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A knock-in mouse model for *KCNQ2*-related epileptic encephalopathy displays spontaneous generalized seizures and cognitive impairment

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Abstract

Objective: Early onset epileptic encephalopathy with suppression-burst is one of the most severe epilepsy phenotypes in human patients. A significant proportion of cases have a genetic origin, and the most frequently mutated gene is *KCNQ2*, encoding Kv7.2, a voltage-dependent potassium channel subunit, leading to so-called *KCNQ2*-related epileptic encephalopathy (*KCNQ2*-REE). To study the pathophysiology of *KCNQ2*-REE in detail and to provide a relevant preclinical model, we generated and described a knock-in mouse model carrying the recurrent p.(Thr274Met) variant.

Methods: We introduced the p.(Thr274Met) variant by homologous recombination in embryonic stem cells, injected into C57Bl/6N blastocysts and implanted in pseudopregnant mice. Mice were then bred with 129Sv Cre-deleter to generate heterozygous mice carrying the p.(Thr274Met), and animals were maintained on the 129Sv genetic background. We studied the development of this new model and performed in vivo electroencephalographic (EEG) recordings, neuroanatomical studies at different time points, and multiple behavioral tests.

Results: The *Kcnq2*$^{Thr274Met/+}$ mice are viable and display generalized spontaneous seizures first observed between postnatal day 20 (P20) and P30. In vivo EEG recordings show that the paroxysmal events observed macroscopically are epileptic seizures. The brain of the *Kcnq2*$^{Thr274Met/+}$ animals does not display major structural defects, similar to humans, and their body weight is normal. *Kcnq2*$^{Thr274Met/+}$ mice have a reduced life span, with a peak of unexpected death occurring for 25% of the animals by 3 months of age. Epileptic seizures were generally not observed when animals grew older. Behavioral characterization reveals important deficits in spatial learning and memory in adults but no gross abnormality during early neurosensory development.

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Significance: Taken together, our results indicate that we have generated a relevant model to study the pathophysiology of *KCNQ2*-related epileptic encephalopathy and perform preclinical research for that devastating and currently intractable disease.

KEYWORDS
epileptic encephalopathy, *KCNQ2*, mouse model

1 | INTRODUCTION

Early onset epileptic encephalopathies (EOEEs) are rare and intractable devastating epileptic syndromes whose first clinical signs manifest during early stages of postnatal brain development. They are characterized by early life epileptic seizures associated with a developmental impairment of motor, cognitive, and behavioral skills. There is a genetic basis for EOEEs. We and others have identified de novo pathogenic variants in the *KCNQ2* gene encoding the Kv7.2 subunit of the potassium Kv7/M channel, controlling neuronal excitability in the brain and spinal cord via the M current (I_M). Pathogenic variants in the *KCNQ2* gene represent the major cause of EOEEs, leading to the emergence of the concept of *KCNQ2*-related epileptic encephalopathy (*KCNQ2*-REE) to define this condition.[1-4] In a cohort of 103 patients with an EOEE and an initial electroencephalogram (EEG) showing a suppression-burst pattern (ie, paroxysmal bursts of activity interspersed with periods of electrical silence), managed at Marseille University Hospital by two of us (M.M. and L.V.), a pathogenic or probably pathogenic variant was identified in 57 (55%), and among these, 22 have a pathogenic variant in the *KCNQ2* gene (38%; unpublished observation).

KCNQ2-REE patients have a remarkably homogeneous phenotype at the beginning of the epilepsy. It emerges during the first week of life, with frequent asymmetric tonic seizures resulting in abnormal muscle contractions and apnea. EEG often shows a pattern called "suppression-burst." This is a highly recognizable EEG pattern, reflecting the profound and permanent alteration of brain function. This stormy phase of tonic seizures and abnormal EEG pattern lasts 2 to 15 weeks and usually gives place to a calmer period of rare seizures and significant amelioration of the EEG. Despite this apparently positive evolution in terms of seizures, developmental processes are definitively altered and lead to a severe and global neurological impairment. The vast majority of patients have no informative language, autistic behavior, or significant motor impairment such as tetraplegia, spasticity, ataxia, global hypotonia, or dystonia.[3] The current knowledge on *Kcnq2* function and dysfunction has largely been obtained in heterologous setups (eg, transfected cells, transient transfections in vivo) or mice harboring genetic variants nonrelevant to *KCNQ2*-REE. Here, we describe the first *Kcnq2* knock-in mouse model produced using a recurrent variant causing *KCNQ2*-REE. We show that this new model faithfully reproduces the expected phenotype and that it is relevant both to study *KCNQ2*-related epileptic encephalopathy and to develop much-needed therapeutic approaches for that devastating and currently intractable epileptic encephalopathy.

Key Points

- Generation of a new mouse model carrying a variant described in several patients with *KCNQ2*-related epileptic encephalopathy
- *Kcnq2*$^{Thr274Met/+}$ mice display generalized spontaneous seizures from P20, similar to human neonates
- Early sensorimotor development, paw coordination, and breathing pattern are normal
- *Kcnq2*$^{Thr274Met/+}$ brains do not display major structural malformations
- *Kcnq2*$^{Thr274Met/+}$ mice show important spatial learning and memory deficits

2 | MATERIALS AND METHODS

All experiments involving animals were carried out in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/UE) related to laboratory animals used for research purposes. The study was approved by ethics committee CE14 of the Faculty of Medicine of Marseille (APAFIS #4244-2016022413197476 and APAFIS #13906-2018030514509653). The principles outlined in the ARRIVE guidelines including the 3R concept were considered when planning the experiments.

2.1 | Gene targeting

Detailed information is provided as Supporting Information.

2.2 | Behavioral testing

We measured cognitive abilities with two learning tasks (Morris Water Maze [MWM][5] and Barnes Maze [BM][6]) and exploratory behavior (Hole Board test)[7,8] in the knock-in and wild-type (WT) mice. We screened a possible impairment of neurodevelopment over a follow-up study measuring the age of appearance of the adult responses[6] from birth to day 15. Detailed information on the setup of all the tests performed in this article is provided in Table 1 and as Supporting Information. All the tests were performed by an experimenter blind to the genotype.

