Acta Pharmacologica Sinica www.nature.com/aps

REVIEW ARTICLE

# Channelopathy of small- and intermediate-conductance $Ca^{2+}$-activated $K^+$ channels

Young-Woo Nam[1], Myles Downey[1], Mohammad Asikur Rahman[1], Meng Cui[2] and Miao Zhang[1]

Small- and intermediate-conductance $Ca^{2+}$-activated $K^+$ ($K_{Ca}2.x/K_{Ca}3.1$ also called SK/IK) channels are gated exclusively by intracellular $Ca^{2+}$. The $Ca^{2+}$ binding protein calmodulin confers sub-micromolar $Ca^{2+}$ sensitivity to the channel-calmodulin complex. The calmodulin C-lobe is constitutively associated with the proximal C-terminus of the channel. Interactions between calmodulin N-lobe and the channel S4-S5 linker are $Ca^{2+}$-dependent, which subsequently trigger conformational changes in the channel pore and open the gate. *KCNN* genes encode four subtypes, including *KCNN1* for $K_{Ca}2.1$ (SK1), *KCNN2* for $K_{Ca}2.2$ (SK2), *KCNN3* for $K_{Ca}2.3$ (SK3), and *KCNN4* for $K_{Ca}3.1$ (IK). The three $K_{Ca}2.x$ channel subtypes are expressed in the central nervous system and the heart. The $K_{Ca}3.1$ subtype is expressed in the erythrocytes and the lymphocytes, among other peripheral tissues. The impact of dysfunctional $K_{Ca}2.x/K_{Ca}3.1$ channels on human health has not been well documented. Human loss-of-function $K_{Ca}2.2$ mutations have been linked with neurodevelopmental disorders. Human gain-of-function mutations that increase the apparent $Ca^{2+}$ sensitivity of $K_{Ca}2.3$ and $K_{Ca}3.1$ channels have been associated with Zimmermann-Laband syndrome and hereditary xerocytosis, respectively. This review article discusses the physiological significance of $K_{Ca}2.x/K_{Ca}3.1$ channels, the pathophysiology of the diseases linked with $K_{Ca}2.x/K_{Ca}3.1$ mutations, the structure–function relationship of the mutant $K_{Ca}2.x/K_{Ca}3.1$ channels, and potential pharmacological therapeutics for the $K_{Ca}2.x/K_{Ca}3.1$ channelopathy.

**Keywords:** channelopathy; $K_{Ca}2.2$ channels; $K_{Ca}2.3$ channels; $K_{Ca}3.1$ channels; Zimmermann-Laband syndrome; hereditary xerocytosis

*Acta Pharmacologica Sinica* (2023) 44:259–267; https://doi.org/10.1038/s41401-022-00935-1

## INTRODUCTION

The pore-forming α-subunits of $K_{Ca}2.x/K_{Ca}3.1$ channels are encoded by the *KCNN* genes, including *KCNN1* for $K_{Ca}2.1$ (SK1), *KCNN2* for $K_{Ca}2.2$ (SK2), *KCNN3* for $K_{Ca}2.3$ (SK3), and *KCNN4* for $K_{Ca}3.1$ (IK or SK4) channels. The single-channel conductance values of $K_{Ca}2.x$ and $K_{Ca}3.1$ channels are ~10 pS and ~40 pS in symmetrical $K^+$ solutions, which are much smaller than that of $K_{Ca}1.1$ (BK) channels (~200 pS) channels [1]. These channels were hence named small-conductance ($K_{Ca}2.x$ also called SK) and intermediate-conductance ($K_{Ca}3.1$ also called IK) $Ca^{2+}$-activated $K^+$ channels. $K_{Ca}2.x/K_{Ca}3.1$ channels are voltage independent and are activated exclusively by intracellular $Ca^{2+}$. The sub-micromolar sensitivity to $Ca^{2+}$ of $K_{Ca}2.x/K_{Ca}3.1$ channels is attributed to the $Ca^{2+}$-binding protein calmodulin (CaM) constitutively associated with the channels [2]. $K_{Ca}2.x$ channels are activated by $Ca^{2+}$ with $EC_{50}$ values ranging from 300 to 750 nM, while $K_{Ca}3.1$ channels exhibit apparent $Ca^{2+}$ sensitivity of 100–400 nM [3]. Elevated intracellular $Ca^{2+}$ levels cause conformational changes in the channel-CaM complex and result in $K^+$ outflow from excitable and non-excitable cells.

## CHANNEL STRUCTURE

$K_{Ca}2.x/K_{Ca}3.1$ channels assemble as a homotetramer of four α-subunits of each subtype, although functional heterotetrameric channels among subtypes have also been reported [4, 5]. Each pore-forming α-subunit consists of six transmembrane domains denoted S1–S6. The selective permeability of $K^+$ ions is attributed to the selectivity filter that sits between the S5 and S6 transmembrane domains in the channel pore. The $K_{Ca}2.x/K_{Ca}3.1$ channel subtypes are highly homologous in their six transmembrane domains, but the amino acid sequence and length at their cytoplasmic N- and C- termini vary among the subtypes [6, 7].

Within the four $K_{Ca}2.x/K_{Ca}3.1$ channel subtypes, atomistic structures are only available for the $K_{Ca}3.1$ channel. The full-length cryogenic electron microscopy (cryo-EM) structures of $K_{Ca}3.1$ channels have been determined in the absence and presence of $Ca^{2+}$, shedding light on a $Ca^{2+}$/CaM gating mechanism for these channels [8]. In the absence of $Ca^{2+}$, the C-lobe of CaM binds to the HA/HB helices in the proximal channel C-terminus, the N-lobe of CaM is highly flexible, and the channel pore is closed. When bound with $Ca^{2+}$, the N-lobe of CaM becomes well-structured and interacts with the linker between the S4 and S5 transmembrane domains (S4–S5 linker) of a neighboring α-subunit. The interaction between the $Ca^{2+}$-bound CaM N-lobe and the S4-S5 linker triggers movement of the S6 transmembrane domain and the opening of the channel pore.

