KCNMA1-Linked Channelopathy

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ABSTRACT

KCNMA1 encodes the pore-forming α subunit of the 'Big K^+' (BK) large conductance calcium and voltage-activated K^+ channel ($K_{Ca}1.1$). BK channels are widely distributed across many tissues, including both excitable and non-excitable cells. Expression levels are highest in brain and muscle, where the channels are critical regulators of neuronal excitability and muscle contractility. A global deletion in mouse (*KCNMA1*$^{-/-}$) is viable but exhibits pathophysiology in many organ systems. Yet despite the important roles for BK channels in animal models, the consequences of dysfunctional BK channels in humans is not well-characterized. Here, we summarize 16 rare *KCNMA1* mutations identified in 37 patients dating back to 2005, with an array of clinically defined pathological phenotypes collectively referred to as '*KCNMA1*-linked channelopathy.' These mutations encompass gain of function (GOF) and loss of function (LOF) alterations in BK channel activity, as well as several variants of unknown significance (VUS). Human *KCNMA1* mutations are primarily associated with neurological conditions, including seizures, movement disorders, developmental delay, and intellectual disability. Due to the recent identification of additional patients, the spectrum of symptoms associated with *KCNMA1* mutations has expanded but remains primarily defined by brain and muscle dysfunction. Emerging evidence suggests the functional BK channel alterations produced by different *KCNMA1* alleles may associate with semi-distinct patient symptoms, such as paroxysmal non-kinesigenic dyskinesia (PNKD) with GOF and ataxia with LOF. However, due to the *de novo* origins for the majority of *KCNMA1* mutations identified to date, and the phenotypic variability exhibited by patients, additional evidence is required to establish causality in most cases. The symptomatic picture developing from patients with *KCNMA1*-linked channelopathy highlights the importance of better understanding the roles BK channels play in regulating cell excitability. Establishing causality between *KCNMA1*-linked BK channel dysfunction and specific patient symptoms may reveal new treatment approaches with the potential to increase therapeutic efficacy over current standard regimens.

INTRODUCTION

Ion channels are ubiquitously expressed throughout the body and perform key membrane transport processes crucial to normal physiological function. Pathogenic alterations to ion channel activity can disrupt homeostatic and physiological functions leading to disorders such as hemiplegic migraines, epilepsy, or cardiac arrhythmias, collectively referred to as 'channelopathies' (LiBrenner and Wilcox, 2012;Kim, 2014;Meredith, 2015). Channelopathies are rare monogenetic disorders stemming from inherited or *de novo* mutations in genes encoding the functional components of ion channels. Advancements in whole exome sequencing (WES) have contributed to a growing list of diagnosable monogenetic channelopathies (Kim, 2014), yet the molecular basis for these genetic mutations and how they produce clinical phenotypes are not fully established in many cases. Current understandings of channelopathies are limited by several factors, such as deficiencies in: 1) the direct evidence for causality due to a very limited number of patients and lack of genetic pedigree analysis, 2) the functional data for the classification of mutant channel properties, and 3) the tissue loci and nature of aberrant excitability linked to abnormal patient phenotypes. This review will focus on one channelopathy involving the large conductance Ca^{2+} and voltage-activated K^+ (BK) channel, encoded by *KCNMA1*. The hallmark clinical presentation of *KCNMA1*-linked channelopathy is neurological dysfunction, including seizures, movement disorders, developmental delay, and intellectual disability.

The *KCNMA1* gene, located on human chromosome 10q22.3, produces the pore-forming α-subunit of the BK channel (Dworetzky et al., 1994;Pallanck and Ganetzky, 1994;McCobb et al., 1995). BK channels, named for their 'Big K^+' conductance (> 100 pS), are members of the voltage-gated K^+ channel family and mediate K^+ efflux from excitable and non-excitable cells (Latorre and Miller, 1983;Neyton and Miller, 1988). BK channels assemble as homotetramers of the *KCNMA1* gene product. Each α-subunit consists of 7 transmembrane domains (S0 – S6), and a large intracellular C-terminus (**Figure 1**). The extracellular N-terminus and presence of the S0 transmembrane segment differs between BK and other voltage-gated K^+ channels and confers interactions with accessory proteins (β and γ subunits)(Wallner et al., 1996;Morrow et al., 2006;Yan and Aldrich, 2010;2012). The S1-S4 segments contain positively charged residues and constitute the voltage sensing domain, while the S5-S6 segments form the pore domain which houses the highly K^+ selective conduction pathway (Meera et al., 1997;Stefani et al., 1997;Horrigan et al., 1999;Horrigan and Aldrich, 2002;Yang et al., 2015). The intracellular C-terminus of the BK channel encompasses the gating ring, a structure comprised of two regulators of K^+ conductance

(RCK) domains. RCK1 and RCK2 each contain two distinct high-affinity Ca^{2+} binding sites, mediating the allosteric gating of BK channels (Wang and Sigworth, 2009;Lee and Cui, 2010; Xia et al., 2002;Yuan et al., 2010; Yuan et al., 2011;Zhang et al., 2010). The human BK channel structure was determined in 2010 (Yuan et al., 2010), providing a basis for understanding the location of specific residues, such as mutations, with respect to functional domains.

Multiple activation mechanisms are involved in opening the channels *in vivo* (Hou et al., 2009). Under physiological conditions in excitable cells, the BK channel activation requires both membrane depolarization and an increase in intracellular Ca^{2+} to micromolar levels for activation (Yang et al., 2015). These high concentrations of Ca^{2+} are attained in local microdomains through tight functional coupling with voltage-gated Ca^{2+} channels or channels that release Ca^{2+} from intracellular stores (Fakler and Adelman, 2008). Different tissues exhibit BK currents of varying voltage and Ca^{2+}-dependent activation properties, underlying the complex roles for BK channels in regulating excitability. This cell and tissue-specific regulation is achieved via extensive alternative splicing of *KCNMA1* transcripts, assembly with modulatory β- and γ-regulatory subunits, and a variety of post-translational modifications (Kyle and Braun, 2014;Latorre et al., 2017;Gonzalez-Perez and Lingle, 2019). These mechanisms work together to tailor the voltage and Ca^{2+}-dependence of channel activation, as well as the kinetics of activation and deactivation gating, for the cell-specific function of BK channels in excitability or K^+ transport.

