharmacologic activation of either mGlu1 or mGlu5 receptors reduces the frequency of SWDs.

IGE/GGEs are the most common types of inherited epilepsy and include at least four well-established epilepsy syndromes, namely, CAE, JAE, JME, and EGTC, all of them characterized by the presence of SWDs/polyspikes. Despite its high heritability of 80%, the genetic background is still largely unknown.

To assess the potential impact of thrombospondin and the z2δ-1 subunit in the pathophysiology of absence epilepsy in humans, we checked a large cohort of patients affected by IGE/GGE. This analysis revealed that genetic variants of THBS1 were significantly more frequent in patients affected by IGE/GGE than in nonepileptic controls, whereas the frequency of CACNA2D1 was unchanged. Syndromic stratification analysis failed to show that variants in both genes were specifically enriched in any of the IGE/GGE syndromes, including CAE, which is in line with the view that IGE/GGE is a spectrum of epilepsy syndromes with variable age at onset associated with common EEG traits generated by pathologic oscillations in a corticothalamiccortical network with a cortical origin.

In conclusion, we showed that expression of thrombospondin-1 gene is reduced in the ventrobasal thalamus of WAG/Rij rats, which model absence epilepsy in humans. This reduction precedes the onset of absence seizures, linking thrombospondin-1 to the pathogenesis of absence epilepsy in WAG/Rij rats. Moreover, a potential role for thrombospondin-1 gene is reduced in the ventrobasal thalamus of WAG/Rij rats, which model absence epilepsy in humans.

**DISCLOSURE**

None of the authors has any conflict of interest 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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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical data overview of the investigated IGE/GGE cohort.

Table S2. Statistical analysis of THBS1 and CACNA2D1 variants observed in CAE patients and in controls from the ExAC collection (http://exac.broadinstitute.org/).