## CHANNEL PHYSIOLOGY

$K_{Ca}2.x$ channel subtypes expressed in neurons contribute to the medium afterhyperpolarization and regulate neuronal firing

[1]Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, CA 92618, USA and [2]Department of Pharmaceutical Sciences, Northeastern University School of Pharmacy, Boston, MA 02115, USA
Correspondence: Miao Zhang (zhang@chapman.edu)

Received: 1 May 2022 Accepted: 31 May 2022
Published online: 17 June 2022

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SPRINGER NATURE

frequency [9]. Intracellular $Ca^{2+}$ levels may increase as a result of $Ca^{2+}$ release from the endoplasmic reticulum through ryanodine receptors or $IP_3$ receptors, as well as $Ca^{2+}$ influx through the voltage- or ligand-gated $Ca^{2+}$-permeable channels in neurons [9]. $K_{Ca}2.x$ channels activated by the elevated $Ca^{2+}$ levels dampen the neuronal firing frequency, as a mechanism for regulating neuronal excitability by $Ca^{2+}$ signaling [10, 11].

One well-studied role of $K_{Ca}2.x$ channels in the central nervous system is their involvement in the long-term potentiation (LTP). In hippocampal neurons, activating muscarinic acetylcholine receptors increases phosphorylation of the $K_{Ca}2.2$-CaM complex, reduces the channel activity, thus lifting their negative influence on LTP [12, 13]. In animal studies, inhibition of $K_{Ca}2.x$ channels improves, while positive modulation of $K_{Ca}2.x$ channels impairs learning and memory [9], echoing the essential role of $K_{Ca}2.x$ channels in LTP. Detailed information on the physiological role of $K_{Ca}2.x$ channels in learning and memory can be found in an excellent review by ref. [9].

$K_{Ca}2.x$ channels also play a vital role in controlling the regular tonic firing in cerebellar Purkinje neurons [14]. The $K_{Ca}2.2$ channel is the predominant subtype expressed in cerebellar Purkinje neurons [15–17]. The $K_{Ca}2.2$ channel is one of the principal ion channels involved in cerebellar Purkinje neuron pacemaking [14]. Dysfunctional $K_{Ca}2.2$ channel activity has been linked with movement phenotypes including tremors and ataxias in rodents [18] and humans [19]. Positive modulation of $K_{Ca}2.2$ channels has been proposed as a potential therapeutic strategy for ataxias [20].

$K_{Ca}2.x$ channels are also expressed in the heart. A higher level of $K_{Ca}2.x$ channel protein is expressed in the atria, pace-making cells, and Purkinje fibers than in the ventricles of a normal heart [21–23]. $K_{Ca}2.2$ knockout mice exhibit significantly prolonged action potential duration (APD), while overexpression of $K_{Ca}2.2$ channels in atrioventricular nodal cells shortens APD [23]. $K_{Ca}2.2$ [24] and $K_{Ca}2.3$ [25] gene polymorphisms have also been linked with atrial fibrillation through genome-wide association studies (GWAS). There is a very recent review on cardiac $K_{Ca}2.x$ channels by ref. [26].

In the vascular endothelium, two-channel subtypes, $K_{Ca}2.3$ and $K_{Ca}3.1$ are expressed [27]. Intracellular $Ca^{2+}$ levels increase in response to mechano- or chemo-stimuli, activating $K_{Ca}2.3$ and $K_{Ca}3.1$ channels. The opening of $K_{Ca}2.3$ and $K_{Ca}3.1$ channels allows $K^+$ efflux and hyperpolarizes vascular endothelial cells. The hyperpolarization then spreads to the underlying vascular smooth muscle, leading to blood vessel dilation, a phenomenon called endothelium-dependent hyperpolarization (EDH) mediated vasodilation. Mice with a genetic deficit of $K_{Ca}2.3$ and $K_{Ca}3.1$ channels exhibit hypertension [28, 29]. There is a detailed review article by Wulff et al. on the endothelial $K_{Ca}2.3$ and $K_{Ca}3.1$ channels in blood pressure regulation [27].

$K_{Ca}3.1$ channel subtype is different from $K_{Ca}2.x$ channels in many ways, including their peripheral expression including the erythrocytes [30] and lymphocytes [31]. In T-lymphocyte, the $K_{Ca}3.1$ channel contributes to electrochemical gradients for $Ca^{2+}$ influx, which is critical for the proliferation of T cells [32]. Together with the mechanosensitive PIEZO1 channel, the $K_{Ca}3.1$ channel (also called the Gardos channel) regulates red blood cell volume [33].

## CHANNEL MUTATIONS AND GENETIC DISORDERS

To the best of our knowledge, there is no reported human mutation of $K_{Ca}2.1$ channels linked with genetic disorders. Loss-of-function (LOF) $K_{Ca}2.2$ mutations are associated with neurodevelopmental disorders including cerebellar ataxias [19] and tremors [18, 34]. Gain-of-function (GOF) $K_{Ca}2.3$ mutations are linked with Zimmermann-Laband syndrome (ZLS) [35–37] and idiopathic non-cirrhotic portal hypertension (INCPH) [38], while GOF $K_{Ca}3.1$ mutations are associated with a subset of hereditary xerocytosis (HX) [39–43].

### LOF $K_{Ca}2.2$ mutations and neurodevelopmental disorders

A LOF r$K_{Ca}$2.2_I289N mutation that diminishes $K_{Ca}2.2$ channel activity has been linked with tremors in rats [18]. Meanwhile, its corresponding h$K_{Ca}$2.2_I288S mutation is causative for neurodevelopmental disorders in humans [19]. In addition, h$K_{Ca}$2.2_L321del, h$K_{Ca}$2.2_I359M, h$K_{Ca}$2.2_Y361C, h$K_{Ca}$2.2_G362S, h$K_{Ca}$2.2_L388V, and h$K_{Ca}$2.2_L432P mutations of the human *KCNN2* gene cause neurodevelopmental disorders including cerebellar ataxia, motor and language developmental delay, and intellectual disability [19]. A h$K_{Ca}$2.2_G371E mutation has been linked with tremulous myoclonus-dystonia in humans [34].

Among these mutations, r$K_{Ca}$2.2_I289N [18], h$K_{Ca}$2.2_L321del [19], h$K_{Ca}$2.2_I359M [19], h$K_{Ca}$2.2_G362S [19], h$K_{Ca}$2.2_L388V [19], and h$K_{Ca}$2.2_L432P [19] lead to either reduced or complete loss of channel activity. This became clear when examined via heterologous expression of these mutant channels in cell lines (Table 1). The remaining mutations, h$K_{Ca}$2.2_I288S [19], h$K_{Ca}$2.2_Y361C [19], and h$K_{Ca}$2.2_G371E [34] have not been studied for their effects on channel activity. Mutations that introduce early stop codons, h$K_{Ca}$2.2_Y160* [19], and h$K_{Ca}$2.2_Y267* [19] are assumed to cause premature truncation of the channel protein and are thus classified as LOF.

