Clinical/Scientific Notes

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CENTRAL SLEEP APNEA AND ATAXIA CAUSED BY BRAINSTEM LESION DUE TO CHRONIC NEUROLEPTOSPIROSIS

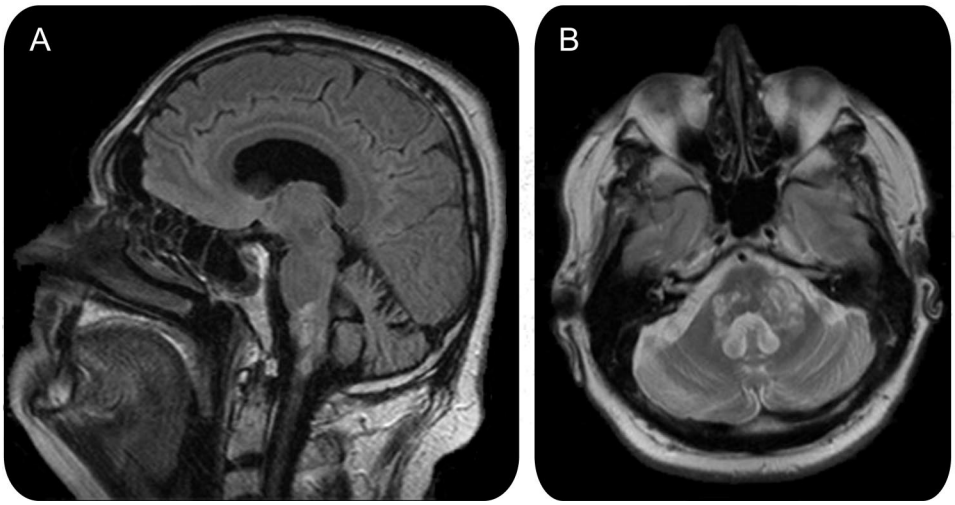
A 37-year-old man developed high fever with expectoration of blood and dyspnea. On examination, he had mild conjunctival injection. He was diagnosed with pneumonia and treated with empiric IV antibiotics. One week later, he developed dysarthria and ataxia; brain CT showed normal results and he was discharged. In the next month, he developed apneas and mild snoring, with frequent awakenings, nonrestorative sleep, choking, and shortness of breath. He was diagnosed with central sleep apnea. At this point brain MRI was performed (figure) to reveal a heterogeneous process that was hyperintense on T2 and fluid-attenuated inversion recovery sequences with signs of perifocal edema located in medulla oblongata and caudal part of the pons, more pronounced on the left side and spreading to the middle cerebellar peduncle. After contrast application there was inhomogeneous postcontrast enhancement indicating disruption of the blood–brain barrier. Neurologic examination revealed dysarthria, dysphagia, absent palatal reflexes, left hemiparesis (muscle strength 4/5) with brisk reflexes 3+, and positive Babinski sign. He had severe dysmetria of all 4 limbs and truncal ataxia.

Laboratory examination revealed normal erythrocyte sedimentation rate and C-reactive protein, complete blood count, electrolytes, and liver function tests. CSF analysis showed normal cell count, mildly elevated proteins (0.52 g/L), and positive oligoclonal bands, which were identical in serum and CSF. Serology for syphilis, HIV, hepatitis B and C, *Candida*, *Aspergillus* and *Cryptococcus*, *Borrelia burgdorferi*, morbilli, rubella, varicella zoster virus, herpes simplex virus 1 and 2, human herpesvirus 6, mumps, Epstein-Barr virus, cytomegalovirus, adenoviruses, *Toxoplasma*, *Mycoplasma pneumoniae,* and tuberculosis were unremarkable in both serum and CSF. Extensive testing for tumor markers and immunologic tests were negative.

The patient was treated with corticosteroids, but without any improvement. In the next 2 years, mild worsening of the symptoms occurred, but repeated brain MRIs were without changes. He was empirically treated with antimycotics, without any improvement.

Upon careful review of the patient's history, we found the patient was working in the Agency for Water

Figure **Brain MRI**



(A) Fluid-attenuated inversion recovery (FLAIR) coronal sequences. (B) T2 transverse sequences showing a heterogeneous process hyperintense on T2 and FLAIR sequences with signs of perifocal edema.

