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Linking Respiratory Challenges in KCNQ2 Encephalopathy to "Phox2b" Neurons in the Retrotrapezoid Nucleus

Phox2b-Expressing Neurons Contribute to Breathing Problems in Kcnq2 Loss- and Gain-of-Function Encephalopathy Models

Soto-Perez J, Cleary CM, Sobrinho CR, Mulkey SB, Carroll JL, Tzingounis AV, Mulkey DK. *Nat Commun.* 2023;14:8059. doi:10.1038/s41467-023-43834-7

Loss- and gain-of-function variants in the gene encoding KCNQ2 channels are a common cause of developmental and epileptic encephalopathy, a condition characterized by seizures, developmental delays, breathing problems, and early mortality. To understand how KCNQ2 dysfunction impacts behavior in a mouse model, we focus on the control of breathing by neurons expressing the transcription factor Phox2b which includes respiratory neurons in the ventral parafacial region. We find Phox2b-expressing ventral parafacial neurons express Kcnq2 in the absence of other Kcnq isoforms, thus clarifying why disruption of Kcnq2 but not other channel isoforms results in breathing problems. We also find that *Kcnq2* deletion or expression of a recurrent gain-of-function variant R201C in Phox2b-expressing neurons increases baseline breathing or decreases the central chemoreflex, respectively, in mice during the light/inactive state. These results uncover mechanisms underlying breathing abnormalities in KCNQ2 encephalopathy and highlight an unappreciated vulnerability of Phox2b-expressing ventral parafacial neurons to KCNQ2 pathogenic variants.

Commentary

KCNQ2 encodes Kv7.2 protein subunits, which form both homotetrameric and heterotetrameric (alongside KCNQ3) voltage-gated potassium channels. These channels generate a slow-activating potassium current, also known as the M current, that is crucial for regulating neuronal excitability by preventing repetitive neuronal firing. *KCNQ2* mutations primarily manifest in 2 clinical conditions: epilepsy and abnormal respiration. Both loss-of-function (LoF)[1] and gain-of-function (GoF)[2] mutations of *KCNQ2* lead to neuronal hyperexcitability and increase susceptibility to spontaneous firing and seizures. Notably, a GoF *KCNQ2* mutation (R201C) is implicated in respiratory dysfunction, including profound hypoventilation and apnea, comorbid with epilepsy.[3] As KCNQ2 is broadly expressed in the brain, understanding the cellular mechanisms of how KCNQ2 dysfunction leads to respiratory disturbances is essential for guiding therapeutic intervention to alleviate respiratory symptoms associated with KCNQ2 channelopathies. There is a great similarity between the breathing phenotype associated with the *KCNQ2* GoF variant and congenital central hypoventilation syndrome, which is caused by variants in the paired-like homeobox 2b (*Phox2b*) transcription factor. Therefore, the chemosensitivity regulatory role of KCNQ2 in Phox2b-expressing cells, including a sparse collection of neurons ($\sim$700 in mice and $\sim$2000 in rats) in the chemosensitive retrotrapezoid nucleus (RTN), merits further examination. Notably, a recent study underscores the indispensable role of maintaining Phox2b expression for lifelong chemosensory control,[4] with implications for potentially treating breathing problems in KCNQ2 encephalopathies by targeting Phox2b-expressing neurons in RTN.

The current study by Soto-Perez and colleagues proposed that alterations in KCNQ2 function within Phox2b+ RTN neurons could be responsible for the observed respiratory dysfunction in individuals with *KCNQ2* mutations.[5] By reanalyzing existing single-cell sequencing dataset and probing Kcnq2 at transcript and protein levels in the RTN of mice, the authors first found that RTN Phox2b+ neurons preferentially express Kcnq2 but not other Kcnq isoforms, including Kcnq3. Subsequently, by generating transgenic mice that carry Phox2b+ neuron-specific Kcnq2 conditional knockout (cKO) and GoF mutations, the authors demonstrated these mice exhibit distinct baseline breathing and CO_2 ventilatory responses, depending on the diurnal rhythms. Utilizing field and whole-cell patch clamping techniques, the authors further illustrated how the Kcnq2 GoF variant alters the intrinsic firing properties of the RTN Phox2b+ neurons, aligning with observed respiratory outcomes. These findings together potentially explain the

cellular mechanisms of abnormal respiration seen in humans with *KCNQ2* mutations. Overall, this study represents a significant advancement in our understanding of the intricate relationship between Phox2b+ RTN neuronal function and clinical respiratory manifestations in *KCNQ2* encephalopathies. These findings also shed light on rescuing breathing problems by restoring KCNQ2 function in RTN neurons.