Most human $K_{Ca}2.2$ mutations are de novo variants (except h$K_{Ca}$2.2_G371E [34] and h$K_{Ca}$2.2_L432P [19]). Patients have heterozygous *KCNN2* alleles. $K_{Ca}2.2$ channels are tetramers that consist of four subunits encoded by the *KCNN2* gene. r$K_{Ca}$2.2_I289N mutation has been found to suppress the activity

**Table 1.** Effects of pathogenic $K_{Ca}2.2$ mutations on channel activity.

| Species | Mutation | $K_{Ca}2.2$ current | Electrophysiological recordings | Cells |
|---|---|---|---|---|
| human | Y160* [19] | N/A | N/A | N/A |
| mouse | L168P (corresponding to L173 in human) | N/A | N/A | N/A |
| human | Y267* [19] | N/A | N/A | N/A |
| human | I288S [19] | N/A | N/A | N/A |
| rat | I289N [18] | Reduced current | Whole-cell | HEK-293 |
| human | L321del [19] | No current | Whole-cell | CHO-K1 |
| human | I359M [19] | No current | Whole-cell | CHO-K1 |
| human | Y361C [19] | N/A | N/A | N/A |
| human | G362S [19] | No current | Whole-cell | CHO-K1 |
| human | G371E [34] | N/A | N/A | N/A |
| human | L388V [19] | No current | Whole-cell | CHO-K1 |
| human | L432P [19] | No current | Whole-cell | CHO-K1 |

of co-expressed wild-type (WT) $rK_{Ca}2.2$ channels and thus deemed dominant-negative [18]. The dominant-negative effect of $rK_{Ca}2.2$_I289N and its equivalent human $hK_{Ca}2.2$_I288S mutation may contribute in part to the autosomal dominance of these mutant alleles [18]. Other human $K_{Ca}2.2$ mutations in Table 1 are also autosomal dominant. It is unknown whether the subunits carrying other mutations in Table 1 can co-assemble with WT subunits in patients with one WT and one mutant allele, let alone the impact of the mutant channel subunits may have when co-expressed with $K_{Ca}2.2$_WT channels.

There are two pathogenic $K_{Ca}2.2$ mutations reported in mice, one missense $mK_{Ca}2.2$_L168P (jitter mice, Mutagenetix database) and one deletion encompassing exon 1 and exon 2 [44] (frissonnant mice). Unlike in rats and humans, these two pathogenic $K_{Ca}2.2$ variants are recessive in mice, which could be explained by distinct baseline $K_{Ca}2.2$ expression levels, gene regulation, or compensatory mechanisms in response to $K_{Ca}2.2$ haploinsufficiency in different species. It is not clear whether the subunits carrying these mouse mutations can co-assemble with WT subunits.

Movement disorders, including cerebellar ataxia, tremor, or extrapyramidal symptoms, are present in patients carrying $hK_{Ca}2.2$_Y267*, $hK_{Ca}2.2$_I288S, $hK_{Ca}2.2$_I359M, $hK_{Ca}2.2$_Y361C, $hK_{Ca}2.2$_L388V, and $hK_{Ca}2.2$_L432P mutations [19]. The mechanism by which LOF $K_{Ca}2.2$ mutations cause cerebellar ataxia symptoms lies in the cerebellar Purkinje neurons. The $K_{Ca}2.2$ channel is one of the principal ion channels involved in the pacemaking of cerebellar Purkinje neurons [14]. Dysfunction of $K_{Ca}2.2$ channels may lead to the loss of firing precision in cerebellar Purkinje neurons, similar to what has been reported in the Purkinje neurons of episodic ataxia (EA2) [45, 46], spinocerebellar ataxias (SCA1 [47], SCA2 [48], SCA3 [49], and SCA6 [50]), and Huntington's disease [51–53] mouse models. Irregular neuronal firing in cerebellar Purkinje neurons may subsequently lead to $Ca^{2+}$ overload and neurodegeneration [20, 54], which may underlie the pathogenesis of ataxias.

Intellectual disability symptoms are present in patients carrying the mutations in Table 1, except $hK_{Ca}2.2$_G371E [34]. In animal studies, overexpression of $K_{Ca}2.2$ channels affects hippocampal synaptic plasticity and impairs learning and memory [55]. Therefore, it is surprising for the $K_{Ca}2.2$ LOF mutations to cause intellectual disability as well. One possible explanation might be that a compensatory event to the LOF $K_{Ca}2.2$ mutations is responsible for the intellectual disability symptoms of these patients, which will require future studies.

Patients carrying the mutations in Table 1 (except $hK_{Ca}2.2$_L321del [19], $hK_{Ca}2.2$_L432P [19], and $hK_{Ca}2.2$_G371E [34]) show psychiatric symptoms, including autistic features, attention deficit hyperactivity disorder, and even psychotic episodes. GWAS has associated the *KCNN2* gene encoding the $K_{Ca}2.2$ channel with autistic spectrum disorder (ASD) [56, 57]. Expression meta-analysis has shown increased *KCNN2* gene expression in ASD individuals compared with controls [56]. Overexpression of the $K_{Ca}2.2$ channel also underlies cortical dysfunction in a model of PTEN-associated autism [58]. It is thus puzzling that both increased expression and LOF mutations of the $K_{Ca}2.2$ channel are associated with ASD.

GOF $K_{Ca}2.3$ mutations, Zimmermann-Laband syndrome, and idiopathic non-cirrhotic portal hypertension

ZLS [OMIM: 135500] is a rare genetic disorder characterized by gingival enlargement, developmental delay, intellectual disability, together with abnormal fingers, fingernails, nose, and ears. ZLS has been associated with genetic mutations in *KCNH1* (encoding an Eag1 $K^+$ channel) [59], *KCNK4* (encoding a K2P $K^+$ channel) [60], and most recently *KCNN3* (encoding the $K_{Ca}2.3$ channel) [35–37] genes. The $hK_{Ca}2.3$_K269E [35], $hK_{Ca}2.3$_A287S [36], $hK_{Ca}2.3$_G350D [35], $hK_{Ca}2.3$_S436C [35], $hK_{Ca}2.3$_A536T [37], $hK_{Ca}2.3$_V539del [36] and $hK_{Ca}2.3$_V555F [36] mutations of the human *KCNN3* gene cause ZLS [35–37]. The $hK_{Ca}2.3$_V450L [38] mutation is associated with INCPH but not ZLS.

