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CRITICAL REVIEW

Mouse models of *Kcnq2* dysfunctionLucile Brun¹ | Jean-Charles Viemari² | Laurent Villard^{1,3} ¹Aix Marseille Univ, Inserm, MMG, Marseille, France²Aix Marseille Univ, CNRS, INT, Marseille, France³Service de Génétique Médicale, AP-HM, Hôpital de La Timone, Marseille, France**Correspondence**Laurent Villard, Aix Marseille Univ, Inserm, MMG, Marseille, France.
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Abstract

Variants in the Kv7.2 channel subunit encoded by the *KCNQ2* gene cause epileptic disorders ranging from a benign form with self-limited epileptic seizures and normal development to severe forms with intractable epileptic seizures and encephalopathy. The biological mechanisms involved in these neurological diseases are still unclear. The disease remains intractable in patients affected by the severe form. Over the past 20 years, *KCNQ2* models have been developed to elucidate pathological mechanisms and to identify new therapeutic targets. The diversity of *Kcnq2* mouse models has proven invaluable to access neuronal networks and evaluate the associated cognitive deficits. This review summarizes the available models and their contribution to our current understanding of *KCNQ2* epileptic disorders.

KEYWORDS

developmental and epileptic encephalopathy, M current, mouse models, self-limited familial neonatal-infantile epilepsy

1 | INTRODUCTION

The *KCNQ2* and *KCNQ3* genes encode the K_v7.2 and K_v7.3 subunits of voltage-gated potassium channels. The homomeric (K_v7.2) and heteromeric (K_v7.2/K_v7.3) potassium channels generate the M current (I_M).¹ The M current is a slow activating and non-inactivating voltage-gated potassium current that regulates neuronal excitability by preventing repetitive neuronal firing.²⁻⁴ Variants in the *KCNQ2* gene cause self-limited familial neonatal epilepsy (SeLFNE)⁵⁻⁷ or developmental and epileptic encephalopathies (DEEs),⁸ inherited variants being found in self-limited forms, whereas most cases of DEE are caused by de novo variants. Most often, patients with SeLFNE have a good prognosis with seizure remission within the first months of life and a normal neurological outcome.⁹ However, epileptic seizures can occur later in life

in patients with SeLFNE and developmental delay may be present.^{10,11} Patients with *KCNQ2*-related DEE present severe motor and cognitive disabilities together with epileptic seizures and an abnormal electroencephalography (EEG) studies,¹² not seen in patients with SeLFNE. Although patients with SeLFNE can be efficiently treated with various antiepileptic drugs (AEDs) such as phenobarbital or carbamazepine, patients with DEE usually respond poorly to treatment.^{7,13} AEDs given to patients at the onset of seizures may be acting on the potassium channel directly. Consequently it is important to understand the functional consequences of a given variant before initiating treatments because *KCNQ2* variants may have opposite functional consequences. Insights gained from studies using transfected cells such as Chinese Hamster Ovary (CHO) or *Xenopus laevis* oocytes^{14,15} provide a new understanding of the mechanisms at the cellular level,

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and insightful data were obtained. However, transposing these results to humans is challenging, and more investigations are needed at the network and whole organism level. During the last 20 years, *Kcnq2* mouse models have been developed and studied using a battery of tests, from observation of their epileptic or behavioral phenotypes to the study of M current in brain networks. This review aims at providing an overview of available *KCNQ2* mouse models and their characteristics.

2 | KNOCKOUT MODELS

The first *Kcnq2* mouse models were created to study SeLFNE, when it was the only phenotype associated with autosomal dominant genetic variants in patients.⁵ The majority of *KCNQ2* variants causing a SeLFNE phenotype are loss-of-function (LoF) variants. These variants are proposed to lead to the reduction of Kv7.2 subunits by half through frameshifts or deletions. Hence, SeLFNE-mimicking mouse models rely on LoF variants or deletion of *Kcnq2*. Yang and colleagues identified a 300 kb heterozygous deletion of the *Kcnq2* gene obtained by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis.¹⁶ The deletion includes part of the *Kcnq2* and *Chrna4* genes, the latter being a gene also causing an epilepsy phenotype.¹⁷ An identical deletion was discovered later in two patients with SeLFNE.¹⁸ A second SeLFNE model was created through the heterozygous deletion of the *Kcnq2* gene.^{19–21} These mice have modeled for the first time important aspects of a *Kcnq2*-related disorder such as neuronal hyperexcitability and epileptic behavior. Homozygous *Kcnq2* knockout (KO) pups die within a few hours after birth.^{16,21} The cause of death seems to be related to respiratory distress, with abnormal lung alveolar expansion referred to atelectasis and pups described as breathless and turning blue just before dying.¹⁶

To explore the consequences of the absence of Kv7.2 and circumvent the early lethality of homozygous KO animals, a homozygous deletion of *Kcnq2* in late-stage embryos was studied.²² In addition to proving that *Kcnq2* is required for M-channel activity from the embryonic stages, this study showed an increased transcription of the *Kcnq3* and *Kcnq5* genes, suggesting a compensatory mechanism following the loss of *Kcnq2* expression.²² Recently, several models with *Kcnq2* deletion restricted to cortical pyramidal neurons and CA1 excitatory and inhibitory neurons were produced.^{23–26} They showed that *Kcnq2* is required to regulate neuronal excitability through cortical spreading depolarization regulation, the increase of input resistance, action potential number, and medium afterhyperpolarization (mAHP) in pyramidal neurons.^{23–25} The conditional deletion of *Kcnq2* in inhibitory interneurons has also been

Key points

- *KCNQ2*-DEE (developmental and epileptic encephalopathies) mouse models display spontaneous seizures, abnormal electroencephalography (EEG) patterns, M-current decrease, and behavioral deficits.
- *KCNQ2*-DEE mouse models have a variable phenotype depending on the variant nature and genetic background.
- Self-limited familial neonatal epilepsy (SeLFNE) mouse models present lower seizure threshold and behavioral deficits.
- The hippocampus is inconstantly affected in the *KCNQ2*-DEE models.
- *KCNQ2*-DEE mouse model can be used as pre-clinical models.