In humans, BK channels are widely expressed in the body including nervous, muscular, skeletal, endocrine, cardiovascular, digestive, urinary, and reproductive systems (**Figure 2**) (Pallotta et al., 1981;Adams et al., 1982;Becker et al., 1995;McCobb et al., 1995;Hirukawa et al., 2008;Cui et al., 2009;Fagerberg et al., 2014;Contet et al., 2016;Latorre et al., 2017;Gonzalez-Perez and Lingle, 2019). Prior to the identification of *KCNMA1*-linked channelopathy patients, changes in BK channel expression or activity had been implicated in human studies of erectile dysfunction, overactive bladder, hypertension, and premature uterine contraction during pregnancy (Christ et al., 2001;Melman et al., 2007;Tomas et al., 2008;Christ et al., 2009;Grimm and Sansom, 2010;Yang et al., 2013;Li et al., 2014). Moreover, several single nucleotide polymorphisms (SNPs) in *KCNMA1* and genes encoding BK-specific auxiliary β subunits (*KCNMB1-4*) were also linked to human disorders or disease risk, including autism, hypertension and cardiovascular function, and asthma (Gollasch et al., 2002;Laumonnier et al., 2006;Schuckit et al., 2005;Han et al., 2013). Despite

these analyses implicating BK channel function in several pathological conditions, human studies have revealed only a limited picture of what is known about BK channel roles *in vivo*. Extensive investigations in animal models, which are more accessible to the interrogation of specific cell types and tissues, have provided a more complete assessment of the various roles for BK channels in physiology and pathophysiology. Although neurological and muscle phenotypes are well represented, in rodents, BK channel function is more broadly required for a panoply of organ functions that parallels their wide expression across tissues (**Figure 3**). Taken together, these animal studies detail a greater potential for wide-ranging pathophysiological dysfunction in patients carrying mutations in *KCNMA1*.

KCNMA1 Patient Mutations

Sixteen *KCNMA1* mutations in thirty-seven symptomatic patients have been reported in the literature to date (**Figure 1)**. Functional classifications into GOF and LOF effects on channel properties have been based on expression of BK channel cDNAs harboring the patient mutations in non-excitable heterologous cell lines. From these studies, two mutations have been shown to confer GOF properties to BK channels: D434G and N995S (also referred to as N999S or N1053S) (Du et al., 2005;Li et al., 2018; Plante et al., 2019;Zhang et al., 2015). Ten mutations have been classified as LOF (S351Y, G354S, G356R, G375R, C413Y/N449fs, I663V, P805L, and D984N) (Liang et al., 2019) (Carvalho-de-Souza et al., 2016) or putative LOF (premature truncation mutations: Y676Lfs*7 and Arg458Ter) (Tabarki et al., 2016;Yesil et al., 2018). The remaining mutations have not yet been conclusively defined with respect to channel properties (E884K) (Zhang et al., 2015) or have not been demonstrated to produce changes in channel function (K518N, E656A, and N1159S) (Li et al., 2018). The majority of the mutations are *de novo* variants, and patients are heterozygous (**Figure 1**). Since BK channels are comprised of tetramers of the *KCNMA1* gene product, this presents the potential for mitigation of mutant effects by co-assembly with wildtype (WT) subunits in heterozygous patients. However, it is not yet known whether tetramers are actually formed between WT and mutant subunits in patients with one normal and one mutant allele, or how the interaction between these subunits would affect BK channel properties. It is also not well-studied how the native regulatory processes that set channel function *in vivo* would combine with the mutation to affect channel properties. It is possible that some previously established GOF or LOF classifications could change under different experimental conditions than those initially tested. *KCNMA1* patient mutations that

have been characterized in at least one standard condition are described in the following section.

GOF Mutations

D434G - The D434 residue is located in the AC region of the RCK1 domain (**Figure 1**), which contributes to the calcium gating of BK channels (Du et al., 2005). Patch-clamp recordings from Chinese hamster ovary (CHO) cells and *Xenopus laevis* oocytes expressing D434G channels demonstrated increased BK current. The increased current was primarily due to a three- to fivefold increase in Ca^{2+} sensitivity and faster activation compared to wildtype (WT) BK channels. The voltage of half-maximal activation ($V_{1/2}$) for D434G channels was shifted to more negative potentials by 26 mV and 56 mV at 0.1 and 2 μM Ca^{2+}, respectively. These experiments revealed that D434G makes the channels easier to open, and the mutation was identified as the first human GOF *KCNMA*1 allele. The conclusions were confirmed in two independent studies that characterized the effect of D434G on BK/β4 channels and further probed the molecular mechanism behind the aspartate-to-glycine substitution (Wang et al, 2009;Yang et al., 2010). Several putative neurophysiological mechanisms consistent with either hyperexcitation in brain areas such as thalamocortical circuits and the basal ganglia, or disinhibition of GABAergic circuits, were hypothesized to explain the symptoms experienced by patients (Du et al., 2005), but the specific neuronal circuit alterations caused by the D434G mutation remain unknown.