Supply, mainly on water canal maintenance. Then serology for leptospirosis was performed to show positive titers of *Leptospira saxkoebing* in serum (titer 1:100). Repeat serum serology and CSF serology performed 2 weeks later showed positive titers of *L saxkoebing* (titer 1:100). Immunoglobulin M antibodies were negative.

The diagnosis of chronic leptospiral brainstem involvement, possibly arteritis, was made. The patient was treated with corticosteroids again and was stable on 1-year follow up.

Discussion. Leptospirosis is often underdiagnosed because of protean clinical manifestations. This is especially true in cases of neuroleptospirosis. In the 2 largest series of patients with neuroleptospirosis published up to now, the most common presentation included altered sensorium and seizures followed by meningitis, encephalitis, meningoencephalitis, myelitis or myeloradiculoneuritis, acute disseminated encephalomyelitis, and stroke.[1,2] However, all these patients had acute neuroleptospirosis. Even in an acute phase of the disease, CSF findings are nonspecific, and only 23% of patients are positive for antileptospira antibody.[1] Some studies have shown that seropositivity for Leptospira antibodies is 6%–7% in healthy controls, and that these antibodies crossreact with antibodies to *Treponema pallidum* and *B burgdorferi*.[3] Our patient had negative serology for syphilis and borreliosis, and the presence of CSF leptospira antibodies additionally supported the diagnosis.

The mechanisms of chronic neurologic manifestations remain unknown; however, rare pathologic studies have shown that most of the clinical features of neuroleptospirosis are due to capillary endothelial damage and vasculitis.[2] The patient presented had pneumonia in the septic stage of the disease, and brainstem involvement, possibly arteritis, in the immunologic stage of the disease.

It is essential to be aware of leptospirosis in patients with otherwise unexplained neurologic deficits. Careful epidemiologic history and history of previous illnesses is of paramount importance to order appropriate tests in such instances.

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OPTIC NEUROPATHY IN PATIENTS WITH GLIOBLASTOMA RECEIVING BEVACIZUMAB

Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor, was recently approved for treatment of glioblastoma. Initial data indicate increased response rates and progression-free survival compared to historical controls. Despite these promising data, we have identified several cases of severe optic neuropathy in patients with glioblastoma treated with bevacizumab.

Methods. We performed a retrospective record review from 2005 to 2008 to identify adult patients with glioblastoma receiving bevacizumab who developed severe optic neuropathy. Five institutions participated, including the University of Virginia, UCLA, Columbia University, Rush University, and the University of Nebraska. The UCLA patient has already been reported in a larger case series discussing patients with glioblastoma receiving bevacizumab.[1] Age at diagnosis, gender, radiation therapy data, chemotherapeutic regimens including the bevacizumab dosing schedule, ophthalmologic records, CSF results, and MRI were assessed.

Standard protocol approvals, registrations, and patient consents. Each institution provided institutional review board approval. Since data were collected retrospectively without identifiers, institutional review boards did not require patient or surrogate consent.

Results. Six patients (5 women) were identified. Median age at diagnosis was 61 years (range 37 to 68). Following surgery, all patients received fractionated radiation therapy with concomitant temozolomide. One patient received bevacizumab at initial diagnosis; 5 received it at progression. Tumors received 60 Gy delivered in a mean of 30 fractions. Mean radiation dose to the optic chiasm, left optic nerve, and right optic nerve was 5,602.4 cGy, 3,673 cGy, and 3,464.3 cGy (table e-1 on the *Neurology*® Web site at www.neurology.org). Median time from