investigated,^{26,27} and these studies suggested that the M current could remodel excitatory networks. Crispr/Cas9 editing was used to delete *Kcnq2* and *Kcnq3* in hypocretin (Hcr) neurons. The downregulation of the M current increases the hyperexcitability of Hcr neurons and leads to sleep instability during aging in mice.²⁸ Very recently, a *Kcnq2* conditional KO model²⁵ was crossed with *Emx1*-Cre mice to obtain a knock out of *Kcnq2* in forebrain excitatory neurons. The downregulation of *Scn8a* in these mice using antisense oligonucleotides led to an extension of their lifespan (although it did not prevent premature lethality) and a prevention of seizures.²⁹ These results are in good agreement with the efficacy of sodium channel blockers in a proportion of individuals with *KCNQ2* variants.

3 | KNOCK-IN MODELS

Knock-in (KI) models were created by the insertion of variants in the voltage-sensor domain or in the pore loop of Kv7.2 channels, two regions being hotspots for pathogenic variants,³⁰ but also in the C-terminal domain (Figure 1). The p.(A306T) and p.(Y284C) variants were first identified in patients with SeLFNE.⁵ When studied in *Xenopus laevis* oocytes, they reduced I_M amplitude by 20%–40%.³¹ The two variants have been studied in homozygous and heterozygous states. Homozygous *Kcnq2*^{A306T/A306T} and *Kcnq2*^{Y284C/Y284C} mice were the first to present spontaneous generalized tonic-clonic seizures. These seizures are accompanied by a decrease in the M current in CA1 neurons. Heterozygous animals for both variants, which are more similar to the genotype of patients with SeLFNE,

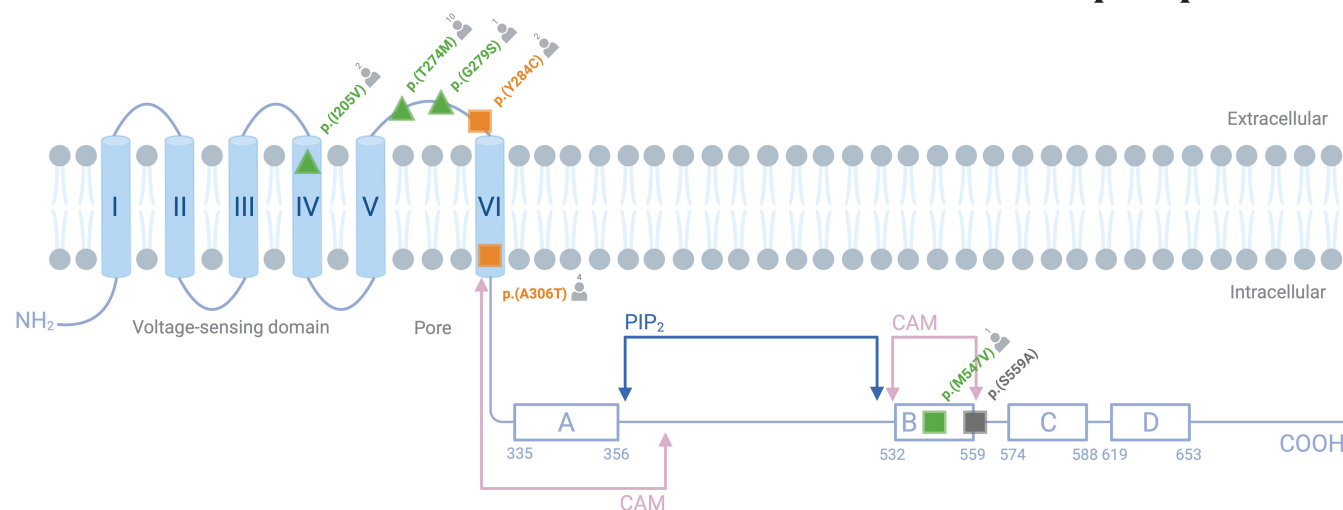


FIGURE 1 Location of pathogenic variants introduced in *Kcnq2* mouse models on a schematic representation of Kv7.2. Topological representation of a single Kv7.2 subunit including the voltage-sensing domain, the six transmembrane segments (I to VI), and the pore loop. The domains interacting with PIP₂ (phosphatidylinositol-[4,5]-biphosphate) and CAM (calmodulin) are indicated, together with the A-D boxes according to Soldovieri and colleagues.⁷⁷ The triangles represent dominant-negative variants, and the squares represent loss-of-function variants. Pathogenic variants found in patients with DEE are in green, those found in patients with SeLFNE are in orange, and the one in gray indicates that it was not found in a patient. The numbers above the silhouettes indicate the number of patients described with this variant in Clinvar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and Weckuysen et al. (2012),⁸ Pisano et al. (2015),¹² for p.(I205V), Weckuysen et al. (2012),⁸ Milh et al. (2013),⁷⁸ Milh et al. (2015),⁷⁹ Millichap et al. (2016),⁸⁰ Hortigüela et al. (2017),⁸¹ Zhang et al. (2017),⁸² Olson et al. (2017)⁸³ for p.(T274M), Sharawat et al. (2019)⁸⁴ for p.(G279S), Singh et al. (1998),⁵ Singh et al. (2003)⁸⁵ for p.(Y284C); Singh et al. (1998),⁵ Singh et al. (2003),⁸⁵ Soldovieri et al. (2014),⁶³ Dimassi et al. (2016)⁸⁶ for p.(A306T), Weckuysen et al. (2012)⁸ for p.(M547V) corresponding to p.(M546V) in *Homo sapiens*.

displayed an interesting low seizure threshold but no profound alterations in M-current amplitude.³²⁻³⁵ Of interest, the heterozygous or homozygous p.(Y284C) variant can enhance γ -aminobutyric acid (GABA) release in CA1 interneurons early after birth and can contribute to an excitatory GABAergic neurotransmission.³⁶

Other variants such as p.(G279S), p.(I205V), p.(T274M), and p.(M547V) were identified in patients with DEE.⁸ Most of these variants have shown, in heterologous models, a dominant negative effect.^{15,31} Mouse models bearing the p.(G279S), p.(T274M), and p.(M547V) variants were generated to model a DEE phenotype: the expression of p.(G279S) was restricted to the cortex and hippocampus,³⁷ whereas the expression of p.(M547V) was restricted to excitatory pyramidal neurons and astrocytes of the forebrain.³⁸ The transgenic model produced by Peters et al.³⁷ was developed prior to the recognition that *KCNQ2* variants could lead to DEE, and was initially used to test the role of *Kcnq2* in neuronal excitability, not its role in disease.