2.3 | Statistical analysis

The distributions were nonnormal for the block of MWM and daily scores of BM as shown by both Kolmogorov-Smirnov and Shapiro-Wilk. The differences between the blocks or between the days in MWM and BM were examined with Friedman nonparametric analysis of variance for related samples. The slope of the learning curve was obtained for each mouse in MWM and BM. We calculated the median value of the four trials for the seven blocks. The between-block difference defined the learning slope.[9] We used Statistical Package for the Social Sciences (v19). The difference was also expressed as an effect size that is the percentage of total variance of the dependent variable associated with between-group differences (η^2). We considered the effect size rather than only the probability to reject the null hypothesis because the use of any model for a disease requires not only a significant difference but also a large difference.

2.4 | Neuroanatomical study

Neuroanatomical study was carried out using eight female and seven male mice for the *Kcnq2*$^{Thr274Met/+}$ genotype and nine female and nine male mice for the *Kcnq2*$^{+/+}$ genotype at 15 weeks old per group defined by sex and genotype. Mouse brain samples were fixed in 4% buffered formalin for 48 hours. Forty brain parameters, consisting of area and length measurements, were taken blind to the genotype across one sagittal section (Table S1). Data were analyzed using a two-tailed Student *t* test assuming equal variance to determine whether a brain region is associated with neuroanatomical defect.

2.5 | Video-electrocorticographic monitoring in freely moving mice

Detailed information is provided as Supporting Information.

3 | RESULTS

3.1 | p.(Thr274Met) variant in human *KCNQ2* gene causes EOEE, and is associated with a suppression-burst EEG

Seven patients harboring p.(Thr274Met) in the *KCNQ2* gene (Hg19 chr20:62071057G > A) and suffering from EOEE were reported (ClinVar variation #167208), confirming the severe impact of this missense variant in multiple affected individuals. One female patient followed at Marseille University

TABLE 1 Tasks used to characterize the phenotype of the mice with the number of mice per genotype and the studied functionality

Task	Sample size		Studied functionality
	Kcnq2$^{Thr274Met/+}$	*Kcnq2*$^{+/+}$	
Morris Water Maze	10	10	Spatial memory/learning with positive reinforcement
Barnes Maze	11	11	Spatial memory/learning with positive reinforcement
Hole Board	8	3	Exploratory behavior and motor stereotypy
PhenoRack	14	9	Spontaneous activity
Neurosensory acquisitions	15	16	See Table S2 for details of studied parameters
Accelerating rotarod	14	14	Foreleg and hindleg coordination
Plethysmography	11	10	Breathing characteristics
Marble-burying test	16	6	Motor stereotypy
Elevated Plus Maze	13	12	Anxietylike measure

Hospital by one of us (M.M.) is a heterozygous carrier of the p.(Thr274Met) variant. Seizures were observed from day 3 to day 6; they were controlled with phenobarbital until day 30; some rare seizures were observed until 6 weeks, the time at which carbamazepine was given. Under carbamazepine, the patient became seizure-free. EEG recording revealed a suppression-burst pattern (Figure 1) and several generalized seizures. Brain magnetic resonance imaging and cardiac echography were normal, and metabolic workup was negative. The diagnosis of *KCNQ2*-REE was confirmed at 6 weeks of age, and phenobarbital was progressively replaced by carbamazepine. At 4 years of age, pyramidal signs emerged in the lower limbs. At the end of the follow-up (6 years of age), she was not ambulatory and not able to sit alone; she had no voluntary movement, no language, and poor nonverbal communication. Additional clinical details are provided as Supporting Information.

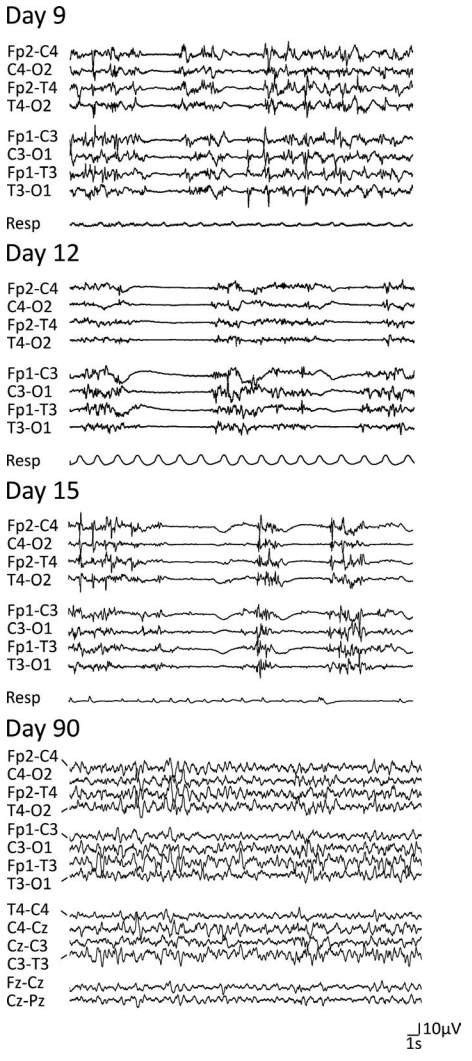


FIGURE 1 Electroencephalographic (EEG) recordings of the female carrying the p.(Thr274Met) variant at day 9, day 12, day 15, and 3 months of life. The first three EEGs display a suppression-burst pattern, with periods of high-amplitude bursts of paroxysmal activity, alternating with periods of flattening of the traces. At 3 months of age, the EEG became continuous, with delta rhythms and no spike

3.2 | Introduction of p.Thr274Met variant into mouse *Kcnq2* gene

The mouse model was generated by homologous recombination in embryonic stem cells using a targeting vector containing regions homologous to the genomic *Kcnq2* sequences and the p.(Thr274Met) variant (Figure 2A,B, Figure S1 and Supplementary Methods). The *Kcnq2*$^{Thr274Met/+}$ animals were maintained and studied on the 129Sv genetic background.