Among these mutations, $hK_{Ca}2.3$_K269E, $hK_{Ca}2.3$_G350D, $hK_{Ca}2.3$_S436C, and $hK_{Ca}2.3$_V450L mutant channels exhibited faster kinetics of current activation by $Ca^{2+}$ upon break-in with whole-cell patch-clamp recordings than the $hK_{Ca}2.3$_WT [35]. Our group quantitatively determined the apparent $Ca^{2+}$ sensitivity of these four mutant channels and the $hK_{Ca}2.3$_V555F mutant. They all exhibited increased apparent $Ca^{2+}$ sensitivity compared with the $hK_{Ca}2.3$_WT in inside-out patch-clamp recordings [61], when examined via heterologous expression of these mutant channels in HEK293 cells. The remaining mutations $hK_{Ca}2.3$_A287S [36], $hK_{Ca}2.3$_A536T [37], and $hK_{Ca}2.3$_V539del [36] have not been studied for their effects on channel activity (Table 2).

The $hK_{Ca}2.3$_V450L [38] genetic mutation in humans is familial, unlike the $hK_{Ca}2.3$_A287S [36] mutation which is unknown. Every other human $K_{Ca}2.3$ mutations in Table 2 are de novo variants. Notably *KCNN3* alleles in the patients are heterozygous. Theoretically, the $K_{Ca}2.3$ subunits carrying the mutations in Table 2 can co-assemble with $K_{Ca}2.3$_WT subunits, and form channel tetramers. It is currently unknown what impact the co-assembling of mutant and WT subunits has on channel function.

The expression of $K_{Ca}2.3$ channels in portal veins has not been reported and the mechanism for $hK_{Ca}2.3$_V450L [38] to cause INCPH is unclear. It is well established that $K_{Ca}2.3$ channels are expressed in arterial endothelial cells [27]. The role of $K_{Ca}2.3$ channels in the regulation of veins is less known [62]. It was also speculated that the $K_{Ca}2.3$ channel might play a role in the homeostasis of liver cells, like what had been reported for the $K_{Ca}3.1$ channel [63]. Increased $K^+$ channel activity in liver cells may cause stress and portal hypertension [35].

Based on the contribution of $K_{Ca}2.3$ channels to the EDH-mediated vasodilation, it has been speculated that mutant $K_{Ca}2.3$

**Table 2.** Effects of pathogenic $K_{Ca}2.3$ mutations on channel activity.

| Species | Mutation | Related disease | Apparent $Ca^{2+}$ sensitivity (μM) | Electrophysiological recordings | Cells |
|---|---|---|---|---|---|
| human | K269E | ZLS [35] | ~0.086 [61] | Whole-cell [35], inside-out [61] | CHO [35], HEK-293 [61] |
| human | A287S | ZLS [36] | N/A | N/A | N/A |
| human | G350D | ZLS [35] | ~0.12 [61] | Whole-cell [35], inside-out [61] | CHO [35], HEK-293 [61] |
| human | S436C | ZLS [35] | ~0.087 [61] | Whole-cell [35], inside-out [61] | CHO [35], HEK-293 [61] |
| human | V450L | INCPH [38] | ~0.15 [61] | Whole-cell [35], inside-out [61] | CHO [35], HEK-293 [61] |
| human | A536T | ZLS [37] | N/A | N/A | N/A |
| human | V539del | ZLS [36] | N/A | N/A | N/A |
| human | V555F | ZLS [36] | ~0.067 [61] | Inside-out [61] | HEK-293 [61] |

The apparent $Ca^{2+}$ sensitivity of the $K_{Ca}2.3$_WT is ~0.30 μM [61].

**Table 3.** Effects of pathogenic $K_{Ca}3.1$ mutations on channel activity.

| Species | Mutation | Related disease | Apparent $Ca^{2+}$ sensitivity (μM) | Electrophysiological recordings | Cells |
|---|---|---|---|---|---|
| human | V282M/E | HX [69] | N/A | N/A | N/A |
| human | S314P | HX [40] | ~0.064 [61] | Whole-cell [40], inside-out [61] | erythrocytes [40], HEK-293 [61] |
| human | A322V | HX [41] | ~0.059 [61] | Whole-cell [41], inside-out [61] | erythrocytes [41], HEK-293 [61] |
| human | R352H | HX [39, 43, 70] | ~0.085 [61] | Whole-cell [39, 43], inside-out [43, 61], two electrode voltage-clamp [43] | erythrocytes [39], HEK-293 [43, 61], xenopus oocytes [43] |

The apparent $Ca^{2+}$ sensitivity of $K_{Ca}3.1$_WT is ~0.27 μM [61].

channels expressed in vascular endothelium may be related to vascular damage during limb development of ZLS patients [35]. Fluid shear stress may trigger exaggerated vasodilation during human embryonic development because of the excessive hyperpolarization due to hypersensitivity to $Ca^{2+}$ of the ZLS-related mutant $K_{Ca}2.3$ channels. In critical phases of embryonic development, the consequent edema and vascular ruptures may lead to distal digital hypoplasia with aplastic or hypoplastic nails and terminal phalanges [35].

Intellectual disability and developmental delay symptoms are also reported in patients carrying the $K_{Ca}2.3$ mutations in Table 2, even though the development delay symptom was reported to be mild in patients carrying the $hK_{Ca}2.3$_ A536T mutation [34]. Overexpression of $K_{Ca}2.3$ channels in mice causes hippocampal shrinkage associated with cognitive impairment [64], suggesting the role of $K_{Ca}2.3$ channels in the central nervous system. It is not a surprise to see intellectual disability and developmental delay in patients carrying GOF $hK_{Ca}2.3$ mutations.