The p.(I205V) variant was introduced transiently using in utero electroporation to model *KCNQ2* dysfunction only.²⁴ The model bearing the p.(T274M) variant was the first to reproduce a physiological context, that is, with two *Kcnq2* alleles, an heterozygous variant, under the expression of their endogenous promoter.³⁹ As observed

in the conditional KO (cKO) model, the p.(I205V) model displayed an increased excitability in L2/3 pyramidal cells.^{24,25} For the other models, a severe phenotype was observed with spontaneous epileptic seizures ranging from partial seizures to generalized tonic-clonic, accompanied by cognitive deficits consisting of learning and memory deficits, hyperactivity, and a decrease in the M current.³⁷⁻⁴⁰ To explore the consequences of M-current suppression for the epileptic phenotype, a homozygous model was created bearing the variant p.(S559A), which attenuates the neurotransmitter-induced M-current suppression.⁴¹ In addition, studies of the p.(G279S) variant allowed highlighting of the role of *Kcnq2*-related neuronal hyperexcitability in the forebrain for the perception of visceral pain,⁴² on the fear memory trace,⁴³ and to study the relationship between the peptide hormone ghrelin and *Kcnq2* in the context of dopaminergic neurotransmission.⁴⁴ *Kcnq2*-deficient mouse models generated from 2000 to 2022 are presented in Table 1.

4 | LIMITATIONS RELATED TO MODEL DESIGN

Several limitations related to model design need to be mentioned. Initially, the models of *Kcnq2* dysfunction

TABLE 1 *Kcng2*-deficient mouse models created from 2000 to 2022

Reference	Genetic background	Type of model	Genotype	Variant location	Variant effect	Expression of the variant	Mouse model production
Watanabe et al. (2000) ²¹	C57BL/6J	KO	+/-	-	-	Constitutive	Homologous recombination
Robbins et al. (2013) ²²			-/-				
Kim et al. (2020) ¹⁹							
Monni et al. (2022) ²⁰							
Yang et al. (2003) ¹⁶	C57BL/6J	KO	<i>Szt1</i>	C-terminal domain	-	Constitutive	ENU
Otto et al. (2004) ⁴⁸							
Otto et al. (2006) ⁵⁷							
Peters et al. (2005) ³⁷	na	Conditional transgenic	Tg p.(G279S)	Pore	Dominant negative <i>Xenopus laevis</i> oocytes for testing variant effect	Cortex and hippocampus	Transgene with Prnp promoter
Singh et al. (2008) ³⁴	C57BL/6J;	KI	p.(A306T)/+	S6	LoF	Constitutive	Homologous recombination
Otto et al. (2009) ³³	FVB/NJ		p.(A306T)/		20%–40% I_M reduction in <i>Xenopus laevis</i> oocytes (a)		
Tomonoh et al. (2014) ³⁵			p.(A306T)				
Ihara et al. (2016) ³²							
Bi et al. (2011) ⁴²	B6D2F1/Crl	Conditional transgenic	Tg p.(G279S)	Pore	Dominant negative <i>Xenopus laevis</i> oocytes for testing variant effect (c)	Forebrain	Transgene with α CamKII promoter
Shi et al. (2013) ⁴⁴							
Soh et al. (2014) ²⁵	C57BL/6J	Conditional KO	+/-	-	-	Cortical pyramidal neurons	Homologous recombination
Niday et al. (2017) ²⁴							
Aiba et al. (2021) ²³							
Tomonoh et al. (2014) ³⁵	C57BL/6J	KI	p.(Y284C)/+	Pore	LoF	Constitutive	kick-in system
Ihara et al. (2016) ³²			p.(Y284C)/		20%–30% I_M reduction in <i>Xenopus laevis</i> oocytes (a)		
Uchida et al. (2017) ³⁶			p.(Y284C)				
Yiu et al. (2014) ⁴³	C57BL/6NTac X 129S6/SvEvTac	Conditional transgenic	Tg p.(G279S)	Pore	Dominant negative <i>Xenopus laevis</i> oocytes for testing variant effect (c)	Lateral amygdala pyramidal neurons	HSV vector
Niday et al. (2017) ²⁴	CD-1	Transient	Tg p.(I205V)	S4	Dominant negative <i>Xenopus laevis</i> oocytes for testing variant effect (b)	L2/3 pyramidal neurons	In utero electroporation
Greene et al. (2018) ⁴¹	C57BL/6J	KI	p.(S559A)/+	C-terminal domain	LoF Loss of PKC phosphorylation acceptor site	Constitutive	BAC clone
Kosenko et al. (2020) ⁶¹							
Soh et al. (2018) ²⁶	C57BL/6J	Conditional KO	-/-	-	-	PV ⁺ and SST ⁺ interneurons	<i>Kcng1</i> x <i>Emx1-ires-cre</i>
Milh et al. (2020) ³⁹	129Sv	KI	p.(T274M)/+	Pore	Dominant negative 70%–80% I_M reduction in <i>Xenopus laevis</i> oocytes (b)	Constitutive	Homologous recombination
Vermeuil et al. (2020) ⁶²							
Biba et al. (2022) ⁴⁰							