Supplemental data at www.neurology.org

Table Clinical features of the cases

Feature	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	F	M	F	F	F	F
Age at diagnosis, y	37	68	67	48	63	59
Preexisting hypertension	Present	Present	Absent	Absent	Present	Absent
Treatment-induced hypertension	Absent	Absent	Absent	Absent	Absent	Absent
Preexisting diabetes mellitus	Absent	Absent	Absent	Absent	Absent	Absent
Tumor location	Left temporal	Right temporal	Left temporal	Right frontal	Right temporal/parietal	Left frontal
Mean target volume (Gy)	59.4	56.6	59.4	60	60	66
Fractions	33	30	30	30	30	33
Optic chiasm mean dose (cGy)	5,602.4	5,585	4,177	6,218	5,966.3	3,503.2
Left optic nerve mean dose (cGy)	4,302.4	3,073	3,959	3,843	2,241.6	3,503
Right optic chiasm mean dose (cGy)	3,616	5,309	2,318	3,843	3,312.6	2,250
Months after radiation prior to visual decline	11.5	15.5	10.5	8	9.5	7
Bevacizumab doses prior to visual decline	17	8	6	18	3	7
Affected eye	OU	OU	OS	OS	OS	OU
CSF workup	Negative	Negative	Negative	N/A	Negative	N/A
MRI T1 enhancement	Bilateral optic nerve	None	None	Left optic nerve	None	None
MRI FSE T2	Not commented upon	Normal	Normal	Left optic nerve & chiasm	Left aspect of chiasm	Normal
MRI Gliomatosis	Present	Absent	Absent	Absent	Absent	Absent
Optic nerve pathology for malignancy	N/A	N/A	N/A	Negative	N/A	N/A

FSE = fast spin echo.

the end of radiotherapy to the onset of visual symptoms was 11.5 months (range 7 to 15.5).

Patients received a median of 7.5 doses (range 3–18) of bevacizumab prior to onset of visual symptoms. Bevacizumab was discontinued a median of 1 month after onset of visual symptoms (range 1 week to 10 months). Visual loss subsequently developed unilaterally in 3 patients and bilaterally in 3 patients. The MRI findings for the optic apparatus are presented in the table. Postmortem pathologic specimen of the patient with left optic nerve enhancement displayed no evidence of tumor infiltration with focal vascular hyalinization and mild gliosis consistent with prior radiotherapy. CSF analysis in 4 patients was negative for myelin basic protein, oligoclonal bands, malignant cells, and pleocytosis. Median time from visual symptom onset to no light perception in at least 1 eye was 1.0 month (range 0.5–4.5 months). Over this epoch, 503 patients with glioblastoma received bevacizumab at the 5 institutions for an incidence of 1.2%. In comparison, 1 of 567 glioblastoma patients treated at these institutions without bevacizumab developed severe optic neuropathy (0.2% incidence, $p = 0.056$).

Discussion. Bevacizumab has become a treatment option for recurrent glioblastoma.[1-3] A phase II clinical trial (AVF3708g) assessed 167 patients receiving bevacizumab with and without irinotecan at tumor progression. Two of the patients in the current report were included in this clinical trial. Recognized bevacizumab side effects include arterial thrombosis (twofold increase), hypertension, proteinuria, impaired wound healing, and gastrointestinal perforation; visual loss has not previously been reported.[1,2,4-6]

We report 6 recent patients who developed severe optic neuropathy after bevacizumab treatment. While etiology and mechanism remain uncertain, an association between this rare event and bevacizumab is possible. While not seen in patients treated for non–brain tumor indications, this association appears to require dose independent radiation to the optic apparatus suggesting a priming effect for optic nerve injury. The patients in the current report received standard chemora-

diation, with radiation to the optic apparatus generally considered within tolerance levels. Ophthalmologic assessment in all patients confirmed optic neuropathy of unknown etiology. The MRI of the optic apparatus for each case is unique with 3 patients displaying a normal examination. CSF findings did not support the diagnoses of either neoplastic meningitis or autoimmune demyelination. Gliomatosis cerebri was excluded as only 1 patient displayed this finding on MRI and a separate patient with optic nerve enhancement displayed negative pathology. Radiation-induced optic neuropathy was considered less likely secondary to both the severity and timing of the visual decline relative to the radiation and bevacizumab treatment. Proposed mechanisms may involve arterial thrombosis or upregulation of VEGF and subsequent neovascularization after radiotherapy with delayed ischemia following bevacizumab. An animal study analyzing whether bevacizumab decreases optic nerve tolerance to radiation is currently being devised. Until we understand the mechanistic basis for our findings, patients receiving bevacizumab should be followed closely in order to clarify whether this complication represents drug-related optic neuropathy, coincidental radiation optic neuropathy, or an unusual bevacizumab-related pattern of tumor failure with infiltration of the optic pathways from gliomatosis.