3.3 | Life span and weight of *Kcnq2*$^{T274M/+}$ mice

Kcnq2$^{Thr274Met/+}$ animals have normal weight (Figure S1D) and do not display gross morphological abnormalities when compared to WT littermates. A significant proportion of *Kcnq2*$^{Thr274Met/+}$ mice (approximately 25%) die before 4 months of age, with a peak of mortality evidenced around postnatal day 100 (P100; Figure 2C). Kaplan-Meir survival plot confirms increased early mortality in the *Kcnq2*$^{Thr274Met/+}$ mice (Figure 2D). The half-life of the *Kcnq2*$^{Thr274Met/+}$ mouse population is P221, whereas it is P425 for the population of *Kcnq2*$^{+/+}$ mice. In a period of 2 years of breeding in our laboratory, we observed 192 spontaneous deaths (age range = P19-P356), of which 175 were heterozygous knock-in animals (91%, no difference between males and females). When death follows a seizure, heterozygous knock-in animals are stiff, with extended limbs and a strongly curved spine. This is very typical and distinct from a dead mouse seen elsewhere in our colony. We scored such a posture as "death following a seizure" (see next section).

3.4 | Brain morphology of *Kcnq2*$^{T274M/+}$ mice

To examine finer-scale neuromorphological features in the *Kcnq2*$^{Thr274Met/+}$ mouse, we used a recently developed and highly robust approach for the assessment of 40 brain parameters across 22 distinct brain regions (Table S1). This consisted of a systematic quantification of the same sagittal brain region at lateral +0.60 mm.[10] Seven *Kcnq2*$^{Thr274Met/+}$ and nine *Kcnq2*$^{+/+}$ male mice, and nine female mice of each genotype, were analyzed at 15 weeks of age. In males, moderate macrocephaly was identified in the heterozygous knock-in mice, with an overall tendency for areas to be larger in size when compared to WT mice (Figure 3A,B). The total brain area and height along the dorsoventral axis was larger by a