TABLE 1 (Continued)

Reference	Genetic background	Type of model	Genotype	Variant location	Variant effect	Expression of the variant	Mouse model production
Kim et al. (2021) ³⁸	C57BL/6J	Conditional transgenic	Tg p.(M547V)	C-terminal domain	LoF 75%–80% I _M reduction in <i>Xenopus laevis</i> . oocytes (b)	Excitatory pyramidal neurons and astrocytes	BAC clone
Jing et al. (2022) ²⁷	C57BL/6J	Conditional KO	–/–	–	–	PV ⁺ interneurons	<i>Kcnq2^{fl}</i> x PV-Cre
Li et al. (2022) ²⁸	C57BL/6J	Conditional KO	+/-	–	–	Hcrt neurons	Crispr/Cas9

Note: (a) Ref. [31] (b) Ref. [15] (c) Ref. [37].

Abbreviations: ENU, *N*-ethyl-*N*-nitrosourea; HSV, herpes simplex virus; KI, knock-in; KO, knockout; na, not available; Prnp, prion protein promoter; PV, parvalbumin; Tg, transgenic.

were used to mimic SeLFNE and the expression of *Kcnq2* was manipulated to reproduce haploinsufficiency. Although the constitutive heterozygous deletion of *Kcnq2* was the first and most widely used model, the *Szt1* model was also used despite that it is also haploinsufficient for the *Chrna4* gene, another gene involved in epilepsy, making it difficult to compare of the results obtained in these two models. An additional difficulty was introduced when these KO models were made homozygous (not relevant to the human pathologies) or when the deletion of *Kcnq2* was restricted in time or space (e.g., first postnatal week, parvalbumin positive (PV+) or Hcrt expressing neurons).

Several knock-in models also have limitations when the *Kcnq2* gene is expressed under a different promoter or when it is expressed from a transgene delivering additional copies of the gene in addition to the two endogenous alleles. For a tetrameric channel such as the M channel (two Kv7.2 copies and two Kv7.3 copies), the presence of a non-physiological number of alleles within the cell is likely to alter the stoichiometry of the mutant and wild-type (WT) subunits. In addition, for the p.(G279S) model, the transgene was integrated on the X chromosome, introducing an additional bias, with differences between males and females and possibly between females too depending on their X-chromosome inactivation status. Of interest, one of the prominent brain morphology abnormalities seen in transgenic (Tg) models (i.e., hippocampal defects) was seen only in this model, maybe due to its genetic architecture. In addition, variability is also expected when the transgene carrying the mutant *Kcnq2* allele is delivered in the brain using in utero electroporation. In this case, the number and location of the cells receiving the transgene will be different from one animal to the next. Finally, the genetic background can also have a strong influence on the results, especially for behavioral studies or the susceptibility to epileptic seizures. At least six different genetic backgrounds were used in the different models (see Table 1). These limitations need to be taken into account when the results obtained with the different models are compared.

5 | EPILEPTIC PHENOTYPE

KCNQ2-related disorders are characterized by the occurrence of early seizures that can be accompanied by developmental delay.^{13,45} Spontaneous cortical hyperactivity and epileptic seizures are thus expected in *Kcnq2* mouse models. In several models, however, the induction of seizures required the use of proconvulsant drugs or electrostimulation. These methods are generally used in WT animals to induce *status epilepticus* and to screen AEDs.⁴⁶ In addition, they were used to evaluate

the correlation between a *Kcnq2* variant and the seizure threshold in vivo.

5.1 | Spontaneous epileptiform activity

The presence of spontaneous seizures, an abnormal EEG, or hyperactivity in mouse models are indicators of cortical hyperexcitability. Consistent with the severe nature of dominant-negative variants found in patients with DEE, the Tg *Kcnq2*^{G279S}, *Kcnq2*^{T274M/+}, and Tg *Kcnq2*^{M547} mice displayed a spontaneous epileptic phenotype.³⁷⁻³⁹ Spontaneous generalized tonic-clonic seizures were observed in these models using infra-red videos and/or during EEG recording.

In the *Kcnq2*^{T274M/+} model, all seizures are generalized tonic-clonic and most are fatal: 91% of spontaneous deaths concerned *Kcnq2* heterozygous knock-in animals.³⁹ A peak of mortality was observed at postnatal day 100 (P100), with disappearance of epileptic seizures afterwards for the heterozygous survivors, supporting a relationship between the occurrence of seizures and the death of mutant animals.³⁹ The spontaneous disappearance of epileptic seizures in this model suggests a compensatory mechanism possibly mediated via gene expression modulation, in accordance with previous work performed in *Kcnq2* KO animals.²²

The *cKcnq2*^{M547/+} mice showed spontaneous seizures such as dorsal extensions and violent jumping and running between P60 and P85.³⁸ Surprisingly, a high mortality rate was observed in *cKcnq2*^{M547/+} at P10 but it was not observed from P21 onwards. The particularly high expression of the gene in the postnatal period has been correlated with the period of increased sensitivity to epileptic seizures leading to the death of the young animals.³⁸

Partial seizures were observed in Tg *Kcnq2*^{G279S} mice, occasionally followed by spontaneous generalized tonic-clonic seizures, and most animals showed hyperactivity when placed in a novel environment. Hyperactivity leads to high-amplitude sharp-wave activity associated with partial seizures with asymmetric short-limb extensions or head bobs.³⁷ Of interest, the conditional model deficient for *Kcnq2* in pyramidal neurons²⁵ also showed partial seizures with rearing, jumping, and falling. The EEG analysis revealed that the *Kcnq2* cKO mice exhibited episodes of polyspikes events, documenting cortical hyperexcitability showing the importance of Kv7.2 in pyramidal neurons.²⁵ This type of “silent seizure” only seen during EEG recording with no motor correlates is found in the youngest patients.⁴⁵

Concerning the p.(A306T) and p.(Y284C) SeLFNE variants, spontaneous epileptiform activity was observed only in homozygous animals.^{34,35} Both homozygous

models displayed generalized tonic-clonic seizures with forelimb and hindlimb extensions. Hyperactivity was present during interictal phases with frequent generalized interictal cortical discharges for p.(A306T) mice and myoclonic seizures with spike discharge for p.(Y284C) mice. The genetic background may also modulate the severity of the phenotype.⁴⁷ The p.(A306T) variant causes a more severe phenotype on the FVB/NJ background with death occurring after the generalized seizures and an earlier seizure onset compared to the C57BL/6J background.³⁴ These results highlight the difference between SeLFNE and DEE for this aspect of the phenotype. Mice carrying DEE variants present spontaneous seizures with abnormal EEG recordings, whereas SeLFNE variants need to be homozygous to show spontaneous epileptiform activity.

