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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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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 (IM). 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 set-ups (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.

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] and Barnes Maze [BM]) and exploratory behavior (Hole Board test) 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 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. 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 ($\eta^2$). 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.

| Task                        | Sample size | $Kcnq2^{Thr274Met/+}$ | $Kcnq2^{+/+}$ | Studied functionality                   |
|-----------------------------|-------------|-----------------------|---------------|----------------------------------------|
| 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                    | Anxiety-like 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.

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<sup>Thr274Met/+</sup> animals were maintained and studied on the 129Sv genetic background.

3.3 Life span and weight of Kcnq2<sup>T274M/+</sup> mice

Kcnq2<sup>Thr274Met/+</sup> animals have normal weight (Figure S1D) and do not display gross morphological abnormalities when compared to WT littermates. A significant proportion of Kcnq2<sup>Thr274Met/+</sup> 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<sup>Thr274Met/+</sup> mice (Figure 2D). The half-life of the Kcnq2<sup>Thr274Met/+</sup> mouse population is P221, whereas it is P425 for the population of Kcnq2<sup>^+/+</sup> 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<sup>T274M/+</sup> mice

To examine finer-scale neuromorphological features in the Kcnq2<sup>T274M/+</sup> 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. Seven Kcnq2<sup>Thr274Met/+</sup> and nine Kcnq2<sup>^+/+</sup> 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 1](image-url)
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...
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.

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

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**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.
that the observed paroxysmal events correspond to abnormal neuronal activity, in vivo EEG recordings were performed. Twenty-six mice (16 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+; 33.2 ± 2.125 days in WT). Of 16 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ mice; Figure S5). We also investigated the presence of

FIGURE 4 Representative example of the different phases of a spontaneous generalized tonic-clonic seizure observed in the Kcnq2Thr274Met/+ mouse model. These images are extracted from a video where a heterozygous knock-in mouse is housed with a wild-type animal (Video S3).

FIGURE 5 Prefrontal cortex electrocorticogram of a P32 Kcnq2Thr274Met/+ mouse during a seizure. Artifacts are caused by violent seizure movements.
stereotypies in the two groups using the marble-burying test because of the unusual rate of stereotypy in patients with epilepsies. The marble-burying score, calculated according to Thomas et al., 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<sup>Thr274Met/+</sup> and 10 Kcnq2<sup>+/+</sup> in MWM and 11
Kcnq2<sup>Thr274Met/+</sup> and 11 Kcnq2<sup>+/+</sup> 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
hierarchical curve that did not indicate a decrease during the seven
blocks in Kcnq2<sup>Thr274Met/+</sup> mice (within-group, between-blocks
difference: $\chi^2 = 13.80, df = 6, P < .032$), whereas a significant
reduction was seen in Kcnq2<sup>+/+</sup> 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<sup>Thr274Met/+</sup> 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<sup>Thr274Met/+</sup>
mice, whereas a steady decline in the time to reach the shelter
was observed in Kcnq2<sup>+/+</sup> mice (within-group, between-blocks
difference: $\chi^2 = 23.67, df = 6, P < .001$). The slopes of the two
curves (Kcnq2<sup>Thr274Met/+</sup> vs Kcnq2<sup>+/+</sup>) 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<sup>+/+</sup> ($\chi^2 = 41.85, df = 6, P = .001$) but not in Kcnq2<sup>Thr274Met/+</sup>
($\chi^2 = 1.85, df = 6, P < .90$) 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<sup>Thr274Met/+</sup> explored less than Kcnq2<sup>+/+</sup> 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<sup>Thr274Met/+</sup> and Kcnq2<sup>+/+</sup> mice performed equally in
a visual ability test (not shown) as well as in the visible plat-
form version of MWM (Figure 6A), ruling out the contribu-
tion of visual dysfunction in the learning deficits observed in
the Kcnq2<sup>Thr274Met/+</sup> group.