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REFINEMENT OF THE CLINICAL PHENOTYPE IN *MUSK*-RELATED CONGENITAL MYASTHENIC SYNDROMES

Congenital myasthenic syndromes (CMS) are a heterogeneous group of inherited disorders caused by genetic defects that affect transmission at the neuromuscular junction.[1] To date, 10 genes are known to cause CMS if mutated.[2]

Mutations in the muscle specific kinase (*MUSK*) gene have been published in a single family worldwide.[3] Two siblings of this family were reported carrying heteroallelic *MUSK* mutations.

Case reports. We report on CMS caused by a novel homozygous missense mutation in *MUSK* in 5 affected sibs (patients 1–5) from a consanguineous Sudanese family. The father and the maternal grandmother are first-degree cousins.

The patients were followed up for 5 years (ages at the end of follow-up: 9–19.5 years). All studies were carried out with informed consent of the patients' parents and approved by the institutional ethics review board.

All affected individuals demonstrated ptosis at age 1–3 years and fatigability, when walking for a long distance, more pronounced in the evening. At the first examination they had exercise-induced weakness of the deltoid muscle. Four of them had partial ophthalmoparesis (except patient 3). Patients 2 and 3 showed modified Gowers sign and waddling gait or pronounced lordosis, respectively. Treatment with pyridostigmine (30–60 mg/day) led to slight benefit. Increased doses

Table Clinical features of affected siblings with congenital myasthenic syndrome at the end of the 5-year follow-up

	Patient 1/male	Patient 2/male	Patient 3/male	Patient 4/female	Patient 5/female
Age at assessment, y	19.5	18.5	16	13	9
Bilateral ptosis	Yes	Yes	Yes	Yes	Yes
Extraocular muscle weakness	Yes	Yes	Yes	Yes	Yes
Limitation of gaze (degree)*	Lateral gaze (20°)	Upward, lateral, and medial (30°); downward (20°)	Upward (40°); lateral (20°)	Lateral and medial (30°)	Upward, downward, lateral, and medial (20°)
Facial weakness	Mild (myasthenic snarl)	Mild (myasthenic snarl)	Mild (myasthenic snarl)	Mild (myasthenic snarl)	Mild (myasthenic snarl)
Lordosis	Yes	None	Yes	None	None
Proximal muscle weakness in the upper limbs	None	Yes: shoulder abductors (grade 4)	Yes: shoulder abductors (grade 4)	None	None
Gowers sign	Yes (modified)	Yes (modified)	Yes (modified)	None	Yes (modified)
Waddling gait	None	Yes	None	None	None

*Degree of reduction in the maximum range of eye movement.

were reported to result in the "feeling of muscle stiffness" and the medication was discontinued.

Five years later (table), there was some progression, i.e., ophthalmoparesis in all, involving additional directions of gaze in patients 2 and 5 and mild facial weakness. Patient 4 had episodes of nasal speech at the age of 8 years. Patients 1 and 5 had developed modified Gowers sign. Patients 2 and 3 had mild shoulder girdle weakness.

At age 20 years, during pneumonia, Patient 1 developed respiratory failure and required mechanical ventilation through tracheostomy. Antibiotics, pyridostigmine (up to 180 mg/day), and 3,4-DAP (50 mg/day) led to gradual and slow improvement. Higher doses of pyridostigmine or treatment with ephedrine were not tolerated. 3,4-DAP did not add further benefit and was discontinued. One year later, while on pyridostigmine (180 mg/day), the patient still needed bilevel positive airway pressure ventilation during sleep, and had a waddling gait and slight weakness in the neck flexors and extensors.

Direct sequencing of downstream of tyrosine kinase 7 (*DOK7*) and collagenic tail of endplate acetylcholinesterase (*COLQ*) genes revealed no mutations. Haplotype fragment analysis[4] excluded linkage to all known CMS genes except *MUSK*. All affected individuals showed the same, homozygous haplotype for 5 markers tested at the *MUSK* locus. A maximum 2-point lod score of 3.09 and multipoint lod score of 3.91 showed significant linkage for markers used for fine mapping. Direct sequencing of all 14 exons of *MUSK* (gi13162053 for gDNA and AF006464 for mRNA) identified a c.1031C>G transversion homozygously in exon 8 leading to substitution of conserved proline for arginine at position 344 (p.P344R). All patients are homozygous while their parents are heterozygous carriers. The mutation p.P344R was not detected in 100 healthy control individuals of European, African, and North American origin. The PolyPhen algorithm (http://genetics.bwh.harvard.edu/pph/) predicted a "probably damaging" putative effect of the mutation on the MuSK protein.