Gingival hyperplasia was reported in patients carrying mutations in Table 2, except $hK_{Ca}2.3$_ A287S [36], $hK_{Ca}2.3$_ G350D [35], and $hK_{Ca}2.3$_ A536T [37]. GOF mutations in genes encoding $K^+$ channels including *KCNQ1* [65], *KCNH1* [59], *KCNJ8* [66], and *KCNK4* [60] have been associated with hereditary gingival overgrowth. A recent mechanistic study revealed that activation of $K^+$ channels promotes fibrogenic response in hereditary gingival overgrowth via clustering and activation of the small GTP-binding protein Ras [67].

### GOF $K_{Ca}3.1$ mutations and hereditary xerocytosis

HX (OMIM 194380) also known as dehydrated hereditary stomatocytosis, is an autosomal dominant congenital hemolytic anemia characterized by erythrocyte dehydration. The majority of HX cases have been linked to GOF mutations of the mechanosensitive cationic PIEZO1 channel in erythrocytes [68]. A small subset (~10%) of HX (also called the Gardos channelopathy) has been linked with $hK_{Ca}3.1$_V282M [69], $hK_{Ca}3.1$_V282E [69], $hK_{Ca}3.1$_S314P [40], $hK_{Ca}3.1$_A322V [41], and $hK_{Ca}3.1$_R352H [39, 43, 70] mutations in the $K_{Ca}3.1$ channel encoded by the *KCNN4* gene.

Among these mutations, $hK_{Ca}3.1$_S314P, $hK_{Ca}3.1$_A322V, and $hK_{Ca}3.1$_R352H cause hypersensitivity to $Ca^{2+}$ of the mutant channels (Table 3) [43, 61]. The remaining mutations $hK_{Ca}3.1$_V282M [69], and $hK_{Ca}3.1$_V282E [69] have not been studied for their effects on the channel's sensitivity to $Ca^{2+}$.

All the human $K_{Ca}3.1$ mutations in Table 3 are inherited familial variants. All patients carry one WT and one mutant *KCNN4* allele. The $K_{Ca}3.1$ subunits carrying mutations in Table 3 may co-assemble with $K_{Ca}3.1$_WT subunits in the tetrameric channel assembly. The activity of such channels containing both WT and mutant subunits is unknown and requires future studies.

The excessive opening of these mutant $K_{Ca}3.1$ channels could lead to increased $K^+$ efflux, followed by water loss and erythrocyte dehydration [39, 40, 42, 71]. Erythrocytes from patients carrying these $K_{Ca}3.1$ mutations often exhibit decreased $K^+$ content and increased $Na^+$ content, accompanied by increased mean corpuscular hemoglobin concentration resulting from cell dehydration.

## STRUCTURE–FUNCTION RELATIONSHIP OF THE MUTANT CHANNELS

Many of the $K_{Ca}2.x/3.1$ mutation hot spots are located at regions essential for channel gating, including the S4-S5 linker, the selectivity filter, the pore-lining transmembrane S6 domain, and the HA/HB helices. In the S4-S5 linker, $hK_{Ca}2.2$_I288S [19] in humans and its corresponding $rK_{Ca}2.2$_I289N [18] in rats are LOF mutations, while $hK_{Ca}2.3$_S436C and $hK_{Ca}2.3$_V450L are GOF mutations that increase the apparent $Ca^{2+}$ sensitivity [61]. In the cryo-EM structure of $K_{Ca}3.1$ channels, the N-lobe of CaM forms contacts with the S4-S5 linker when bound with $Ca^{2+}$, which pulls the pore-forming transmembrane domains to open the gate [8]. Similar interactions between the CaM N-lobe and the S4-S5 linker are also predicted in the homology models of $K_{Ca}2.2$ [72] and $K_{Ca}2.3$ [61] channels. The binding interfaces between CaM and its substrates are often hydrophobic [73]. As such, $hK_{Ca}2.2$_I288S and $rK_{Ca}2.2$_I289N mutations may decrease hydrophobicity at the interface and impair the interactions between the CaM N-lobe and the S4-S5 linker, leading to LOF mutant $K_{Ca}2.2$ channels (Fig. 1). In contrast, $hK_{Ca}2.3$_V450L increases hydrophobicity at the interface and strengthens the interactions between the CaM N-lobe and the S4-S5 linker (Fig. 2), leading to more efficient channel opening and GOF mutant $K_{Ca}2.3$ channels. It is still not clear how $hK_{Ca}2.3$_S436C mutation causes GOF. But mutating its corresponding serine residue in $hK_{Ca}3.1$ ($hK_{Ca}3.1$_S181) to tryptophan or tyrosine amino acid residue increases the hydrophobicity at the interface and causes hypersensitivity to $Ca^{2+}$ [74].

In the selectivity filter, $hK_{Ca}2.2$_I359M, $hK_{Ca}2.2$_Y361C, $hK_{Ca}2.2$_G362S are LOF mutations (Fig. 1B) [19]. The selectivity filter is well conserved between different $K^+$ channels and mutations in the selectivity filter can often lead to LOF [75], by disrupting the $K^+$ passage.

In the transmembrane S6 domain, $hK_{Ca}2.2$_L388V [19] is a LOF mutation, while $hK_{Ca}2.3$_A536T [37], $hK_{Ca}2.3$_V539del [36], and $hK_{Ca}3.1$_V282M/E [69] are GOF mutations. The V539 residue in $hK_{Ca}2.3$ ($hK_{Ca}2.3$_V539) corresponds to the V282 residue in $hK_{Ca}3.1$ ($hK_{Ca}3.1$_V282). In the cryo-EM structure of $K_{Ca}3.1$, V282 defines the narrowest constriction site of the cytoplasmic gate (Fig. 3) [8]. The replacement of V282 by a glycine residue generates a "leaky" channel that conducts $K^+$ current in the absence of $Ca^{2+}$ [76]. Theoretically, the GOF $hK_{Ca}3.1$_V282E mutation introduces negatively charged residues at the cytoplasmic gate. This may cause electrostatic repulsion and enlargement of the gate, leading to constitutively active channels that leak $K^+$. How $hK_{Ca}3.1$_V282M mutation causes GOF channel activity still requires investigation.