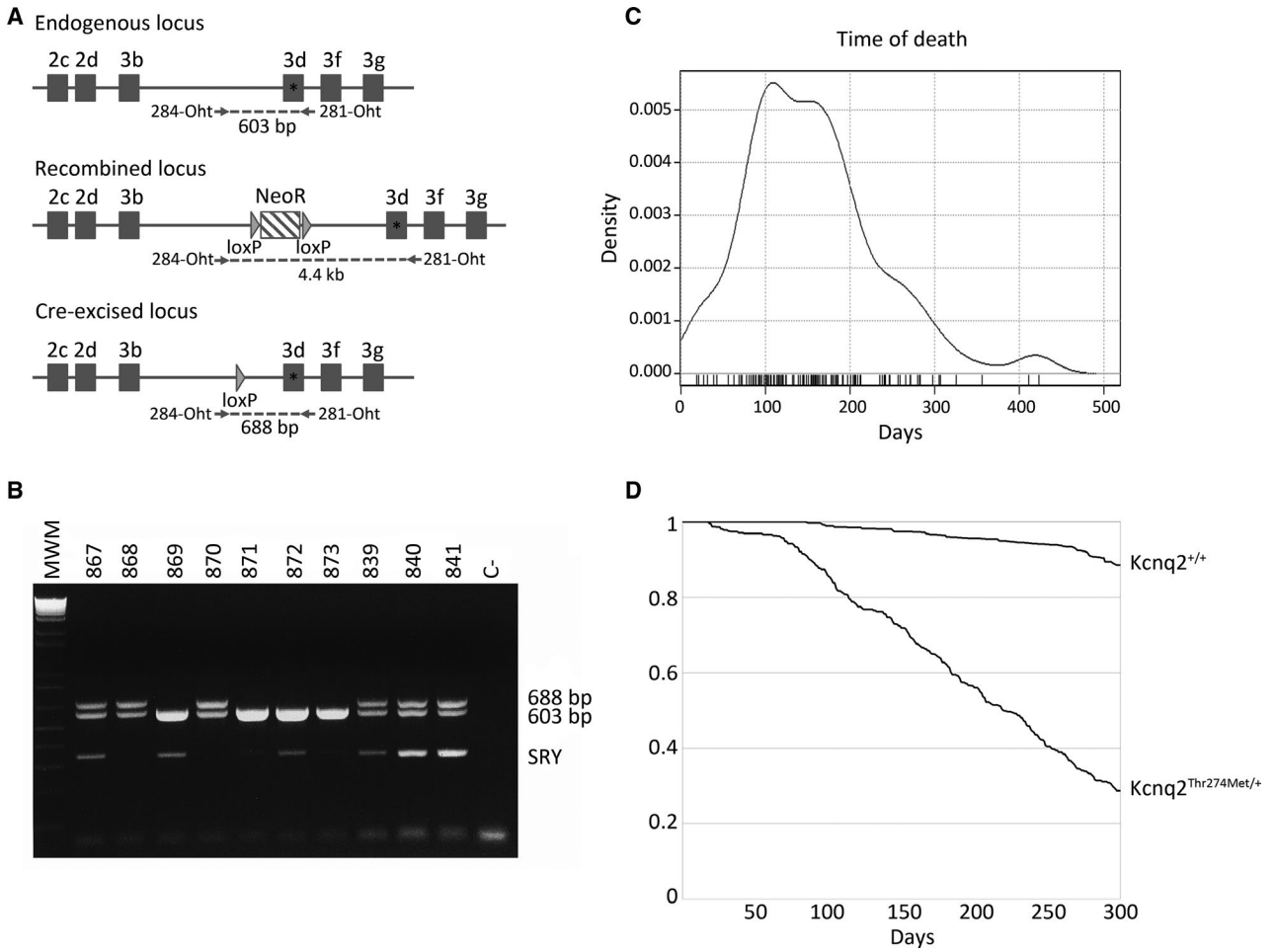


FIGURE 2 A, Schematic representation (not to scale) of the *Kcnq2* endogenous, recombined, and Cre-excised loci. The binding sites of the genotyping primers 284-Oht and 281-Oht are shown. Asterisks show the position of Thr274 in exon 3d of the mouse *Kcnq2* gene. B, Representative example of a genotyping polymerase chain reaction (PCR) using mouse genomic DNA as a template and primers 284-Oht and 281-Oht. PCR without DNA served as a negative control (C−). The results are shown for 10 different animals, including six heterozygous knock-in mice. Multiplexing is performed with SRY genotyping primers to confirm gender. MWM, Molecular Weight Marker. C, Kernel density estimates computed using R, with the time of spontaneous death as a variable. The graph shows the time of death in 175 knock-in animals. Ninety-one percent of spontaneous death in our colony occurred in heterozygous knock-in animals. D, Kaplan-Meier survival plot for the two genotypes from postnatal day 0 (P0) to P300

minimum of 4% ($P < .05$; see Figure 3C for a representative image). Accordingly, the corpus callosum area was significantly larger, by 8% ($P < .05$; Figure 3D), and the superior colliculus by 12% ($P < .005$). Several measures within the hippocampus also showed a tendency and even a significant enlargement in some areas and length (the oriens height was larger by 8%, $P < .05$). Female brains showed little variation from WT animals. Neuroanatomical studies were carried out at earlier stages to study disease progression. We measured the thickness of the motor and somatosensory cortex in coronal sections of prefrontal brain as well as the hippocampus volumes at P14 and P30. We found that, at P14, the thickness of the motor cortex was significantly larger in *Kcnq2*$^{Thr274Met/+}$ mice ($P = .014$), whereas at P30, motor and somatosensory cortices were significantly smaller in *Kcnq2*$^{Thr274Met/+}$ mice ($P = .0014$ and 0.0025, respectively; Figure S2). We did not find any differences between *Kcnq2*$^{+/+}$ and *Kcnq2*$^{Thr274Met/+}$ mice for the global volume of the hippocampus, cornus ammoni (CA), and dentate gyrus areas (Figure S2). These results indicate that there are no major brain morphology defects in the *Kcnq2*$^{Thr274Met/+}$ animals, although subtle gender-specific differences exist.

3.5 | *Kcnq2*$^{T274M/+}$ mouse displays spontaneous generalized tonic-clonic seizures

The home cage behavior was investigated using a 24/24 infrared recording device (Phenorack, Viewpoint). Recordings were performed for 31 heterozygous animals (18 males and 13 females) and compared to 24 WT littermates (11 males, 13 females). Each animal was recorded