N995S/N999S/N1053S – A second GOF mutation is reported in the literature using three different reference sequence numbering schemes but constitutes the same residue substitution (**Figure 1** and **Supplemental Table 1**) (Zhang et al., 2015;Wang et al., 2017;Li et al., 2018;Heim et al., 2019;Plante et al., 2019). In this review, this mutation will be referred to by the numbering scheme in the original publication for the data being discussed. Patch-clamp recordings from HEK293 cells expressing N995S or N999S channels exhibited increased BK current compared to WT (Li et al., 2018;Plante et al., 2019). This increased current was due to a >40mV hyperpolarizing shift in the $V_{1/2}$ (Li et al., 2018;Plante et al., 2019). The mechanism of this shift was proposed to be independent of Ca^{2+}, as the N995S mutation increased BK current when the intracellular Ca^{2+} binding sites were mutated (Li et al., 2018). Additionally, activation of the mutant N995S (N999S) channels was faster and deactivation was slower than WT, correlated with increased mean open times in single channel recordings (Li et al., 2018;Plante et al., 2019). Interestingly, the GOF BK current phenotypes from N999S channels were found to exceed the GOF alterations produced by

D434G (Plante et al., 2019), suggesting that the relative alterations in BK channel properties exhibited by distinct GOF mutations could influence the clinical heterogeneity among patients.

LOF Mutations

Liang et al. (2019) reported 9 unrelated patients affected by 8 distinct *KCNMA1* mutations spanning from the pore domain to end of the intracellular C-terminal gating ring of the BK protein (**Figure 1**). Five mutations abolished BK current in HEK293T patch-clamp recordings: **S351Y** and **G356R** in the pore domain, **G375R** in the S6 domain, **N449fs*** in the AC domain of RCK1, and **I663V** in the loop between RCK1 and RCK2, suggesting these mutations comprise LOF alleles of *KCNMA1* (Liang et al., 2019). Of these 5 mutations, only I663V was evaluated by western blot for protein expression levels. I663V channels had higher molecular weight compared to WT, but additional experiments would be needed to determine whether the size shift was due to changes in post translational modifications and how this relates to loss of BK current. The mechanisms for current abrogation of the other four mutations has not yet been addressed.

The other three mutations, **C413Y**, **P805L**, and **D984N,** reduced the mean amplitude of BK current compared to WT in patch clamp recordings, suggesting a mechanistically distinct LOF phenotype from the prior group. **C413Y** in the AC region of RCK1 and **P805L** located in the loop between S9 and S10 of the gating ring (**Figure 1**) showed shifts in the $V_{1/2}$ values to more positive potentials, with a slope change suggestive of alterations in the voltage and Ca^{2+} sensitivity of the channels (Liang et al., 2019). Both mutations produced smaller current amplitudes compared to WT channels, and the expression level of P805L was decreased in western blot analysis. Interestingly, the patient harboring the C413Y mutation inherited this mutation from his asymptomatic mother, and the N449fs* from his asymptomatic father (Liang et al., 2019). This raises two possibilities, either that each mutation is akin to an autosomal recessive allele, or that co-expression with WT in the heterozygous parents may preclude a pathological phenotype. Finally, the mutation **D984N** located in the loop between S9 and S10 in RCK2 showed no shift in the $V_{1/2}$ at 10μM Ca^{2+}. Other Ca^{2+} concentrations and expression levels were not evaluated, leaving the mechanism for this LOF mutation unresolved.

G354S –Voltage-clamp recordings from Xenopus oocytes expressing the G354S mutant BK channels demonstrated a tenfold reduction in BK current due to slower activation kinetics (Carvalho-de-Souza et al., 2016).

R458Ter and Tyr676Lfs*7 – These mutations were predicated to be LOF allele based on the early termination of the BK channel protein (Tabarki et al., 2016) (Yesil et al., 2018) . **Tyr676Lfs*7** is an autosomal recessive *KCNMA1* duplication mutation (Tabarki et al., 2016). Due to the retention of the tetramerization domain in the C-terminus of the channel, **Tyr676Lfs*7** could potentially reduce current through a dominant negative action, but the functional properties for both mutations remains to be tested.

K518N, E656A, and N1159S – Patch-clamp recordings in HEK293 cells for each of these mutant channels showed no differences in activation kinetics or BK current density compared to WT BK channels suggesting they are benign genetic variants, or VUS, under the tested conditions (Li et al., 2018).

E884K – The functional effect for this mutation has not been shown. However, the patient shares similar symptoms to other GOF and LOF patients including PNKD, developmental delay, and visual impairment (Zhang et al., 2015). Pathogenicity prediction algorithms reported this mutation to be possibly deleterious (Zhang et al., 2015).

Neuronal Mechanisms of BK Channel Dysfunction

The mechanisms by which *KCNMA1* mutations alter BK channel activity to cause patient symptoms is still an open question. In the central nervous system (CNS), BK channels play an important role in neuronal excitability, passing outward K^+ current upon membrane depolarization and increased intracellular Ca^{2+} during the action potential, leading to hyperpolarization of the membrane and decreased excitability (Hille, 2001). BK current shapes action potential waveforms by mediating the repolarization and afterhyperpolarization (AHP) phases (Shao et al., 1999;Faber and Sah, 2002;Gu et al., 2007;Ly et al., 2011;Contet et al., 2016). In the majority of neuronal and muscle contexts where the channels are expressed, activation of BK current reduces neuronal firing rates, presynaptic neurotransmitter release, or muscle contraction or tone (Jaggar et al., 2000;Contet et al., 2016;Tricarico and Mele, 2017). In neurons, this decrease in firing is predominantly due to the contribution of BK current to the afterhyperpolarization (AHP) phase of the action potential (Storm, 1987;Contet et al., 2016). In central neurons linked to neurological phenotypes such as cerebellar Purkinje and dentate gyrus (DG) granule neurons, the β4 subunit slows BK current activation, facilitating its suppressive role in neuronal firing during the AHP (Brenner et al., 2005;Womack et al., 2009;Petrik et al., 2011;Benton et al., 2013). BK channel activation also reduces excitatory synaptic activity at neuromuscular junctions and in central neurons (Robitaille et al., 1993;Raffaelli et al., 2004). In smooth muscle cells, BK channels

hyperpolarize the membrane potential, which in turn decreases Ca^{2+} influx through voltage-gated Ca^{2+} channels and promotes smooth muscle relaxation. These studies suggest that LOF in BK channel activity would broadly produce hyperexcitability, such as that resulting in seizure. Consistent with this, at least half of the patients with putative LOF *KCNMA1* mutations have seizures of some type (**Figure 4**).