In the HA/HB helices, $hK_{Ca}2.2$_L432P [19] is a LOF mutation, while $hK_{Ca}2.3$_V555F, $hK_{Ca}3.1$_S314P, $hK_{Ca}3.1$_A322V, and $hK_{Ca}3.1$_R352H increase the apparent $Ca^{2+}$ sensitivity [61]. The most studied HA/HB helices mutation is the $rK_{Ca}2.2$_V407F mutation corresponding to $hK_{Ca}2.3$_V555F. The $rK_{Ca}2.2$_V407F

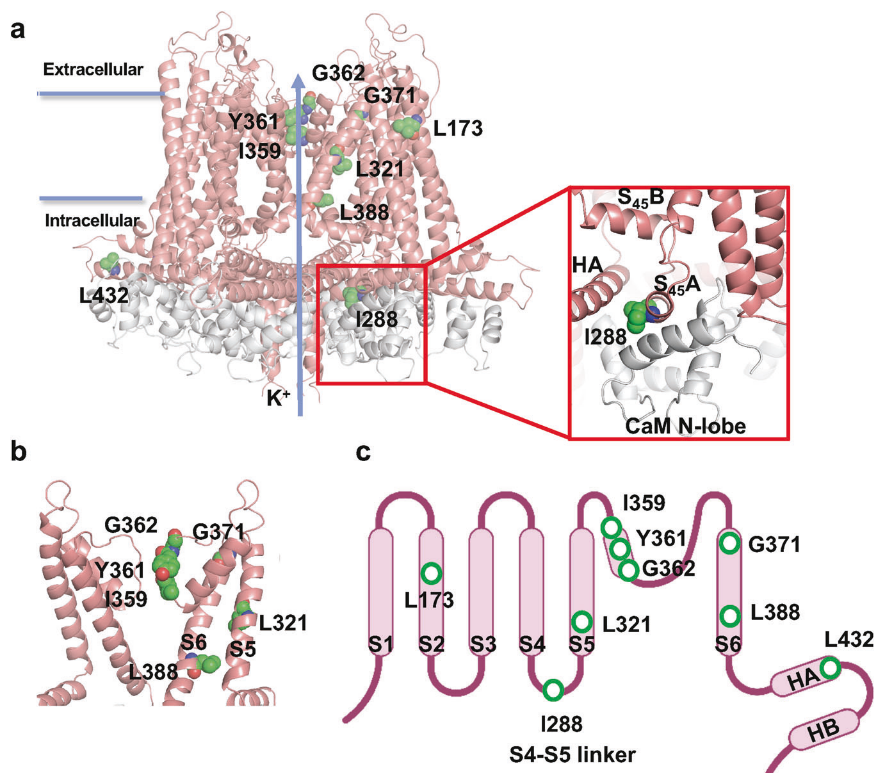


**Fig. 1 $K_{Ca}2.2$ channel structure and LOF mutations.** **a** Human $K_{Ca}2.2$ channel homology model generated using human $K_{Ca}3.1$ channel (PDB: 6cnn) as a template. Pore-forming channel α-subunits are shown in salmon and its accessory CaM is shown in gray. Mutations are shown as spheres with carbon atoms in green, oxygen atoms in red, and nitrogen atoms in dark blue. Mutations in only one of the four α-subunits are shown for clarity. In the inset, the relative position of CaM N-lobe, the S4-S5 linker ($S_{45}A$ and $S_{45}B$ helices), and HA helix is shown. **b** Mutations in selectivity filter and transmembrane S6 domain of channel pore. Mutations in only one of two opposite α-subunits are shown for clarity. **c** Schematic representation of one $K_{Ca}2.2$ channel subunit. Pathogenic LOF mutations are shown as green circles. **a**, **b** were generated using Pymol (Schrödinger LLC). **c** was generated using Biorender.com

mutation increases the apparent $Ca^{2+}$ sensitivity by enhancing the hydrophobic interactions between the proximal end of HA helix, the S4-S5 linker, and the CaM N-lobe, which may pull the transmembrane S6 domain more efficiently during the pore opening [72]. The $hK_{Ca}2.3$_V555F mutation may increase the hydrophobicity at the proximal end of the HA helix of $K_{Ca}2.3$ channels and enhance its interactions with the S4-S5 linker and the CaM N-lobe in a similar fashion (Fig. 2A). The $hK_{Ca}2.2$_L432P, $hK_{Ca}3.1$_S314P, and $hK_{Ca}3.1$_A322V mutations in the distal HA helix, as well as the $hK_{Ca}3.1$_R352H mutation in the HB helix, are at the interface between the HA/HB helices and the CaM C-lobe (Figs. 1 and 3). Their roles in the channel activation by $Ca^{2+}$ are less understood. It seems that changes at the interface between the HA/HB helices and the CaM C-lobe may also affect channel activity.

Two GOF mutations, $hK_{Ca}2.3$_K269E and $hK_{Ca}2.3$_G350D, are speculated to interact with casein kinase 2 (CK2) [35]. The $K_{Ca}2.3$_K269E mutation is equivalent to K121 in $rK_{Ca}2.2$ channels that are essential for the CK2 phosphorylation of the $rK_{Ca}2.2$-CaM complex [77]. The positively charged K121 residue in $rK_{Ca}2.2$ channels is not the phosphorylation site. Without the positively charged residue, CK2 cannot phosphorylate the $rK_{Ca}2.2$-CaM complex effectively [77]. The $rK_{Ca}2.2$_K121A mutation diminished the phosphorylation of the $rK_{Ca}2.2$-CaM complex [77]. These two GOF $hK_{Ca}2.3$ mutations may reduce the phosphorylation and negative modulation by CK2 and thus cause $Ca^{2+}$-hypersensitivity.

One GOF mutation $hK_{Ca}2.3$_A287S is in the transmembrane S1 domain (Fig. 2). The $mK_{Ca}2.2$_L168P mutation identified in jitter mice equivalent to the $hK_{Ca}2.2$_L173P in humans is in the S2 domain (Fig. 1). Transmembrane S1–S4 domains in the voltage-gated $K^+$ ($K_v$) channels are referred to as the voltage-sensing domain [78]. Unlike the $K_v$ channels, $K_{Ca}2.x$/ $K_{Ca}3.1$ channels are voltage independent. Even though a voltage-sensing role of the S1–S4 domains is not expected in $K_{Ca}2.x$/ $K_{Ca}3.1$ channels, their regulatory role in these voltage-independent channels may still need to be elucidated.