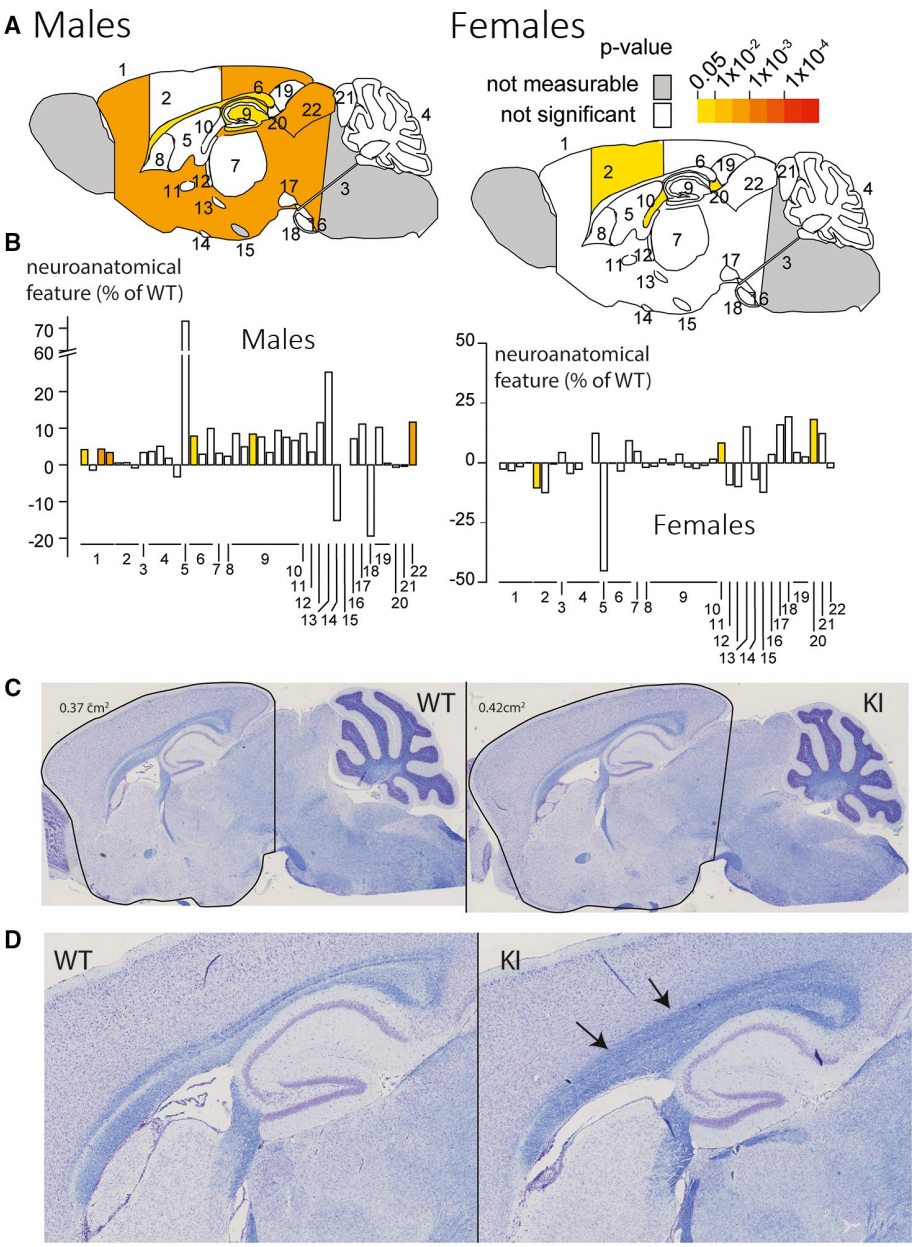


FIGURE 3 A, Schematic representation of a section at lateral +0.60 mm. Colored regions indicate the presence of at least one significant parameter within the brain region at the 0.05 level. White coloring indicates $P > .05$, and gray indicates that there were not enough data to calculate a P value. The left panel is for males, and the right is for females. B, Histograms for males (left) and females (right) showing variation (decreased, minus scale; increased, positive scale) in areas and lengths expressed as percentage of wild type (WT) together with a color map indicating the significance level. Numbers on the x-axis correspond to brain regions illustrated in A, and bars are listed in the order of parameters provided in Table S1. C, Illustrating example of *Kcnq2*$^{+/+}$ (WT) and *Kcnq2*$^{Thr274Met/+}$ (knock-in [KI]) male brain sizes in parasagittal sections double-stained for Nissl and Luxol. D, Representative image of the corpus callosum enlargement in *Kcnq2*$^{Thr274Met/+}$ male brains shown across a parasagittal section

for at least 48 hours, and the data were analyzed blind to the genotype. No significant difference was observed for the distance traveled or the mean velocity of the tested animals (Figure S3). Split analysis for diurnal or nocturnal activity did not reveal any difference between the groups. Exploratory behavior in their home cage was similar to WT littermates and the expected circadian behavior. Our setting, combined with additional direct observations in the colony, led us to observe wild running followed by spontaneous generalized seizures and death in *Kcnq2*$^{Thr274Met/+}$ animals (Figure 4 and Videos S1 to S3). Interestingly, following the occurrence of these generalized seizures, it is possible to rescue the animals before they die using immediate stimulation of forelimbs and hindlimbs (Video S3). To demonstrate



FIGURE 4 Representative example of the different phases of a spontaneous generalized tonic-clonic seizure observed in the *Kcnq2*$^{Thr274Met/+}$ mouse model. These images are extracted from a video where a heterozygous knock-in mouse is housed with a wild-type animal (Video S3)

that the observed paroxysmal events correspond to abnormal neuronal activity, in vivo EEG recordings were performed. Twenty-six mice (16 *Kcnq2*$^{Thr274Met/+}$ and 10 WT, half males, half females) underwent surgical implantation of electrocorticographic (ECoG) electrodes. Because the seizures are usually observed after P30, we performed surgical implantations between P23 and P45 (mean age = 32.8 ± 1.5 days in *Kcnq2*$^{Thr274Met/+}$; 33.2 ± 2.125 days in WT). Of 16 *Kcnq2*$^{Thr274Met/+}$ mice, three had a seizure at the time of the recordings (at P33, P37, and P38, respectively). All of these events were extremely violent, fast, and terminated by the death of the animal. The recording of one of those seizures, from a P32 mouse, is represented in Figure 5. The presence of artifacts during the event reveals the severity of the seizure, as the animal was bumping on the cage walls. After a few seconds, the animal fell on its side with a rigid posture and its ECoG flattened. Of note, four other *Kcnq2*$^{Thr274Met/+}$ mice were found dead on the day after surgery, before we had the opportunity to record them. They had the same rigid posture and extended legs as the one seen after seizures. We investigated hippocampal recordings in nine *Kcnq2*$^{Thr274Met/+}$ and five *Kcnq2*$^{+/+}$ mice, for a total of 119 and 91 hours, respectively. There were an average of 4.75 ± 0.9 hippocampal interictal spikes per hour in *Kcnq2*$^{Thr274Met/+}$ local field potential recordings, which was much more than in *Kcnq2*$^{+/+}$ recordings, with only 0.2 ± 0.22 interictal spikes per hour in *Kcnq2*$^{+/+}$ (Mann-Whitney $U = 2857$, $P < .0001$).

3.6 | *Kcnq2*-REE mouse model has normal early sensorimotor development, paw coordination, and breathing pattern and does not display enhanced stereotypies or anxiety

To determine whether sensory acquisitions were normal in the heterozygous knock-in mice, using a protocol we recently developed,[11] 15 traits featuring the sensory and motor development from P0 to P15 were measured. We considered the day of appearance of a pattern of adult response in the pup (righting, grasping, cliff avoidance, paw placing, geotaxis, auditory and visual onset, hindlimbs crossed extension, and rooting response). Males and females were examined daily (15 *Kcnq2*$^{Thr274Met/+}$ and 16 *Kcnq2*$^{+/+}$ in total). The two genotypes had identical sensory and motor development (Table S2). The righting score and the age at disappearance of the rooting response differed ($P < .03$), but the difference did not reach the 0.01 threshold when corrected for Bonferroni multiple comparisons. Heterozygous knock-in mice behaved similarly to WT on the accelerating rotarod test (Figure S4). Breathing pattern was investigated at P100 using plethysmography and revealed no difference between heterozygous knock-in and WT animals for all measured parameters (mean frequency, hyperventilation, apnea, tidal volume, and respiratory rate; n = 10 *Kcnq2*$^{+/+}$ mice and n = 11 K*cnq2*$^{Thr274Met/+}$ mice; Figure S5). We also investigated the presence of