GOF *KCNMA1* patient mutations are also clearly associated with seizure, raising the possibility that changes in BK channel activity in both directions could alter the balance of excitation and inhibition in the brain. Underlying the potential for bi-directional effects on excitability, BK channels are expressed in both excitatory and inhibitory neurons, and the effects of BK current on firing can vary by neuron type (Contet et al., 2016). Activation of BK current can both decrease and increase neuronal firing rates (Montgomery and Meredith, 2012), a paradox that can be resolved by understanding the voltage- and Ca^{2+}-dependence and gating kinetics of the channels in a specific membrane and cellular context. Predicting the effect of BK channel activation on action potential frequency, for example, requires knowledge of the activation, inactivation, and deactivation properties for the BK channels expressed, and the interaction with other ionic currents in that cell (Jaffe et al., 2011;Ly et al., 2011;Montgomery and Meredith, 2012;Contet et al., 2016). The intrinsic gating properties in turn, are regulated by the subunit composition of the BK channel complex: the α, β, γ subunits of the BK channel and its associated Ca^{2+} source (Berkefeld and Fakler, 2008;Latorre et al., 2017). A pro-excitatory effect of BK channel activation is revealed by BK antagonists that decrease neuronal firing or plateau potentials (Solaro et al., 1995;Jin et al., 2000;Van Goor et al., 2001;Sausbier et al., 2004;Gu et al., 2007;Bell et al., 2008) or reduce sinoatrial node firing and heart rate (Lai et al., 2014), an opposite effect from the typical result of inhibiting BK channels in most excitable cells. Furthermore, BK channel inhibition has been reported to exert anticonvulsant, rather than epileptogenic effects, in picrotoxin and pentylenetetrazole (PTZ) mouse seizure models (Sheehan et al., 2009).

The mechanisms underlying the pro-excitatory effects could simply stem from the increased BK current produced by GOF channels hyperpolarizing the membrane of inhibitory neurons. On the other hand, one property correlated with pro-excitatory BK currents is fast activation. More rapid BK current activation directly increases neuronal firing rates by leading to faster repolarization of the action potential (Jaffe et al., 2011;Ly et al., 2011;Montgomery and Meredith, 2012;Contet et al., 2016), an effect that could link GOF channels to hyperexcitation and increased firing in excitatory neurons. In addition, rapidly

activating BK currents contribute to the fast phase of the AHP, facilitating enhanced Na_v channel recovery from inactivation and decreased activation of other K^+ currents (Van Goor et al., 2001;Gu et al., 2007;Gittis et al., 2010;Wang et al., 2014). Thus, rather than strictly hyperpolarizing membranes due to increased BK current, mutations such as D434G and N995S/N999S/N1053S which activate more rapidly than WT channels (Du et al, 2005;Plante et al, 2019; Li et al., 2018;Wang et al, 2009;Yang et al., 2010), may be capable of directly increasing action potential firing. Thus, the bidirectional and cell-specific effects of BK channels on neuronal excitability suggests that *KCNMA1* mutation-linked dysfunction could disrupt neuronal circuitry and provoke pathologic symptoms via multiple potential mechanisms.

KCNMA1-Linked Channelopathy and the Spectrum of Patient Phenotypes

The clinical symptoms of patients with *KCNMA1*-linked channelopathy have not been comprehensively defined but are principally characterized by seizures and movement disorders. Of the 16 *KCNMA1* mutations reported in the literature to date, 4 mutations have been identified in more than one patient (**Figure 1)**. Comparison of these cases suggests that patients sharing the same mutation do exhibit similar and/or overlapping neurological and neuromuscular phenotypes, in addition to distinct symptoms. Particularly with the *de novo* mutations, this provides evidence for the causative nature of the mutations in patient symptoms. The evidence for symptomatic similarities by mutation is summarized in the following.

The D434G *KCNMA1* mutation was identified in 13 members of a large family with symptoms of a coexistent syndrome of generalized epilepsy and paroxysmal dyskinesia (GEPD) (Du et al., 2005). Within this group, 7 patients developed paroxysmal non-kinesigenic dyskinesia (PNKD), 1 patient developed epilepsy, and 5 patients developed both symptoms (Du et al., 2005). These clinical findings were the first to link *KCNMA1* to disease in humans and provided prima facie confirmation of the BK channel's extensive involvement in CNS function.

The N1053S mutation (also called N995S or N999S) has been identified in 7 unrelated patients from around the world (Zhang et al., 2015;Wang et al., 2017;Li et al., 2018;Heim et al., 2019) who share similar symptoms with the D434G patients. Of these patients, 4 developed early-onset PNKD, 2 developed epileptic seizures, and 1 patient developed both symptoms. In addition, all 7 patients were reported to have developmental delays, 3 patients had intellectual disability, and 1 patient had other symptoms involving CNS

dysfunction – potentially expanding the neurological phenotypes associated with this channelopathy. The symptoms associated with D434G and N1053S mutations constitute the description of phenotype MIM# 609446 ("PKND3") provided by the Online Mendelian Inheritance in Man (OMIM) database (https://omim.org).

The Y676Lfs*7 mutation was identified in 2 siblings from a consanguineous family (Tabarki et al., 2016). Both patients were reported to have the same symptoms starting at a young age which included epileptic seizures, severe developmental delay, and non-progressive cerebellar atrophy (Tabarki et al., 2016). These 2 patient cases constitute the description of phenotype MIM# 617643 provided by the OMIM database (https://omim.org). While seizure and developmental delay are shared phenotypes with D434G and N995S/N999S/N1053S, cerebellar atrophy without paroxysmal dyskinesia is a distinct phenotype and adds to the variability of neurological symptoms associated with this channelopathy.