## HETEROMULTIMER FORMED BY DIFFERENT CHANNEL SUBTYPES

Different $K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ channel subtypes can form heteromultimers in human and mouse atrial myocytes [5]. Heteromultimerization of $K_{Ca}2.1/K_{Ca}3.1$ subtypes [4], $K_{Ca}2.1/K_{Ca}2.2$ subtypes [79, 80], or $K_{Ca}2.1/K_{Ca}2.2/K_{Ca}2.3$ subtypes [81] has also been reported in heterologous expression systems. Truncated channel fragments of $K_{Ca}2.2$ [82] or $K_{Ca}2.3$ [83, 84] can suppress the activity of other co-expressed $K_{Ca}2.x/K_{Ca}3.1$ subtypes in a dominant-negative fashion, implying the potential hetero-multimerization between subtypes.

$K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ subtypes are expressed in the central nervous system [9]. Expression of $K_{Ca}2.1$ and $K_{Ca}2.2$ channels exhibits a partially overlapping distribution pattern in the neocortex and hippocampus. $K_{Ca}2.3$ subtype is predominantly expressed in the basal ganglia, thalamus, and various brain stem nuclei [17]. The distinct and yet partially overlapping expression profiles of the subtypes imply possible heteromultimerization between $K_{Ca}2.x$ subtypes in the central nervous system. The LOF $K_{Ca}2.2$ mutations may impact more than the $K_{Ca}2.2$ subtype itself in human brains. Other $K_{Ca}2.x$ subtypes expressed in the same type of cells or tissues may be dominant-negatively affected as

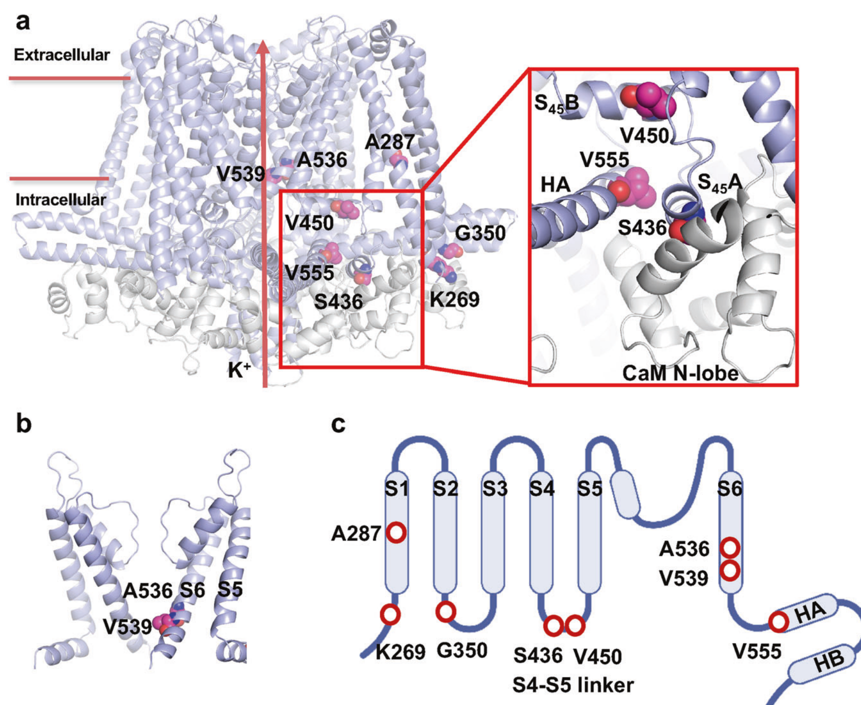


**Fig. 2 $K_{Ca}$2.3 channel structure and GOF mutations.** **a** Human $K_{Ca}$2.3 channel homology model generated using human $K_{Ca}$3.1 channel (PDB: 6cnn) as a template. Pore-forming channel α-subunits are shown in pale blue and its accessory CaM is shown in gray. Mutations are shown as spheres with carbon atoms in magenta, oxygen atoms in red and nitrogen atoms in dark blue. Mutations in only one of the four α-subunits are shown for clarity. In the inset, the relative position of CaM N-lobe, the S4-S5 linker ($S_{45}$A and $S_{45}$B helices), and HA helix are shown. **b** Mutations in the transmembrane S6 domain of channel pore. Mutations in only one of two opposite α-subunits are shown for clarity. **c** Schematic representation of one $K_{Ca}$2.3 channel subunit. Pathogenic GOF mutations are shown as red circles. **a**, **b** were generated using Pymol (Schrödinger LLC). **c** was generated using Biorender.com