4 | DISCUSSION

Several knock-out, knock-in, and transgenic mouse mod-
els 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 (BFNS).<sup>15</sup> Although homozygous ani-
mal died at birth, heterozygous mice displayed enhanced
sensitivity to pentylentetrazol (PTZ) injections, suggest-
ing cortical hyperexcitability. A similar model harboring a
large deletion involving Kcnq2 was generated by N-ethyl-
N-nitosourea (ENU) mutagenesis.<sup>16</sup> Unfortunately, the
deletion contained another epilepsy gene, Chrrna4, compli-
cating its use as a model of BFNS.<sup>17,18</sup> The first mouse model containing a missense variant in Kcnq2 was a trans-
genetic model (over)expressing a human KCNQ2 transgene
containing the p.(Gly279Ser) variant.<sup>19</sup> 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.<sup>20</sup> The study by Peters et al.<sup>19</sup>
was the first to report cognitive and behavioral deficits for
a Kcnq2 missense variant. Spontaneous seizures were obser-
vied 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<sub>Na</sub> inactivation.
Deficits in the probe test of MWM were observed in the
transgenic animals, suggesting hippocampal-dependent
spatial memory dysfunction.<sup>19</sup> We have not observed dis-
appearance or decrease of thickness of the CA1/CA2 region in
the Kcnq2<sup>Thr274Met/+</sup> mouse. The observations of Peters
et al in the hippocampi of the p.(Gly279Ser) transgenic
mouse may not be specific to Kcnq2-related epileptic en-
cephalopathy. 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<sup>21</sup> or Scn1a<sup>22</sup> 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 expres-
sing the p.(Ala306Thr) missense variant causing a BFNS
phenotype in humans was generated and shown to have a
reduced seizure threshold, abnormal hippocampal excitabil-
ity, and altered kindling acquisition rates.<sup>23,24</sup> Spontaneous
seizures were seen only in homozygous animals. Two other
knock-in mice were produced using an alternative knock-
in strategy called “kick-in.”<sup>25</sup> One line harbored the previ-
ously 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. The p.(Ile205Val) variant was transfected into the mouse pyramidal neurons using in utero electroporation, and it induced an increase in excitability, 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, 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 Kcnq2Thr274Met/+ 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.

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. The introduction of the p.(Thr274Met) variant results in cognitive disorders with regard to spatial learning and memory in Kcnq2Thr274Met/+ 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. Hippocampal functions modulate not only spatial memory as measured by MWM and BM, but also exploration, which is reduced in the Kcnq2Thr274Met/+ 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 Kcnq2Thr274Met/+ 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\textsubscript{M} dysfunction. The identification of these adaptive mechanisms will be of uttermost importance to understand the role of I\textsubscript{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 Kcnq2Thr274Met/+ model can be used to refine our understanding of I\textsubscript{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.

References
1. Weckhuysen S, Mandelstam S, Suls A, et al. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol. 2012;71:15–25.
2. Saitsu H, Kato M, Koide A, et al. Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. Ann Neurol. 2012;72:298–300.

3. Milh M, Broutry-Kryza N, Sutera-Sardo J, et al. Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2. Orphanet J Rare Dis. 2013;8:80.

4. Milh M, Lacoste C, Cacciagli P, et al. Variable clinical expression in patients with mosaicism for KCNQ2 mutations. Am J Med Genet. 2015;167A:2314–8.

5. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11:47–60.

6. Barnes CA. Neurological and behavioral investigations of memory failure in aging animals. Int J Neurol. 1987;21:105–60.

7. Makajnuola RO, Hill G, Dow RC, Campbell G, Ashcroft GW. The effects of psychotropic drugs on exploratory and stereotyped behaviour of rats studied on a hole-board. Psychopharmacology. 1977;55:67–74.

8. Caubit X, Gubellini P, Andrieux J, et al. TSHZ3 deletion causes an autism syndrome and defects in cortical projection neurons. Nat Genet. 2016;48:1359–69.

9. Roubertoux PL, Baril N, Cau P, et al. Cognitive and motor profiles associated with partial trisomy. Modeling Down syndrome in mice. Behav Genet. 2017;47:305–22.

10. Collins SC, Wagner C, Gagliardi L, et al. A method for parasagittal sectioning for neuroanatomical quantification of brain structures in the adult mouse. Curr Protoc Mouse Biol. 2018;8:e48.

11. Roubertoux PL, Ghata A, Carlier M. Measuring preweaning sensorial and motor development in the mouse. Curr Protoc Mouse Biol. 2018;8:54–78.