The final mutation reported in the literature that is shared by multiple patients is G375R (Liang et al., 2019). This mutation was identified in 3 unrelated patients who experienced overlapping and heterogenous symptoms. All 3 patients were reported to have a multiple malformation syndrome characterized by facial dysmorphisms, visceral malformations, development delay, intellectual disability, and axial hypotonia (Liang et al., 2019). Additionally, 2 of the 3 patients developed epileptic seizures and one patient had mild cerebral and cerebellar atrophy (Liang et al., 2019). This mutation is associated with the most distinct clinical findings relative to the other 3 mutations, but still demonstrates some phenotypic overlap with the previous mutations regarding neurological and neuromuscular dysfunction.

Of these 4 *KCNMA1* mutations, D434G exhibited autosomal dominant inheritance and was the only mutation identified in patients from successive generations of a pedigree (Du et al., 2005). Y676Lfs*7 demonstrated autosomal recessive inheritance and was identified in a single generation of a pedigree (Tabarki et al., 2016). These hereditable mutations produced very similar symptoms in affected family members, suggesting a causal role. The remaining 2 mutations shared by multiple patients were *de novo* variants (N995S/N999S/N1053S and G375R). In these cases, clinical similarities between patients sharing the same mutations has suggested causality in the absence of comprehensive pedigrees. As a result of the confidence in the *KCNMA1* linkage to patient symptoms, paroxysmal non-kinesigenic dyskinesia-3 with or without generalized epilepsy (PNKD3;

MIM# 609446) is now recognized as distinct type of movement disorder linked to at least two mutations in *KCNMA1* (D434G and N995S/N999S/N1053S).

For mutations where only a single patient defines the clinical presentation, causality must still be investigated. Nine additional *de novo KCNMA1* mutations have been reported in the literature where a single patient represents the known symptomatic information. These mutations include: R458Ter*, G354S, S351Y, G356R, C413Y/N449fs, I6663V, P805L, E884K, and D98N (Zhang et al., 2015;Carvalho-de-Souza et al., 2016;Staisch et al., 2016;Liang et al., 2019) (**Figure 1)**. In these cases, most of the symptoms are similar to those previously described and only a few symptoms do not overlap. For example, 7 of the 9 patients developed a movement disorder, 2 developed seizures, all 9 were reported to have developmental delay, 7 had intellectual disability, and 4 had abnormal brain imaging indicative of cerebellar atrophy. The new symptoms were mostly related to neurological dysfunction and included autistic like features and areflexia (Liang et al., 2019). These clinical findings suggest semi-distinct phenotypes associated with some of these mutations (**Figure 4**), however the single patient sample size makes it difficult to validate this conclusion.

Lastly, 3 additional patients with epilepsy were identified to harbor other *KCNMA1* mutations of unknown significance (K518N, E656A, and N1159S). While patch-clamp recordings from these 3 variants showed benign activity (Li et al., 2018), the shared symptom of epileptic seizure with other pathogenic *KCNMA1* mutations, may indicate these mutations interfere with BK activity and regulation of neuronal excitability through a yet-to-be-defined mechanism.

Across with *KCNMA1*-linked channelopathy patient population, another important outstanding question is whether patient symptoms and phenotypes can be classified with respect to the functional changes in BK channel activity produced by mutations. Patients with both GOF or LOF *KNCMA1* mutations exhibit overlapping symptoms, especially with respect to seizures and neurodevelopment (**Figure 4)**. Thus, combined with the small patient sample size for each mutation, definitive categorization of specific symptoms to increases or decreases in BK channel activity is not yet possible. Moreover, published reports lack detailed clinical description, further limiting the ability to determine if particular symptoms are specific to GOF versus LOF channel mutations. Nevertheless, a few symptoms, primarily movement disorders and muscle dysfunction, appear to be somewhat distinct for GOF versus LOF mutations (**Figure 4)**. Below, we summarize the variability of symptoms pertaining to

seizure, movement disorder, and neurodevelopment for all patients, while highlighting certain symptoms that may segregate with GOF or LOF mutations.

Movement Disorder

A predominant phenotype of *KCNMA1*-linked channelopathy, regardless of the functional classification of the mutation (GOF, LOF, VUS, or, benign), is movement disorder. Twenty-four of the 37 patients were reported to have symptoms related to a movement disorder with variability of the symptomatic presentation among patients. However, there seems to be more apparent segregation by channel functional classification for movement disorder compared to other predominant symptoms like seizures. These symptoms include PNKD (GOF) versus ataxia, axial hypotonia, and tremor (LOF, **Figure 4).**

PNKD was reported in 17 of 20 GOF patients and only 2 of 13 LOF patients (Du et al., 2005;Zhang et al., 2015;Staisch et al., 2016;Wang et al., 2017;Yesil et al., 2018;Heim et al., 2019). A patient with the VUS E884K mutation was also reported to have paroxysmal dyskinesia (Zhang et al., 2015). The paroxysmal dyskinesia symptoms were described as 'non-kinesigenic' due to the lack of a movement trigger, in contrast to the more common paroxysmal kinesigenic dyskinesia (PKD) which is induced by various movements, and other forms of dyskinesia such as exertion-induced dyskinesia. Although the patients were diagnosed with PNKD, the specific types of movements and body parts involved during PNKD attacks were not stereotypical between patients. For example, the patients with D434G GOF mutation experienced episodes of involuntary mouth movements and hand stiffness during attacks (Du et al., 2005). Another patient harboring the G354S LOF mutation was reported to have perioral dyskinesia diagnosed at 18 months (Staisch et al., 2016). One patient with the N1053S mutation was diagnosed with PNKD at 7 months after having paroxysmal dystonic postures (Zhang et al., 2015). The 3 patients with N999S mutations were all diagnosed at different ages with alike paroxysmal drop attacks characterized by behavioral arrest and generalized dystonic posture or stiffening (Heim et al., 2019) (personal communication). The frequency and duration of theses movement attacks were variable, ranging from few to many dozens of attacks every day, and lasting a few seconds to minutes. OMIM defines this movement disorder phenotype as 'PNKD type 3' or 'PNKD3' (MIM# 609446) because *KCNMA1* was the third gene linked to a PNKD phenotype. The other two genes include MR1 located on chromosome 2q35 and PNKD2 on chromosome 2q31, and correspond to PNKD1 (phenotype MIM# 118800) and PNKD2 (phenotype MIM# 611147), respectively (https://omim.org). However, the specific term PKND3 is not systematically

used for patients with PNKD and *KCNMA1* mutations in the literature, suggesting existing and new patients expressing similar symptoms should be reevaluated for this diagnosis.