5.2 | The electroconvulsive threshold (ECT) test

The ECT test consists of delivering high-frequency pulses (e.g., ≥60 Hz) using transcorneal electrodes.¹⁶ The different stimulation protocols including high-frequency sinusoidal wave stimulation or low-frequency rectangular wave stimulation correspond to different seizures type associated with different brain-area activation.⁴⁷ Thresholds for clonic, tonic, or psychomotor (partial) seizures were determined in *Kcnq2* mouse models and compared to WT mice. Heterozygous *Szt1*, *Kcnq2*^{A306T/+}, and *Kcnq2*^{+/-} animals showed a lower seizure threshold compared to their WT counterparts.^{16,20,34,48} Of interest, the seizure threshold was lower for mutant and WT females compared to males of the same genotype, suggesting that the seizure threshold is genotype and gender dependent.^{16,48} Currently, no patient study in the literature documents a difference for the epileptic phenotype between men and women for *KCNQ2*-related disorders. The seizure threshold is also age dependent and 10%–16% of patients with SeLFNE still have seizures later in life.¹⁰ Singh and colleagues evaluated seizure thresholds in aging *Kcnq2*^{A306T/+} mice from P140 to P382. These older heterozygous mutant mice had a reduced seizure threshold compared to their WT counterpart, supporting the hypothesis that this SeLFNE variant causes seizure susceptibility in adulthood.³⁴ The sensitivity to AEDs in *Kcnq2* models was also tested using the ECT test. The proconvulsant linopirdine (LPD) and the anticonvulsant retigabine (RTB) can, respectively, block and activate the M current.^{49,50} They were used to show that *Szt1* mice are more sensitive to the proconvulsant LPD in the clonic model, and less sensitive to the anticonvulsant properties of RTB in the partial psychomotor model, showing a different sensitivity according to the brain areas considered.⁴⁸

5.3 | Chemically induced seizures

Pentylenetetrazol (PTZ), a GABA-A receptor antagonist, is used widely to study seizures in animals.⁵¹ In *Kcnq2*^{+/-} mice, a smaller number of PTZ injections is needed to trigger generalized seizures compared to WT, indicating that a 50% reduction of Kv7.2 increases the susceptibility to drug-induced seizures.²¹

SeLFNE models harboring heterozygous p.(Y284C) or p.(A306T) variants were challenged by PTZ and kainic acid (KA),³⁵ an analog of glutamate.⁵² With both drugs, treated mice displayed an increase in seizure susceptibility and a higher seizure severity compared to WT. After KA treatment, the EEG of *Kcnq2*^{Y284C/+} mice reveals longer and very frequent spike bursts compared to *Kcnq2*^{A306T/+} that are not different from WT.³² As observed in homozygous mice, the p.(Y284C) variant seems to cause a more severe phenotype than p.(A306T). The different location of these variants in the Kv7.2 protein can critically influence the neuronal firing and the disease mechanism.¹⁵ In these heterozygous models, RTB is more effective than phenobarbital (PB) in attenuating KA-induced seizures and ameliorating the EEG.³²

6 | STUDY OF THE M CURRENT

Kv7 channels have emerged as critical regulators of hippocampal CA1 pyramidal neurons, cortical pyramidal neurons, but also PV⁺ interneurons excitability and spiking behavior.^{24,25,26,53} In excitatory neurons, Kv7 channels help to set the action potential (AP) threshold and contribute to the postburst afterhyperpolarization, which limits repetitive firing following bursts of AP.⁵⁴⁻⁵⁶ Blockade of Kv7 channels modulates spiking activity by converting simple spikes to high-frequency bursts and attenuating spike-frequency adaptation (SFA).^{57,58}

Results obtained using the *Szt1* mice model were the first direct evidence of decreased neuronal I_M amplitude and current density in an animal model of *Kcnq2* dysfunction. In the *Szt1* mice, the reduction in I_M amplitude and SFA in CA1 neurons are consistent with the previously described decreased seizure threshold and altered pharmacology sensitivity.⁴⁸

The Tg *Kcnq2*^{G279S} DEE model revealed that medium afterhyperpolarization (mAHP), a potassium-mediated hyperpolarization that is typically activated following a brief surge of spiking activity, is mediated by Kv7.2/3 channels.³⁷ Like the mAHP, the M current, a potassium conductance that increases as neurons approach the AP threshold,⁵⁹ is also primarily mediated by Kv7.2/Kv7.3 heteromeric channel and is extremely sensitive to the loss of Kv7.2. In contrast to the mAHP, the M current is also

sensitive to the loss of Kv7.3.²⁵ cKO mice showed that Kv7.2 subunits are required for the mAHP, whereas Kv7.3 are not.²⁵ Introduction of the *Kcnq2* dominant-negative variant p.(I205V) into L2/3 pyramidal neurons results in a hyperexcitable phenotype similar to the cKO, even in the presence of an intact mAHP.²⁴ The p.(I205V) variant shifts the voltage-activation curve of Kv7.2/Kv7.3 channels to more depolarized membrane potentials, preventing any significant activation of the channel at subthreshold membrane potentials, like in neurons lacking Kv7.2 subunits.