Discussion. Here we report the second family known so far with a CMS due to a mutation in the *MUSK* gene. Mutated proline 344 is located in the Frizzled-like cysteine-rich domain, a part of the extracellular domain of MuSK. MuSK is thought to be the key component that orchestrates neuromuscular junction formation.[5]

The combined phenotypic data from the 2 kinships with *MUSK* mutations highlight ptosis from an early age and fatigability on walking or exercise. Respiratory crises were experienced neonatally in the French family but later in life in one of our patients. Muscle weakness is proximal in the extremities and in the neck extensors in some patients or more generalized during exacerbation. Incomplete ophthalmoparesis is found in both families although later in the French patient. Episodes of bulbar weakness were rare but experienced in both families. Acetylcholinesterase inhibitors combined with 3,4-diaminopyridine were of some, but limited benefit in our patients.

Ophthalmoparesis is frequently incomplete or absent in CMS with *DOK7*, rapsyn (*RAPSN*), choline acetyltransferase (*CHAT*), and *COLQ* mutations, while it is early and usually fixed in patients with receptor deficiency due to epsilon subunit of acetylcholine receptor (*CHRNE*) mutations.[6] Respiratory crises are characteristic in patients with *CHAT* mutations and experienced in *RAPSN*, *DOK7,* and *COLQ* related

CMS.[1] Limb-girdle weakness is usually found in *DOK7* patients and in some patients with *RAPSN* mutations.[7]

The identification of further CMS patients with *MUSK* mutations may be required to fully establish the MuSK-related CMS phenotype.

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LACK OF REPLICATION OF ASSOCIATION BETWEEN *SCN1A* SNP AND FEBRILE SEIZURES

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Febrile seizures (FS) affect 3% of children aged 6 months to 6 years and have a strong genetic component with recurrence risk ratios of 3–5 in first-degree relatives.[1] In rare families with autosomal dominant FS, mutations in the sodium channel alpha 1 subunit gene, *SCN1A,* have been identified.[2,3] However, FS usually show complex inheritance with a polygenic basis. The search for susceptibility variants has been slow, so the recent report of a common splice site single nucleotide polymorphism (SNP) in *SCN1A* (IVS5N + 5 G>A, rs3812718) is notable. Two patient groups were studied: the first, adults with focal epilepsy with and without a history of FS (n = 90 and 486 respectively); the second, children with FS (n = 144). Patients homozygous for the A allele were reported to have a genotype relative risk of ~3.0 for FS.[4] The rs3812718 SNP is a plausible candidate for FS as it influences relative expression of neonatal and adult transcripts of *SCN1A,* which plays a key role in membrane excitability.[5]

To confirm the association between rs3812718 and FS, independent replication studies are vital.[6] We tested the primary hypothesis of the original study, that an AA genotype at this *SCN1A* polymorphism confers increased risk of FS. For this, we recruited 558 unrelated Australian (predominantly Caucasian) patients from epilepsy clinics at 2 tertiary hospitals in Melbourne. The criteria for a diagnosis of FS were identical to that of the original study. Patients with an afebrile seizure history prior to the onset of FS were excluded. The genotyping methodology has been described previously.[7] Approval was obtained from the Human Research Ethics Committees of Austin and Melbourne Health.

The cohort of 558 patients comprised 76 (14%) cases with and 482 (86%) without a history of FS. A total of 382 (68%) patients had idiopathic generalized epilepsies; 137 (25%) had focal epilepsies; 12 (2%) had both idiopathic generalized and focal epilepsies; 17 (3%) had unclassified epilepsy; and 10 (2%) had only ever experienced FS.