12. Bercum FM, Rodgers KM, Benison AM, et al. Maternal stress combined with terbutaline leads to comorbid autistic-like behavior and epilepsy in a rat model. J Neurol. 2015;3:15894–902.

13. Ravizza T, Onat FY, Brooks-Kayal AR, et al. WONOEP appraisal: biomarkers of epilepsy-associated comorbidities. Epilepsia 2017;58:331–42.

14. Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology. 2009;204:361–73.

15. Watanabe H, Nagata E, Kosakai A, et al. Disruption of the epilepsy KCNQ2 gene results in neural hyperexcitability. J Neurochem. 2000;75:28–33.

16. Yang Y, Beyer BJ, Otto JF, et al. Spontaneous deletion of epilepsy gene orthologs in a mutant mouse with a low electroconvulsive threshold. Hum Mol Genet. 2003;12:975–84.

17. Otto JF, Yang Y, Frankel WN, Wilcox KS, White HS. Mice carrying the szt1 mutation exhibit increased seizure susceptibility and altered sensitivity to compounds acting at the m-channel. Epilepsia. 2004;45:1009–16.

18. Otto JF, Yang Y, Frankel WN, White HS, Wilcox KS. A spontaneous mutation involving Kcnq2 (Kv7.2) reduces M-current density and spike frequency adaptation in mouse CA1 neurons. J Neurosci. 2006;26:2053–9.

19. Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. Nat Neurosci. 2005;8:51–60.

20. Schroeder BC, Kubisch C, Stein V, Jentsch TJ. Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K+ channels causes epilepsy. Nature. 1998;396:687–90.

21. Kearney JA, Yang Y, Beyer B, et al. Severe epilepsy resulting from genetic interaction between Scn2a and Kcnq2. Hum Mol Genet. 2006;15:1043–8.

22. Hawkins NA, Martin MS, Frankel WN, Kearney JA, Escayg A. Neuronal voltage-gated ion channels are genetic modifiers of generalized epilepsy with febrile seizures plus. Neurobiol Dis. 2011;41:655–60.

23. Otto JF, Singh NA, Dahle EJ, et al. Electroconvulsive seizure thresholds and kindling acquisition rates are altered in mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions. Epilepsia. 2009;50:1752–9.

24. Singh NA, Otto JF, Dahle EJ, et al. Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. J Physiol. 2008;586:3405–23.

25. Tomonoh Y, Deshimaru M, Araki K, et al. The kick-in system: a novel rapid knock-in strategy. PLoS One. 2014;9:e88549.

26. Uchida T, Lossin C, Ihara Y, et al. Abnormal g-aminobutyric acid neurotransmission in a Kcnq2 model of early onset epilepsy. Epilepsia. 2017;58:1430–9.

27. Niday Z, Hawkins VE, Soh H, Mulkey DK, Tzingounis AV. Epilepsy-associated KCNQ2 channels regulate multiple intrinsic properties of layer 2/3 pyramidal neurons. J Neurosci. 2017;37:576–86.

28. Greene DL, Kosenko A, Hoshi N. Attenuating M-current suppression in vivo by a mutant Kcnq2 gene knock-in reduces seizure burden and prevents status epilepticus-induced neuronal death and epileptogenesis. Epilepsia. 2018;59:1908–18.

29. Yamatogi Y, Ohtahara S. Early-infantile epileptic encephalopathy with suppression-bursts, Ohtahara syndrome; its overview referring to our 16 cases. Brain Dev. 2002;24:13–23.

30. Orhan G, Bock M, Schepers D, et al. Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. Ann Neurol. 2014;75:382–94.

31. Remmelink E, Chau U, Smit AB, Verhage M, Loos M. A one-week 5-choice serial reaction time task to measure impulsivity and attention in adult and adolescent mice. Sci Rep. 2017;7:42519.

32. Eichenbaum H. Time (and space) in the hippocampus. Curr Opin Behav Sci. 2017;17:65–70.

33. Lever C, Burton S, O’Keefe J. Rearing on hind legs, environmental novelty, and the hippocampal formation. Rev Neurosci. 2006;17:111–33.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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