In good agreement with the results obtained studying seizures, heterozygous p.(G279S) and p.(T274M) DEE mouse models showed a significant decrease of I_M and an increase of neuronal excitability in hippocampal and cortical cells, respectively,^{37,40} whereas SeLFNE p.(A306T) and p.(Y284C) models showed a decrease in I_M only when the variants were homozygous.^{34,35} DEE variants cause the most important loss of M current with less than 50% of current density, whereas SeLFNE variants lead to an M-current density of 50% to 100%,⁶⁰ supporting a proportional relationship between the severity of epileptic behavior and the decrease in I_M. According to these results, restoring the M current could be an efficient approach to improve the epileptic phenotype.

To explore the consequences of M-current suppression for learning and memory, a homozygous model carrying the p.(S559A) variant was used.⁶¹ This variant affects the protein kinase C phosphorylation acceptor site and attenuates the neurotransmitter-induced M-current suppression, a mechanism able to transiently increase neuronal excitability. The results of this study show that the M current is involved in the consolidation of specific types of memory such as long-term object recognition, but not location or contextual memory.⁶¹ This model also showed a resistance to chemoconvulsant-induced seizures and prevents status epilepticus-induced neuronal death and epileptogenesis.⁴¹ Recent studies highlighted the role of *Kcnq2* in inhibitory interneurons. The conditional deletion of Kv7.2/3 channels in parvalbumin-expressing interneurons changed their firing properties, as expected, an induced a remodeling of fast excitatory activity.²⁶ Accordingly, removing *Kcnq2* from parvalbumin-expressing interneurons improved the antiseizure efficacy of RTB.²⁷ In the substantia nigra pars compacta, the ghrelin hormone enhances firing of nigral dopaminergic neurons by inhibiting Kv7 channels,⁴⁴ and in aged mice, Hcrt neurons showed hyperexcitability with lower Kv7.2 expression and impaired M current.²⁸ The neuronal hyperexcitability seen in different cell types in the cerebral cortex of *Kcnq2* models is also visible in the spinal cord, where the M current was shown to help set the speed of locomotion.⁶²

The consequences of *Kcnq2* dysfunction for different cell types are summarized in Table 2.

TABLE 2 Consequences of *Kcnq2* dysfunction according to the cell type

Reference	Experiment	Cell type	Observations
Peters et al. (2005) ³⁷	Brain slice	CA1 pyramidal neurons	Increase excitability, reduced SFA and attenuated mAHP in p.(G279S) neurons
Otto et al. (2006) ⁵⁷	Brain slice	CA1 pyramidal neurons	Reduction of M-current density and SFA in Szt1 neurons
Singh et al. (2008) ³⁴	Brain slice	CA1 pyramidal neurons	Reduction in amplitude and increased deactivation kinetics in p.(A306T) neurons
Bi et al. (2011) ⁴²	Brain slice	CA1 pyramidal neurons	Reduction of M-current, increase AP number, depolarizing shift of resting membrane potential, lower spike firing threshold and greater input resistance in p.(G279S) transgenic neurons
Soh et al. (2014) ²⁵	Brain slice	CA1 pyramidal neurons	Increase excitability with decreased mAHP and prolonged afterdepolarization in <i>Kcnq2</i> -null neurons
Robbins et al. (2013) ²²	Primary neurons culture	Sympathetic ganglia neurons	Increase <i>Kcnq3</i> and <i>Kcnq5</i> transcription in <i>Kcnq2</i> -null neurons
Tomonoh et al. (2014) ³⁵	Brain slice	Hippocampal pyramidal neurons	Reduction of M-current in p.(Y284C) neurons
Shi et al. (2013) ⁴⁴	Brain slice	Nigral dopaminergic neurons	Inhibition of ghrelin-induced hyperexcitability in p.(G279S) neurons
Yiu et al. (2014) ⁴³	Brain slice	Lateral amygdala pyramidal neurons	Increase of firing rate of lateral amygdala pyramidal neurons
Niday et al. (2017) ²⁴	Brain slice	L2/3 pyramidal neurons	Reduction of mAHP, increased AP number and frequency, increased input resistance in <i>Kcnq2</i> -null neurons Right-shifted conductance-to-voltage relationship in p.(I205V) neurons
Uchida et al. (2017) ³⁶	Brain slice	CA1 excitatory GABAergic interneurons	Reduction of M-current density, increased AP frequency, increased GPSC frequency in p.(Y284C) neurons
Greene et al. (2018) ⁴¹	Primary neurons culture	Cortical neurons	Normal basal M-current, reduced M-current suppression when challenged by oxotremorine-M in p.(S559A) neurons
Soh et al. (2018) ²⁶	Brain slice	CA1 and L2/3 PV ⁺ and SST ⁺ interneurons	Increase the AP number following suprathreshold depolarizing current pulses only in <i>Kcnq2</i> -null PV ⁺ interneurons
Verneuil et al. (2020) ⁶²	Brain slice	L1–L2 ventromedial interneurons	Decrease M-current in CPG neurons with higher spiking frequency and a more depolarized resting membrane potential in p.(T274M) neurons
Aiba et al. (2021)	Brain slice	L2/3 pyramidal neurons	Spreading depolarization in cKO neurons
Biba et al. (2022) ⁴⁰	Brain slice	L2/3 and 5 pyramidal neurons	Reduction of M-current density and conductance and neuronal hyperexcitability and increase in the frequency of spontaneous network-driven events mediated by GABA receptors in p.(T274M) neurons
Jing et al. (2022) ²⁷	Brain slice	PV ⁺ interneurons	Reduction of M-current amplitude in <i>Kcnq2</i> -null PV ⁺ interneurons
Li et al. (2022) ²⁸	Brain slice	Hcrt neurons	Depolarized RMP and spontaneous firing activity in higher proportion in <i>Kcnq2</i> -null Hcrt neurons

Abbreviations: AP, action potential; CA1, cornu ammonis 1; CPG, central pattern generator; GPSC, GABAergic postsynaptic currents; Hcrt, hypocretin; mAHP, medium afterhyperpolarization; PV, parvalbumin; RMP, resting membrane potential; SFA, spike frequency adaptation.