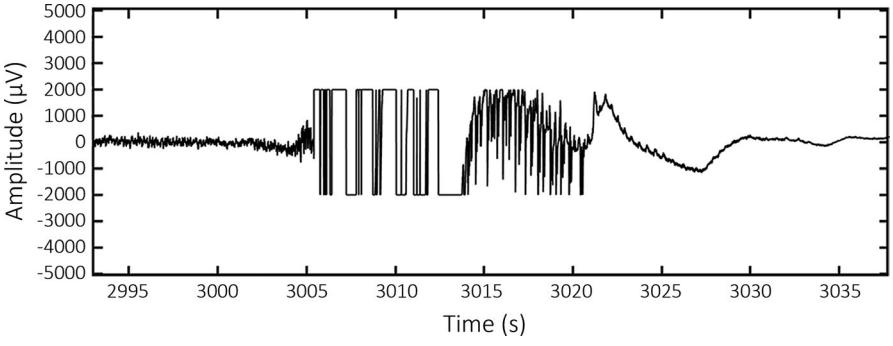


FIGURE 5 Prefrontal cortex electrocorticogram of a P32 *Kcnq2*$^{Thr274Met/+}$ mouse during a seizure. Artifacts are caused by violent seizure movements

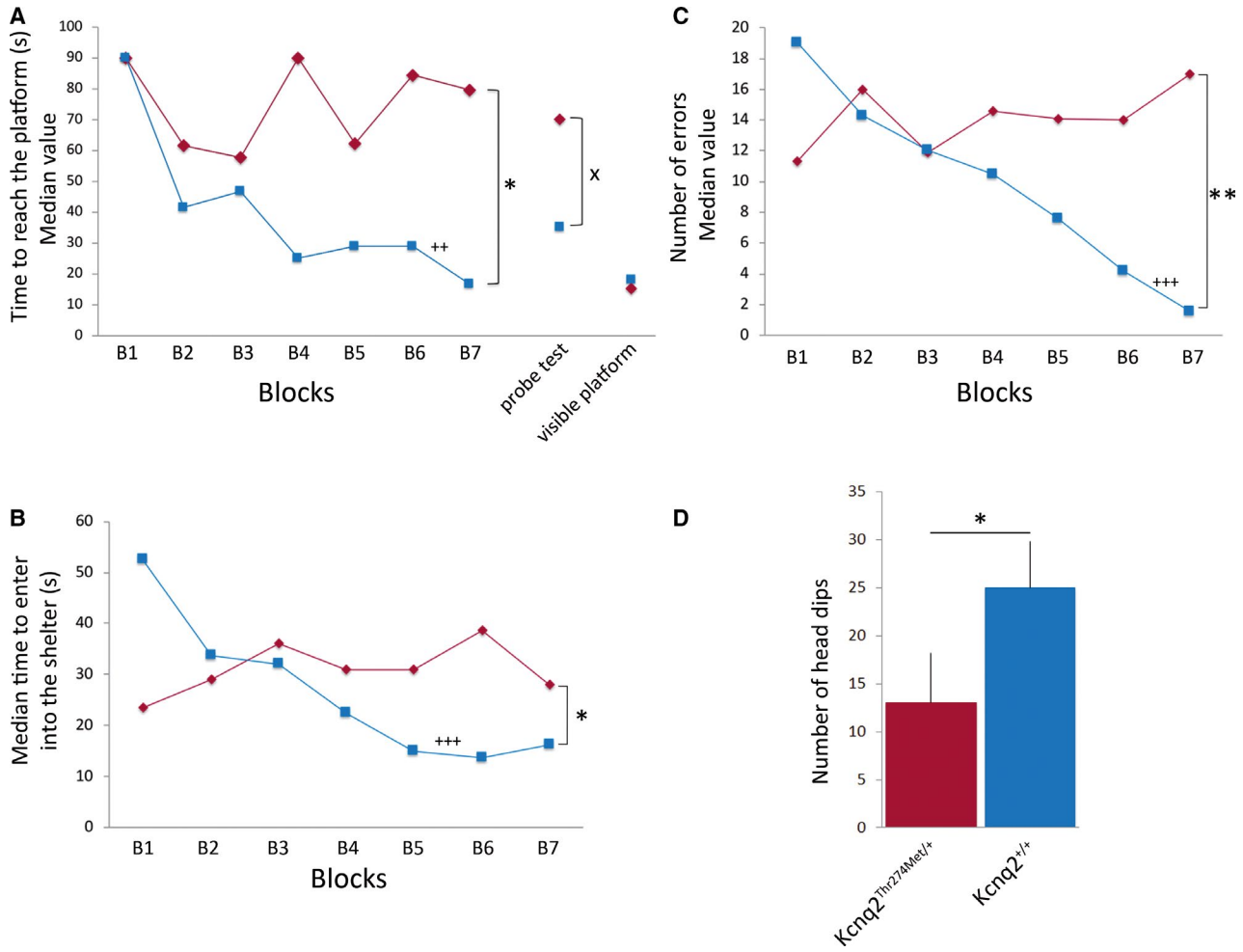


FIGURE 6 Cognitive differences between *Kcnq2*$^{Thr274Met/+}$ and *Kcnq2*$^{+/+}$ mice. A, Time to reach the platform in the Morris Water Maze task. The *Kcnq2*$^{+/+}$ wild-type mice (WT) are represented by blue squares, and *Kcnq2*$^{Thr274Met/+}$ mice (KI) are represented by red diamonds. The time is expressed in seconds, and the figure indicates the median value of a group of 10 mice for each block. Plus signs beside the curve indicate the significance of the within-group, between-blocks variation (χ^2, $^{++}P < .001$). The asterisk beside the left bracket indicates the learning slopes difference (t, $^{*}P < .01$), and the x beside the right bracket indicates the probe test difference (t, $^{x}P < .01$). B, Time to enter into the shelter in the Barnes Maze task. Time is expressed in seconds, and the figure shows the median value of a group of 11 mice for each block. Plus signs beside the curve indicate the significance of the within-group, between-blocks variation (χ^2, $^{+++}P < .001$). The asterisk beside the bracket indicates the learning slopes difference (t, $^{*}P < .01$). C, Number of errors before entering into the shelter in the Barnes Maze task. The median value of the blocks is reported. Plus signs beside the curve indicate the significance of the within-group, between-blocks variation (χ^2, $^{+++}P < .001$). Asterisks beside the bracket indicate the learning slopes difference (t, $^{**}P < .001$). D, Number of head dips in the Hole Board test. Data are given as mean ± SEM for the KI mice and WT mice. t, $^{*}P < .01$