Beyond the technical innovation and clinical significance of the above findings, this study prompts new research directions. Firstly, Phox2b is widely expressed across many lower brainstem nuclei critical for breathing regulation.[6] RTN only constitutes a subset of the cell population that expresses Phox2b in the central nervous system. It is likely that this nucleus represents only a small fraction of all Phox2b+ autonomic neurons.[6] The use of Phox2b mediated cre-recombinase approach enables conditional gene expression across the chain of all Phox2b+ neurons involved in the integration of peripheral and central chemoreception, including those in the locus coeruleus, nucleus solitary tract, dorsolateral pons, carotid body, and their primary afferents. Further study is required to ensure the direct link between the breathing disorder and mutated KCNQ2 channels in Phox2b+ RTN neurons. This is especially pertinent considering that RTN neurons are also biochemically diverse, exemplified by the facts that ~40% of Phox2b+ RTN neurons lack KCNQ2 expression and ~10% KCNQ2+ cells within RTN are devoid of Phox2b expression, thus eluding targeting through the Phox2b cre-dependent recombinase approach. Secondly, concerning the discrepancy in findings related to the pharmacological inhibition of KCNQ2 in vitro (bath application of a pan-KCNQ channel blocker, ML252, improves the firing response of patched neurons) versus in vivo studies (systemic ML252 injection has little effect on respiratory function), it remains possible that KCNQ2 expressing Phox2b-RTN neurons and those outside the RTN play a complementary role for complex breathing control. Apart from the potential leaky KCNQ2 effects, the bioavailability and efficacy of systemically delivered ML252 also require further examination. Thirdly, Dr Mulkey's group has previously demonstrated the association between Phox2b+ RTN neuronal function and breathing abnormalities in Pitt-Hopkins syndrome, which is caused by heterozygous hypomorphic or null mutation or deletion of the transcription factor 4 (TCF4) gene.[7] Given that TCF4 does not directly regulate KCNQ2, it is essential to examine the intersecting role of Phox2b+ RTN neurons in regulating breathing, regardless of specific pathogenic mutations. This entails understanding their vulnerability to the mutations leading to breathing disorders.

To fully comprehend the respiratory regulation by KCNQ2 in RTN neurons, the role of vigilance states represents a crucial missing piece. The authors conducted baseline breathing recordings of central chemoreflex during light versus dark cycles, revealing that KCNQ2 associated respiratory abnormalities were observed predominantly during the light cycle. Considering the mice are typically asleep during the light cycle, it is highly possible that the specific vigilance state during recording can profoundly influence breathing and CO_2 sensing outcomes. Importantly, sleep disturbances are reported in 67% of KCNQ2-related encephalopathies[8] and are also found in animal models with disruption of Kcnq2/Kcnq3 genes.[9] Therefore, the respiratory abnormalities observed in KCNQ2 cKO and GoF mice could be direct consequences of altered sleep patterns. Conducting a rigorous polysomnogram recording in parallel with breathing and CO_2 ventilatory responses during both light and dark cycles will help untangle the relationships between KCNQ2 function, respiratory regulation, and sleep disturbances.

Sudden unexpected death in epilepsy (SUDEP) is the most catastrophic seizure-related outcome. Impairments of the brain, heart, and breathing all contribute to this fatal event. The 5 members of the voltage-gated potassium channel KCNQ family play pivotal roles across neuronal, respiratory, and cardiac domains, sparking increasing interest in studying their roles in SUDEP regulation and prevention. Among these channels, KCNQ1 carries the cardiac slow delayed-rectifier current and repolarizes the cardiac action potential. Notably, mutations of *KCNQ1* increase the risk of SUDEP, possibly through cardiac mechanisms. The KCNQ2–5 channels are predominantly expressed in the brain and modulate neuronal excitability. The Ala306Val variant in a highly conserved transmembrane domain of KCNQ2 has been associated with SUDEP.[10] Many GoF of *KCNQ2* variants have also been linked to early mortality, though the exact cause of death remains unclear. Elucidating the brainstem cellular mechanism of KCNQ2 in modulating breathing and chemosensitivity is significant because seizure-induced respiratory arrest and central apnea are commonly observed in epileptic individuals and animal models of SUDEP. Theoretically, cell-type specific manipulation of KCNQ2 expression and activity can provide a viable approach to correcting respiratory abnormality, which holds great promise for preventing SUDEP and saving lives.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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