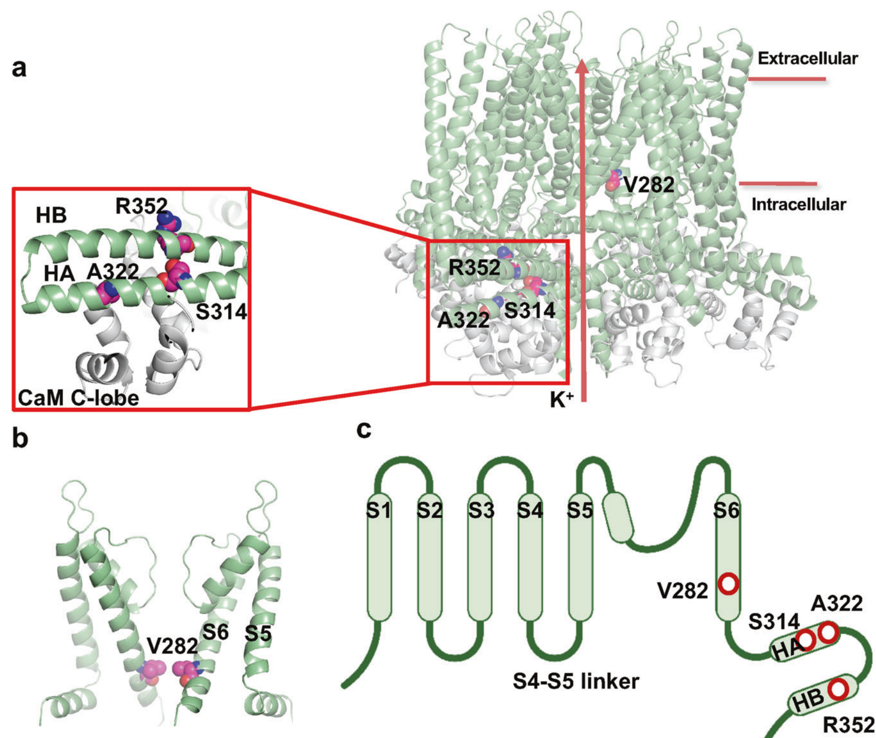


**Fig. 3 $K_{Ca}$3.1 channel structure and GOF mutations.** **a** Human $K_{Ca}$3.1 channel cryo-EM structure (PDB: 6cnn). Pore-forming channel α-subunits are shown in pale green, and its accessory CaM is shown in gray. Mutations are shown as spheres with carbon atoms in magenta, oxygen atoms in red and nitrogen atoms in dark blue. Mutations in only one of the four α-subunits are shown for clarity. In the inset, the relative position of CaM C-lobe and HA/HB helices is shown. **b** The V282 residue defines the narrowest site of the cytoplasmic gate. **c** Schematic representation of one $K_{Ca}$3.1 channel subunit. Pathogenic GOF mutations are shown as red circles. **a** and **b** were generated using Pymol (Schrödinger LLC). **c** was generated using Biorender.com

**Table 4.** Small molecule modulators of $K_{Ca}$2.x and $K_{Ca}$3.1 channels.

| | Compound | Potency | Subtype-selectivity |
|---|---|---|---|
| $K_{Ca}$3.1 negative modulators | Senicapoc [86, 92] | ~11 nM | ~1000-fold over $K_{Ca}$2.x |
| | TRAM-34 [92–94] | ~20 nM | ~1000-fold over $K_{Ca}$2.x |
| $K_{Ca}$3.1 positive modulators | SKA-111 [95] | ~0.11 μM | ~120-fold over $K_{Ca}$2.x |
| $K_{Ca}$2.x negative modulators | AP14145 [88] | ~1.1 μM | ≫10-fold over $K_{Ca}$3.1 |
| | NS8593[96] | ~0.6 μM | ≫10-fold over $K_{Ca}$3.1 |
| $K_{Ca}$2.2/$K_{Ca}$2.3 positive modulators | CyPPA [89] | ~14 μM on $K_{Ca}$2.2<br>~5.6 μM on $K_{Ca}$2.3 | inactive on $K_{Ca}$2.1 and $K_{Ca}$3.1 |
| | NS13001 [97] | ~1.8 μM on $K_{Ca}$2.2<br>~0.14 μM on $K_{Ca}$2.3 | inactive on $K_{Ca}$2.1 and $K_{Ca}$3.1 |
| | Compound 2q [91] | ~0.64 μM on $K_{Ca}$2.2<br>~0.60 μM on $K_{Ca}$2.3 | inactive on $K_{Ca}$2.1 and $K_{Ca}$3.1 |
| $K_{Ca}$2.x/$K_{Ca}$3.1 negative modulators | RA-2 [98] | ~17 nM | Non-selective |
| $K_{Ca}$2.x/$K_{Ca}$3.1 positive modulators | NS309 [10, 99] | ~0.62 μM on $K_{Ca}$2.x<br>~0.01 μM on $K_{Ca}$3.1 | Non-selective |
| | SKA-31 [100] | ~1.9-2.9 μM on $K_{Ca}$2.x<br>~0.26 μM on $K_{Ca}$3.1 | Non-selective |

well. The psychiatric and neurological symptoms observed in patients carrying $K_{Ca}$2.2 LOF mutations may arise from the impaired activity of both $K_{Ca}$2.2 and other co-assembled $K_{Ca}$2.x subtypes. Similarly, the central nervous system symptoms of patients carrying GOF $K_{Ca}$2.3 mutations may be attributed to the elevated activity of both $K_{Ca}$2.3 and other co-assembled $K_{Ca}$2.x subtypes, which will require future studies.

## POTENTIAL PHARMACOLOGICAL THERAPEUTIC STRATEGY

Genomic editing done by CRISPR/Cas9 may offer the ultimate cure for these genetic disorders when the technology matures [85]. Until then, pharmacological therapy may fill in the gap. The pharmacology for $K_{Ca}$2.x/$K_{Ca}$3.1 channel subtype has been well developed [3]. Small molecule positive and negative modulators with differential subtype-selectivity are available (Table 4). Senicapoc [86] inhibits $K_{Ca}$3.1 channels with $IC_{50}$ values of ~11 nM, and selectivity of ~1000-fold for $K_{Ca}$3.1 channels over $K_{Ca}$2.x channel subtypes. Senicapoc has exhibited excellent pharmacokinetic properties in humans [87] and is being studied in a clinical trial (ClinicalTrials.gov Identifier: NCT04372498) for HX patients carrying GOF $K_{Ca}$3.1 channel mutations.

For treating ZLS and INCPH related to GOF $K_{Ca}$2.3 mutations, negative modulators will be needed. AP14145 is equipotent in inhibiting $K_{Ca}$2.2 and $K_{Ca}$2.3, but is not effective on $K_{Ca}$3.1 channels [88]. We tested AP14145 on the ZLS- and INCPH-related mutant $K_{Ca}$2.3 channels. The inhibitory effect of AP14145 on the mutant channels is somewhat weaker than on the $K_{Ca}$2.3_WT channels [62].

For neurodevelopmental disorders related to LOF $K_{Ca}$2.2 mutations, positive modulators might be beneficial. There is a prototype positive modulator, CyPPA, that potentiates the activity of $K_{Ca}$2.2 and $K_{Ca}$2.3 channels selectively [89]. CyPPA binds to a putative binding pocket at the interface between the HA/HB helices and the constitutively associated CaM [90]. We performed chemical modification of CyPPA and developed several more potent and more selective positive modulators [91]. It is unknown how useful these positive modulators are. More research is needed to determine their effectiveness.

## ACKNOWLEDGEMENTS

We are grateful to Lucia Basilio, Young Hur, Misa Nguyen, Nadeed Naguib, Elyn Lam, and Nikita Dave for their helpful suggestions. M.Z. was supported by a Scientist Development Grant 13SDG16150007 from American Heart Association, a YI-SCA grant from National Ataxia Foundation, and a grant 4R33NS101182-03 from NIH.

## AUTHOR CONTRIBUTIONS

M.C. and M.Z. conceptualized the project. All authors contributed to the manuscript and the figures.

## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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