stereotypies in the two groups using the marble-burying test because of the unusual rate of stereotypy in patients with epilepsies.12,13 The test quantifies repetitive and perseverating behavior. The marble-burying score, calculated according to Thomas et al,[14] was identical in the two groups (17.21 ± 9.58 and 14.42 ± 7.17 in *Kcnq2*$^{Thr274Met/+}$ and *Kcnq2*$^{+/+}$ mice respectively, $P < .57$). The difference between the knock-in mice (4.67 ± 3.85) and the controls (5.12 ± 4.79) did not reach the threshold, and the difference remained unchanged when the three measures were calculated with the nonstereotyped dips as covariate ($P < .31$). This result shows that the number of stereotypies in adult animals is not increased by the *Kcnq2*$^{Thr274Met/+}$ genotype. The Elevated Plus Maze was also used, and the results suggest that anxiety did not contribute to the observed learning deficits (Figure S6).

3.7 | Cognition is affected in *Kcnq2*$^{T274M/+}$ mouse

The MWM and BM procedures measure the ability of rodents to solve learning problems using spatial cues. MWM measures the ability to find a submerged resting platform concealed beneath opaque water, whereas BM measures the ability of a mouse to find a path to a shelter to avoid a brightly

illuminated area. The two exercises require the use of extra-maze visual cues, which are provided in the room. To avoid the influence of gender on the results, exclusively males were tested (10 *Kcnq2*$^{Thr274Met/+}$ and 10 *Kcnq2*$^{+/+}$ in MWM and 11 *Kcnq2*$^{Thr274Met/+}$ and 11 *Kcnq2*$^{+/+}$ in BM) during seven blocks. One block consisted of four trials in MWM and two trials in BM. Examination of the distributions (shape and variance) was oriented toward nonparametric statistics. We selected a $P < .01$ threshold after correction for multiple comparisons.

The median time to reach the platform in MWM followed a hieratic curve that did not indicate a decrease during the seven blocks in *Kcnq2*$^{Thr274Met/+}$ mice (within-group, between-blocks difference: $\chi^2 = 13.80$, $df = 6$, $P < .032$), whereas a significant reduction was seen in *Kcnq2*$^{+/+}$ mice ($\chi^2 = 26$, $df = 6$, $P < .001$; Figure 6A). The slopes of the two curves differ ($t = 3.149$, $df = 18$, $P < .01$), with a large effect size ($\eta^2 = 0.33$). The probe test confirms the learning deficit in *Kcnq2*$^{Thr274Met/+}$ mice ($t = 2.49$, $df = 18$, $P < .011$), the effect size ($\eta^2 = 0.28$) being large (Figure 6A). The median time to reach the shelter in BM did not indicate a progression (within-group, between-blocks difference: $\chi^2 = 2.094$, $df = 6$, $P = .901$) in the *Kcnq2*$^{Thr274Met/+}$ mice, whereas a steady decline in the time to reach the shelter was observed in *Kcnq2*$^{+/+}$ mice (within-group, between-blocks difference: $\chi^2 = 23.67$, $df = 6$, $P < .001$). The slopes of the two curves (*Kcnq2*$^{Thr274Met/+}$ vs *Kcnq2*$^{+/+}$) differed ($t = 4.112$, $df = 20$, $P \leq .001$), with a large effect size ($\eta^2 = 0.48$; Figure 6B). We also measured the number of errors. An error was automatically counted when mice stopped for 2 seconds or more in the area corresponding to a dead end. The number of errors decreased in *Kcnq2*$^{+/+}$ ($\chi^2 = 41.85$, $df = 6$, $P = .001$) but not in *Kcnq2*$^{Thr274Met/+}$ ($\chi^2 = 1.85$, $df = 6$, $P < .90$) mice, ending in a slope difference ($t = 5.12$, $df = 20$, $P < .001$) with a large effect size ($\eta^2 = 0.54$; Figure 6C). The walking and the swimming speeds in MWM and BM did not differ between the groups ($P = .68$) and were not correlated with learning scores in the two tasks ($r^2 = 0.01$ with time to reach the hidden platform, $r^2 = 0.002$ with time to enter into the shelter, and $r^2 = 0.001$ with the number of errors).

Kcnq2$^{Thr274Met/+}$ explored less than *Kcnq2*$^{+/+}$ mice during the Hole Board task ($t = 2.89$, $df = 22$, $P = .005$, $\eta^2 = 0.56$; Figure 6D), but we found no correlation with the learning measures done in MWM and BM.

Kcnq2$^{Thr274Met/+}$ and *Kcnq2*$^{+/+}$ mice performed equally in a visual ability test (not shown) as well as in the visible platform version of MWM (Figure 6A), ruling out the contribution of visual dysfunction in the learning deficits observed in the *Kcnq2*$^{Thr274Met/+}$ group.