7 | BEHAVIORAL AND COGNITIVE PHENOTYPES

One of the major differences between SeLFNE and DEE patients is psychomotor development, which is severely altered in the latter group. Patients with SeLFNE have been reported sporadically to have delayed psychomotor development, intellectual disability, and neurological symptoms,^{9,63} showing the existence of rare clinical overlaps.

Cognitive abilities are altered in the *Kcnq2*^{T274M/+}, Tg *Kcnq2*^{G279S}, and Tg *Kcnq2*^{M547V} DEE mouse models with learning and hippocampus-dependent spatial memory deficits for all.³⁷⁻³⁹ Of note, the hippocampal abnormalities seen by Peters and colleagues have not been seen in other models of *Kcnq2* dysfunction. The hyperexcitability due to the reduction of Kv7.2 in the hippocampus seems to be responsible for these learning and memory deficits. Of interest, the mouse model bearing the p.(S559A) variant, which attenuates neurotransmitter-induced M-current suppression,⁴¹ has an intact hippocampus-mediated object location memory. However, this variant impairs the consolidation of object recognition memory, which is rescued with the Kv7 channel blocker XE991.⁶¹ Recently, *Kcnq2*^{+/-} mice were shown to have a normal behavior in a fear-induced learning and memory assay or an object location and recognition memory assay,⁶⁴ whereas the formation of fear memory was shown to be increased in a conditional transgenic model carrying the p.(G279S) variant.⁴³ Although the mechanisms involved remain unclear, and the results somewhat contradictory, these studies show a relationship between the *Kcnq2*/M-current function and specific forms of memory.

In addition, Tg *Kcnq2*^{G279S}, *Kcnq2*^{+/-}, and Tg *Kcnq2*^{M547V} mice displayed marked hyperactivity with an increased locomotor activity.^{19,37,38} Tests such as open-field or elevated plus-maze showed reduced anxiety in *Kcnq2*^{+/-} and Tg *Kcnq2*^{M547V} mice.^{19,38} A relationship was assumed between the prefrontal cortex (PFC), where *Kcnq2* is expressed, and the amygdala, which is responsible for increasing anxiety and the perception of fear.⁶⁵ An altered balance of excitation and inhibition in PFC may also be involved in the social deficits seen in *Kcnq2*^{+/-} and Tg *Kcnq2*^{M547V} mice by a social avoidance test, and an increased social dominance and aggression for *Kcnq2*^{+/-} mice.^{19,66} Furthermore, *Kcnq2*^{+/-} and Tg *Kcnq2*^{M547V} mice displayed an increase in repetitive and compulsive-like behaviors like self-grooming or increased burying of marbles.^{19,38} The repetitive self-grooming can be correlated with an alteration of the activity of the striatum where Kv7.2 is expressed.^{67,68} These behavioral abnormalities are intriguing when present in the *Kcnq2*^{+/-} SeLFNE model, since severe impairments of neurological development associated with cognitive, intellectual, and behavioral deficits including

hallmarks of autism spectrum disorder are almost found only in DEE patients.^{69,70} The discrepancy between the behavioral phenotype of SeLFNE mice and SeLFNE patients reveals that the ablation of Kv7.2 causes pathological mechanisms that could be different between humans and mice. On the other hand, the cognitive deficits observed in DEE-mouse models mimic the *KCNQ2*-related developmental encephalopathies. However, there are differences likely relying on the nature of the variant and the genetic background.

Data obtained using *KCNQ2*-mouse models and described in this review are summarized in Figure 2.

8 | DISCUSSION

The diagnosis of SeLFNE or DEE is essentially made at birth following the occurrence of neonatal seizures and the presence of abnormal EEG.⁷¹ Patients with SeLFNE have a normal neurological development and are sensitive to AEDs with seizure remission within the first months of life.⁹ The clinical features of DEE are different, with refractory seizures and a severe neurological disorder. From 1998 to 2012, cellular and mouse models were created to study the mechanisms of SeLFNE.^{16,21,34} After 2012, with the discovery of *KCNQ2*-related DEE,⁸ the research projects have been extended to missense variants having a dominant-negative effect.³⁷⁻³⁹ The consequences of *KCNQ2* variants for I_M were first studied using transfected cells such as CHO or *Xenopus laevis* oocytes.^{14,15,31} These functional tests are needed to determine the impact of the pathogenic variant for the M current (LoF, GoF, or dominant-negative LoF) because it has strong implications for the treatment of the patients. Without this knowledge, prescription of AED may be ineffective or even deleterious for the patient. These heterologous cellular models hence continue to serve as simple readouts. Nowadays, however, the induced pluripotent stem cells (iPSc) technology allows for a wider range of functional studies.⁷² When differentiated into patients' neurons, these cells allow the study of molecular mechanisms in a relevant cell type and performance of high-throughput screening of new drugs. Mouse models are also required to better understand the pathological mechanisms involved in *KCNQ2*-related disorders and to study new therapeutic approaches, allowing to study clinical manifestations such as seizures and to perform EEG recordings thereby providing clinically relevant readouts.