Table IVS5N + 5 G>A (rs3812718) genotypic frequencies in an Australian population

Test	Genotype	Case, n (%)	Control, n (%)	*p* Value	(OR, 95% CI)*
FS with all epilepsy (n = 76) vs no FS with all epilepsy (n = 482)	AA	27 (35)	152 (32)	0.41	(1.33, 0.7–2.6)
	AG	34 (45)	218 (45)		(1.23, 0.7–2.2)
	GG	15 (20)	112 (23)		(1.16, 0.6–2.2)
Focal with FS and FS only (n = 23)† vs focal without FS (n = 124)‡	AA	10 (43)	33 (27)	0.24	(1.82, 0.6–5.9)
	AG	8 (35)	61 (49)		(1.15, 0.4–3.4)
	GG	5 (22)	30 (24)		(0.79, 0.2–2.6)
FS with all epilepsy (n = 76) vs European controls (n = 701)	AA	27 (35)	187 (27)	0.15	(1.57, 0.8–3.0)
	AG	34 (45)	351 (50)		(1.23, 0.7–2.2)
	GG	15 (20)	163 (23)		(1.05, 0.6–2.0)

*First OR (AA vs GG); second OR (AA + AG vs GG); third OR (AG vs GG).
†Combining focal epilepsy patients with FS (n = 13) and the pure FS cases (n = 10).
‡Patients who had both generalized and focal epilepsies (n = 12) were excluded from this analysis.
CI = confidence interval; FS = febrile seizures; OR = odds ratio.

To ensure statistical consistency with the original findings,[4] we utilized the Armitage trend test. No other *SCN1A* polymorphisms were examined.

Results. Our Australian study did not replicate the association reported between the AA genotype of the rs3812718 *SCN1A* SNP and increased risk of FS despite using identical FS classification, statistical methodology, and a similar sample size[4] (table). We tested the primary hypothesis of the original study in a cohort of mixed idiopathic generalized and focal epilepsy patients who had a confirmed history of FS. We then performed a secondary post hoc analysis to more closely replicate the population of the original study. We compared our focal epilepsy and FS group (including n = 10 pure FS cases) with our focal epilepsy control group (table). Finally, we compared the Australian FS group (n = 76) with the European control group (n = 701) as described in the original article.[4] On all occasions the results were negative; however, we did not have the sample size required to test the hypothesis in a pure FS group.

Because our Australian cohort consisted of predominantly patients with generalized epilepsy, we also evaluated a pure focal epilepsy cohort from the EPIGEN consortium.[7] SNP *SCN1A* rs922224, which is in complete linkage disequilibrium with rs3812718 ($r^2 = 1$) in Western European populations, was studied. Focal epilepsy patients (n = 1,589) from the United Kingdom, Ireland, Belgium, and the United States showed no difference between those with (n = 232) and those without a history of FS (n = 1,357) ($p = 0.9$, odds ratio AA vs GG homozygotes = 1.0, 95% confidence interval 0.7–1.5) or focal epilepsy with FS vs population controls (n = 1,806) ($p = 0.3$, odds ratio AA vs GG = 1.2, 95% confidence interval 0.8–1.8).

Our failure to replicate the original findings raises the possibility that the original observation was a false positive.

**Members of the EPIGEN Consortium are listed in the appendix.*

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APPENDIX

The EPIGEN Consortium contribution to this work was coordinated by Dr. S.M. Sisodiya. The Consortium additionally comprises the following: C. Depondt, M. Pandolfo (Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium); G. Cavalleri, N. Delanty, S. Alhusaini (The Department of Clinical Neurological Sciences and Molecular and Cellular Therapeutics, RCSI Research Institute, Royal College of Surgeons in Ireland); C. Doherty (Division of Neurology, Beaumont Hospital, Dublin, Ireland); E.L. Heinzen, D.B. Goldstein, R. Radtke, T.J. Urban (Institute for Genome Sciences and Policy, Center for Human Genome Variation, Duke University, Durham, NC); C. Catarino, D. Kasperaviciute, S.K. Tate (Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London, UK). C.D., M.P., N.D., S.A., C.D., R.R., C.C., S.M.S., and S.K.T. participated in the collection of the EPIGEN data. G.L.C., E.L.H., D.B.G., T.J.U., and D.K. analyzed the EPIGEN data. The Australian authors (S.P., I.E.S., T.J.O., and S.F.B.) were not part of the EPIGEN consortium.

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