A movement disorder distinct from PNKD, and noted only in LOF patients, was ataxia (**Figure 4)** (Carvalho-de-Souza et al., 2016;Liang et al., 2019), a phenotype also well-described in BK channel 'knockout' mice harboring a LOF deletion in the *KCNMA1* gene (Meredith et al., 2004;Sausbier et al., 2004;Chen et al., 2010). In patients, the ataxic symptoms included lack of coordination, unbalanced gait, and spasticity of lower extremities. Three of the 5 ataxic patients also had cerebellar atrophy diagnosed by brain MRI (Carvalho-de-Souza et al., 2016;Liang et al., 2019), which was another clinical finding distinct for LOF patients. Studies of *KCNMA1*$^{-/-}$ mouse models showed reduced basal firing from cerebellar Purkinje neurons and impaired cerebellar signaling and motor coordination, which may offer a possible mechanism for the ataxic symptoms in these patients (Sausbier et al., 2004). In addition, BK channel inhibitors produce ataxia when ingested or injected (Imlach et al., 2008;McManus and Rothberg, 2014;Hoshi and Heinemann, 2016;Kaczorowski and Garcia, 2016), supporting association between BK channel LOF and ataxic movement disorder.

Baseline axial hypotonia is another motor dysfunction symptom most prominent with LOF mutations (**Figure 4**). The axial hypotonia reported in 8 of 13 LOF patients ranged from mild to severe and was independent from PNKD associated symptoms noted in GOF patients (Tabarki et al., 2016;Liang et al., 2019). Additionally, tremor was noted in 2 LOF patients, and is a phenotype shared with BK channel KO mice (Sausbier et al., 2004;Imlach et al., 2008), but specific details describing this symptom were not provided (Yesil et al., 2018;Liang et al., 2019). Further diagnostic studies are required to determine if these movement disorder attacks are due to mutant BK channel activity within the affected muscle groups, or whether they are secondary to CNS dysfunction, as both mechanisms are seen in a variety of channelopathies associated with movement disorders (Graves and Hanna, 2005).

Seizure Disorder

Seizure is another predominant symptom associated with both GOF and LOF *KCNMA1* mutations. Eighteen of the 37 patients described in this review developed epileptic seizures. However, there was considerable variability among the characteristics of the seizures such as age of onset, type, EEG pattern, frequency and duration, and response to medications. None of these characteristics are clearly delineated by the mutation type. Both GOF and LOF mutations are associated with overlapping seizure phenotypes including myoclonic (Tabarki et al., 2016;Li et al., 2018), generalized tonic-clonic (GTCS) (Du et al.,

2005;Tabarki et al., 2016;Yesil et al., 2018), and absence seizures (Du et al., 2005;Li et al., 2018;Yesil et al., 2018;Liang et al., 2019) (**Figure 4).** Interestingly, all 9 GOF patients experienced absence seizures whereas only 3 of 6 LOF patients experienced them. Atonic seizures and status epilepticus were only reported in 2 different patients expressing LOF mutations (Yesil et al., 2018;Liang et al., 2019). Ten of the 18 patients were reported to have abnormal inter-ictal EEG findings ranging from mild background slowing to generalized spike wave complexes to Lennox-Gastaut patterning (Du et al., 2005;Tabarki et al., 2016;Li et al., 2018;Yesil et al., 2018). Age of onset for seizure also varied between patients regardless of the mutation type. Of the 13 family members expressing a D434G GOF mutation, 6 were diagnosed with epilepsy at ages ranging from 6 months and 9 years (Du et al., 2005). Three of the seven patients expressing a N995S or N999S GOF mutation began experiencing seizures at 20 months, 6 years, and 9 years of age (Li et al., 2018;Heim et al., 2019) (personal communication), and the two siblings with the same Y676Lfs*7 putative LOF mutation had seizures beginning at one year of age (Tabarki et al., 2016). As in the general population, it is likely that seizure heterogeneity with this subset of patients is heavily influenced by additional factors (Steinlein, 2008).

Neurodevelopmental and Cognitive Phenotypes

Patients with both GOF and LOF *KCNMA1* alleles showed developmental delay and intellectual disability (**Figure 4)**. Of the 37 patients reported in the literature, 21 were described as having developmental delay (Zhang et al., 2015;Carvalho-de-Souza et al., 2016;Wang et al., 2017;Li et al., 2018;Yesil et al., 2018;Heim et al., 2019;Liang et al., 2019) and 12 were noted to have intellectual disability (Zhang et al., 2015;Carvalho-de-Souza et al., 2016;Wang et al., 2017;Heim et al., 2019;Liang et al., 2019). The developmental delays ranged from mild to severe and included psychomotor symptoms such as delayed/unstable sitting, inability to support head or walk unassisted, speech delay, and global developmental delay (Zhang et al., 2015;Tabarki et al., 2016;Heim et al., 2019;Liang et al., 2019). A subset of neurodevelopmental symptoms has only been described in patients with LOF *KCNMA1* alleles (**Figure 4**). One patient had hearing impairment (Liang et al., 2019), and multiple patients demonstrated visual impairment due to nystagmus, strabismus, and macular coloboma (Carvalho-de-Souza et al., 2016;Liang et al., 2019). Two patients had autism disorder or features (Heim et al., 2019;Liang et al., 2019) (personal communication), which together with mental retardation was previously associated with a potential LOF SNP in *KCNMA1* (Laumonnier et al., 2006).