4 | DISCUSSION

Several knock-out, knock-in, and transgenic mouse models for *Kcnq2* have previously been generated. However, none of them reproduced a pathogenic variant causing a phenotype of epileptic encephalopathy in several human patients. A knock-out model was generated to mimic *KCNQ2* haploinsufficiency known to cause benign familial neonatal seizures (BFNSs).[15] Although homozygous animals died at birth, heterozygous mice displayed enhanced sensitivity to pentylenetetrazol (PTZ) injections, suggesting cortical hyperexcitability. A similar model harboring a large deletion involving *Kcnq2* was generated by N-ethyl-N-nitrosourea (ENU) mutagenesis.[16] Unfortunately, the deletion contained another epilepsy gene, *Chrna4*, complicating its use as a model of BFNS.[17,18] The first mouse model containing a missense variant in *Kcnq2* was a transgenic model (over)expressing a human *KCNQ2* transgene containing the p.(Gly279Ser) variant.[19] The transgene was shown to be inserted on the mouse X chromosome. The missense variant caused a dominant negative effect in *Xenopus laevis* oocytes. It is located in the pore region of the Kcnq2 subunit and was modeled upon variants found in the *KCNQ1* gene and causing long-QT syndrome through a dominant negative effect.[20] The study by Peters et al[19] was the first to report cognitive and behavioral deficits for a *Kcnq2* missense variant. Spontaneous seizures were observed in three of 64 mice. Spontaneous home cage activity and activity recorded in the open field were increased in transgenic males. Hippocampal abnormalities were present (loss of Timm-stained mossy fibers). A critical period was identified in the first postnatal week of life to induce the behavioral and hippocampal changes upon I_M inactivation. Deficits in the probe test of MWM were observed in the transgenic animals, suggesting hippocampal-dependent spatial memory dysfunction.[19] We have not observed dispersion or decrease of thickness of the CA1/CA2 region in the *Kcnq2*$^{Thr274Met/+}$ mouse. The observations of Peters et al in the hippocampi of the p.(Gly279Ser) transgenic mouse may not be specific to *Kcnq2*-related epileptic encephalopathy. The second *Kcnq2* missense variant studied in the mouse was an ENU-generated loss-of-function variant p.(Val182Met) used to exacerbate the phenotype of a Scn2a[21] or Scn1a[22] mouse model of epilepsy. The p.(Val182Met) variant in *KCNQ2* has, since then, been reported once in a patient with an unknown phenotype (ClinVar variation #427046). A knock-in mouse expressing the p.(Ala306Thr) missense variant causing a BFNS phenotype in humans was generated and shown to have a reduced seizure threshold, abnormal hippocampal excitability, and altered kindling acquisition rates.[23,24] Spontaneous seizures were seen only in homozygous animals. Two other knock-in mice were produced using an alternative knock-in strategy called "kick-in."[25] One line harbored the previously studied p.(Ala306Thr) variant and the second line a p.(Tyr284Cys) variant shown to cause a BFNS phenotype in humans. In these two lines, brain morphology, EEG, and open field and rotarod tests were normal, but mutant

animals showed increased severity for PTZ-induced seizures.[25,26] The p.(Ile205Val) variant was transfected into the mouse pyramidal neurons using in utero electroporation, and it induced an increase in excitability,[27] similar to the depletion of Kcnq2 from the same cells. Recently, homozygous mice carrying the p.(Ser559Ala) variant, involved in phosphorylation of Kcnq2 by protein kinase C,[28] were shown to have better resistance to chemically induced seizures and preserved neuronal integrity.

Hence, to the best of our knowledge, our model is the first knock-in mouse harboring a recurrent variant known to cause *KCNQ2*-REE in several human patients. The characterization of the *Kcnq2*$^{Thr274Met/+}$ mouse reveals that it faithfully reproduces what is expected based on the human phenotype: no gross morphological brain alterations, no neurosensory alterations before the onset of seizures occurring at P20 followed by a high rate of unexpected death in epilepsy, and important cognitive difficulties. At this stage, we cannot exclude the possibility that subtle seizures also occur during early development and have not been noticed by our analyses. There is currently no demonstration of an increased rate of sudden unexpected death in epilepsy in Kcnq2 encephalopathy, and more data are clearly needed to study this point in greater detail. However, the life span of patients affected by Ohtahara syndrome was shown to be reduced.[29]

The p.(Thr274Met) variant has been studied using transfected *X. laevis* oocytes and was shown to induce a dominant negative effect, with a 70%–80% reduction of the WT current when cotransfected with WT subunits in a 1:1 ratio.[30] The introduction of the p.(Thr274Met) variant results in cognitive disorders with regard to spatial learning and memory in *Kcnq2*$^{Thr274Met/+}$ mice. They perform poorly on MWM and BM. These results support the implication of p.Thr274Met in the hippocampus functions in lines with previous conclusions.[19] Hippocampal functions modulate not only spatial memory as measured by MWM and BM,[31,32] but also exploration,[33] which is reduced in the *Kcnq2*$^{Thr274Met/+}$ mice. The impairment of the cognitive processes reported here seems independent from motor and sensory factors. The large effect sizes reported here are in the pathological range and confirm the validity of p.Thr274Met mice as a model of *KCNQ2*-related epileptic encephalopathy.

Most interestingly, the *Kcnq2*$^{Thr274Met/+}$ animals have a transient epileptic phenotype, and seizures are only exceptionally observed after P100. These results, combined with the observation in patients that the EEG normalizes and seizures will generally cease after a few months, suggest that compensatory mechanisms occur in the mutant cells to cope with I_M dysfunction. The identification of these adaptive mechanisms will be of uttermost importance to understand the role of I_M in the generation of seizures and the epileptic phenotype. It will also be key to understanding what cellular or molecular components help shift neuronal excitability below the seizure threshold. Once identified, these components will provide candidates for the development of new treatments. The *Kcnq2*$^{Thr274Met/+}$ model can be used to refine our understanding of I_M function and dysfunction. It can also be used as a preclinical model to develop treatments targeting seizure repetition as early as possible during the disease process, if not from the beginning. We believe that the treatments, if they are effective, should have an impact on the life span (half of the heterozygous mice die by P221) and improve the score of the mice on MWM or BM. Other deficits will be investigated in the future with additional tests, and we will also try to trigger seizures on demand using specific stimulations such as light or sound. The presence of generalized cortical hyperexcitability and the recurrence of multiple seizures are thought to contribute strongly to neurological deterioration in patients. Providing an early solution to stop the seizures will hopefully preserve patients' neurological development.

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CONFLICT OF INTEREST

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 online in the Supporting Information section.

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