In addition, AEDs have been used in *Kcnq2* models and they have provided new insights for the understanding of the pathophysiology of *KCNQ2*-related epilepsy. In *Kcnq2*^{Y284C/+} and *Kcnq2*^{A306T/+} mouse models, RTB ameliorated KA-induced seizures in terms of seizure

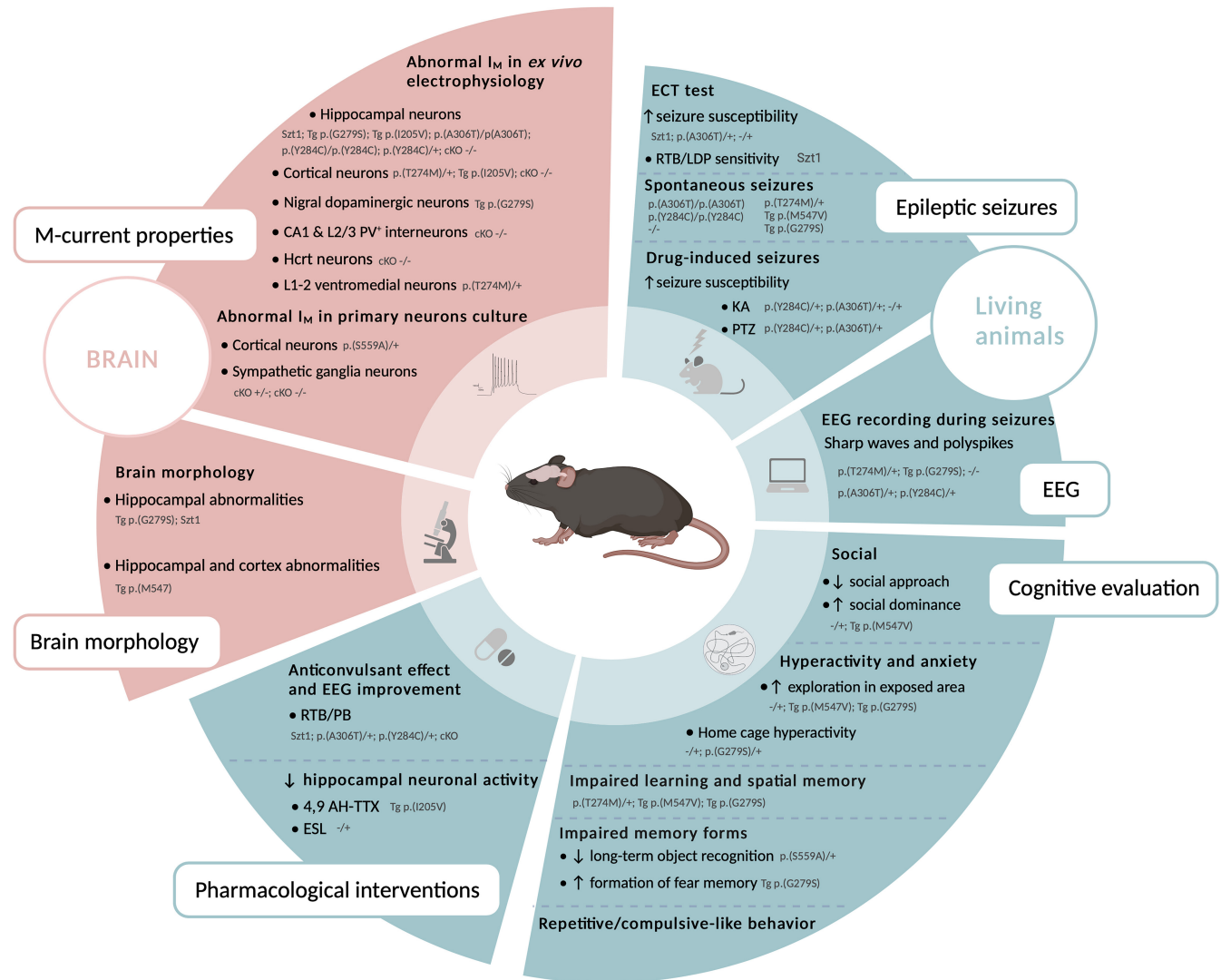


FIGURE 2 Phenotypes in *Kcnq2*-deficient mouse models. This figure summarizes the data (black lettering) collected in the different *Kcnq2* mouse models (gray lettering). The pink sections of the figure present the data collected in the models' brain and the blue sections present the data collected using living animals. AH-TTX, anhydro-tetrodotoxin; ECT, electroconvulsive threshold; EEG, electroencephalography; ESL, eslicarbazepine acetate; KA, kainic acid; LPD, linopirdine; PB, phenobarbital; PTZ, pentylenetetrazol; RTB, retigabine.

frequency and spike bursts on EEG.³² However, first-line treatments for *KCNQ2*-related epilepsy are usually based on the administration of blockers of the persistent sodium current such as phenytoin or carbamazepine. Reduction of the Nav1.6 function with 4,9 anhydro-tetrodotoxin (4,9 AH-TTX) leads to a decrease in the excitability of neurons at a near-control level and a decrease in the number and frequency of AP in Tg *Kcnq2*^{L205V} mice.²⁴ Thus Nav1.6 represents a potential target for *KCNQ2*-related hyperexcitability. More recently, eslicarbazepine acetate (ESL), another voltage-gated sodium channel blocker, already approved as monotherapy for focal-onset seizures in adult, was tested in the *Kcnq2*^{+/-} model.²⁰ Both 4,9 AH-TTX and ESL result in decreased neuronal excitability following blockade of the M current. The higher

seizure threshold in treated mice compared to mutated mice shows that ESL as a novel AED is also effective in *KCNQ2*-related disorders and offers a new possibility for patients with seizures that are refractory to other AEDs.

Seizure control is an unquestionable necessity, but even in cases where seizures can be controlled by drugs, cognitive/motor deficits persist in *KCNQ2*-DEE.⁷³ To tackle the multiple dimensions of *KCNQ2*-DEE, gene therapy approaches are promising. Such approaches are beginning to emerge for other DEEs such as *SCN2A*,⁷⁴ *DNM1*,⁷⁵ or *SCN8A*-DEE.⁷⁶ Used alone or in combination with pharmacology, these promising strategies use mouse models to demonstrate their efficacy *in vivo*. These *Kcnq2*-mouse models combined with the use of iPSc technology will be key to support

the preclinical development and hopefully provide the much-needed treatments to the patients affected by *KCNQ2* dysfunction.

AUTHOR CONTRIBUTIONS

The three authors have drafted, revised, and approved the manuscript.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL STATEMENT

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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