Lessons from Animal Studies Linking Changes in BK Channel Function to Neurological Phenotypes

Within the rodent brain, high levels of BK channel expression are detected in the neocortex, basal ganglia, hippocampus, thalamus, habenula and its tract to the interpeduncular nucleus in the midbrain, cerebellum, vestibular nuclei in the hindbrain, and olfactory system (Knaus et al., 1996;Wanner et al., 1999;Misonou et al., 2006;Sausbier et al., 2006b;Yue et al., 2014). Animals with functional deficiency of BK channels displayed behaviors that can recapitulate some of the movement symptoms in patients with putative LOF *KCNMA1* mutations. Pharmacological inhibition of BK channels with lolitrem B or paxilline induced tremor and ataxia in mice and livestock (Imlach et al., 2008), and constitutive deletion of *KCNMA1* (*KCNMA1*$^{-/-}$) produced several types of motor impairments such as ataxia, tremor, impaired motor coordination, reduced muscle strength, and abnormal eye blink reflex in mice (Meredith et al., 2004;Sausbier et al., 2004;Imlach et al., 2008;Typlt et al., 2013a). The motor impairments exhibited by *KCNMA1*$^{-/-}$ mice suggested cerebellar dysfunction. Consistent with this, *KCNMA1*$^{-/-}$ Purkinje neurons showed significantly reduced basal firing activity and increased short term transmission depression at synapses on deep cerebellar nuclei (DCN) and disrupted olivo-cerebellar feedback (Sausbier et al., 2004) (Chen et al., 2010). The direct influence of GOF mutations in BK channel function on motor control has not yet been investigated, but mice harboring a strong (non-patient derived) GOF mutation that increases voltage-dependent gating of the BK channel (R207Q) have grossly normal locomotor behavior (Montgomery and Meredith, 2012). Taken together, the results from animal models demonstrate that BK channel LOF is associated with movement disorder phenotypes that are also observed in human patients, and further suggest the potential for segregation of distinct motor dysfunction with LOF versus GOF BK channel alterations.

Unlike motor dysfunction, both LOF and GOF alterations in BK channel activity have been linked with the seizure pathophysiology in animal studies. In a chronic temporal lobe epileptic rat model, expression of the BK channel α-subunit encoded by *KCNMA1* was downregulated in hippocampus and cortex (Ermolinsky et al., 2008;Pacheco Otalora et al., 2008), suggesting a link between seizure and LOF in BK channel activity. BK channel expression and BK current levels were also reduced in the inferior colliculus during alcohol withdrawal periods in a rat model of alcohol withdrawal seizures (N'Gouemo et al., 2009). However, a direct causal relationship between LOF alterations in BK channel activity and epileptogenesis has not yet been reported.

There is a more solid correlation between GOF alterations in BK channel activity and seizure. Deletion of regulatory β4 subunit (*KCNMB4*$^{-/-}$), which slows BK channel activation, produced temporal lobe seizures cortex in mice, associated with hyperactive firing in dentate granule (DG) cells in the hippocampus (Brenner et al., 2005). In addition, kainic acid-induced seizure susceptibility was increased in β4 heterozygous mice (Whitmire et al., 2017). Furthermore, in a model of fragile X syndrome, enhancement of BK channel activity in *KCNMB4*$^{-/-}$ mice was associated with alterations in epileptiform activity in hippocampal slices (Deng and Klyachko, 2016). Several other studies also show situations where increased BK channel expression was associated with hyperexcitability and seizure activity. Elevated cell surface expression and activity of BK channels via mutation of CRL4A^{CRBN} ubiquitin ligase increased susceptibility to spontaneous and PTZ-induced seizures in mice (Liu et al., 2014). Upregulation of BK α and downregulation of β4 protein were reported after seizure induction in rats (Savina et al., 2014), and β4 mRNA was downregulated in DG cells in the hippocampus following pilocarpine-induced seizure (Whitmire et al., 2017). BK currents in somatosensory cortex were significantly increased after picrotoxin-induced seizure in mice, and the increased neuronal firing activity was normalized with BK channel inhibitors (Shruti et al., 2008). Similarly, BK blockers normalized DG cell firing rates that were increased during pilocarpine-induced seizure episodes in rats (Mehranfard et al., 2014;2015). These animal studies show that GOF alterations in BK channel activity, either via increased expression or due to loss of the slow gating imparted by the β4 subunit, alters the balance of neuronal activity in the brain. GOF *KCNMA1* mutations affecting BK channel activity in mechanistically similar ways could be causative in *KCNMA1*-linked patient seizures.

Inheritance and Prevalence of KCNMA-Linked Channelopathy

The majority of the pathogenic *KCNMA1* mutations reported in the literature occurred *de novo*, however few mutations exhibit autosomal recessive or autosomal dominant inheritance patterns (**Figure 1**). To date, the literature reports a total of 16 mutations identified in 37 symptomatic patients (Du et al., 2005;Zhang et al., 2015;Carvalho-de-Souza et al., 2016;Tabarki et al., 2016;Wang et al., 2017;Li et al., 2018;Yesil et al., 2018;Heim et al., 2019;Liang et al., 2019). Seventeen patients have *de novo* mutations, which constitute 9/16 *KCNMA1* mutations. Four patients inherited an autosomal recessive mutation, constituting 3/16 mutations. Thirteen patients (1 family) inherited a single (1/16) autosomal dominant mutation. Additionally, there are 3 benign *KCNMA1* genetic variants (3/16)

identified in 3 patients with epilepsy (Li et al., 2018). One of these 3 variants exhibited an autosomal recessive inheritance pattern, but the inheritance patterns for the other two variants were not determined.

KCNMA1-linked channelopathy is a very rare syndrome. The allele frequency of certain pathogenic *KCNMA1* mutations in larger populations is estimated to be less than 1:100,000 in the Genome Aggregation Database (D434G = $3x10^{-5}$ and Y676Lfs*7 = $4x10^{-6}$) (Lek et al., 2016). However, the allele frequency in larger populations is unknown for majority of the pathogenic *KCNMA1* mutations owing to the rarity of this syndrome. **Supplemental Table 1** provides information regarding the number of missense SNPs present in *KCNMA1* and putative pathogenic mutations reported by different large-scale sequencing databases.

Diagnosis and Treatment of KCNMA1-Linked Channelopathy

Epilepsy and movement disorders are known to be caused by several different medical conditions, a subset of which are the result of monogenetic channelopathies (Brenner and Wilcox, 2012;Kim, 2014). Traditionally, if a patient presents with epileptic seizures and movement attacks with otherwise unremarkable brain imaging and normal neurological function between episodes, then a genetic channelopathy is a leading consideration and genetic testing is recommended to search for causative genetic mutations. The D434G mutation was initially mapped via microsatellite linkage analysis (Du et al., 2005); however, causative genetic findings in neurological disorders are more routinely established now by single gene analysis, panels of multiple gene tests for disorders which share a specific phenotype, or by high-powered whole exome sequencing (WES) or whole genome sequencing (WGS) (Pulst, 1999). When patient symptoms are extensive and a test for multiple pathogenic genetic mutations is desired, WES is typically used instead of WGS (Kong et al., 2018). WES is less costly than WGS, and the majority of pathogenic variants are found within the coding regions or splice sites of proteins. However, WES is prone to incomplete coverage of certain loci, variability in coverage from person to person, and has susceptibility to false positives and negatives. Of the 37 patients described in the literature, 21 were identified to have a *KCNMA1* mutation using WES (Staisch et al., 2016;Tabarki et al., 2016;Li et al., 2018;Yesil et al., 2018;Heim et al., 2019;Liang et al., 2019).

On the other hand, when the clinical symptoms are well-defined or stereotypical such as GEPD, as is the case with some patients with *KCNMA1*-linked channelopathy, a limited set of specific candidate genes are assayed. At present, 29 commercial gene panels include

KCNMA1 (**Supplemental Table 2)**. Of note, the genes included with each panel are frequently updated as additional genetic mutations are associated with specific medical conditions. Three of the 37 patients with *KCNMA1* mutations were identified using epilepsy- and paroxysmal dyskinesia-specific gene panels (Zhang et al., 2015;Wang et al., 2017).

Development of treatment regimens specific for *KCNMA1*-linked channelopathy ('precision medicine') is currently limited by several important factors, including 1) lack of FDA-approved selective BK channel pharmacological modulators, 2) lack of stereotypical clinical presentations with respect to core symptoms (movement disorder and seizure), 3) lack of established causality between alterations in channel activity and patient symptoms, and 4) inadequate medical assessment identifying the loci for symptoms in patients. Thus, the majority *KCNMA1*-linked channelopathy patients with seizures have been treated with anti-seizure medications (ASMs) selected with the same general approach as for any non-syndromic epilepsy. Several *KCNMA1* patient studies commented on the efficacy of various ASMs and demonstrate the range of patient responses may range from positive, to no response, to negative.

Sodium valproate and/or lamotrigine were effective in treating the absence seizures in the proband with the GOF D434G mutation (Du et al., 2005), as well as the absence, generalized tonic-clonic (GTCS), and atonic seizures in a patient with the LOF R458Ter* mutation (Yesil et al., 2018), and myoclonic seizures in a patient with homozygous LOF Tyr676Leufs*7 mutation (Tabarki et al., 2016). These two ASMs were also used to treat absence seizures in two patients with the LOF G375R mutation, but efficacy of the treatments was not reported (Liang et al., 2019). The second patient with the homozygous Tyr676Leufs*7 mutation and myoclonic seizures and GTCS was treated effectively with sodium valproate and levetiracetam (Tabarki et al., 2016). Levetiracetam alone was effective to treat atypical absence and myoclonic seizures in two patients with the GOF N995S mutation (Li et al., 2018). However, one of these patients was trialed on various ASMs prior to levetiracetam; treatment with oxcarbazepine, and sodium valproate produced negative side-effects, sultiame and perampanel did not have any effects, and zonisamide monotherapy reduced the seizure frequency, but failed to stop the myoclonic seizures. Notably, this patient experienced significant worsening of seizures with ethosuxmide, the drug of choice for the absence seizures seen in childhood absence epilepsy. A dramatic worsening of absence seizures at very low doses of ethosuximide also occurred in a GOF N999S patient, reported in (Heim et al., 2019), while levetiracetam was not tolerated due to side effects (author S.K. personal observation). The patient with a LOF D984N mutation was diagnosed with status

epilepticus and treated with several ASMs including clobazam, ethosuximide, lamotrigine, zonisamide, sodium valproate, phenobarbital, and lacosamide, but the therapeutic efficacy was not known (Liang et al., 2019).

Paroxysmal dyskinesias are commonly categorized into three main types (kinesigenic or PKD, non-kinesigenic or PNKD, and exercise-induced or PED), and each type has been shown to respond differently to medications (Unterberger and Trinka, 2008). PNKD, the dominant type associated with *KCNMA1*-linked channelopathy, is commonly described as refractory to medications. However, benzodiazepines, particularly clonazepam, and some ASMs have been shown to be effective in some PNKD patients (Unterberger and Trinka, 2008). Additional therapies used to treat other movement disorders, such as acetazolamide in episodic ataxia, have demonstrated positive effects in some patients with PNKD (Silveira-Moriyama et al., 2018). Acetazolamide is a carbonic anhydrase inhibitor and has been suggested as a possible direct agonist of BK channels in rat cerebellar neurons and skeletal muscle (Tricarico et al., 2004;Abbasi et al., 2014;Tricarico and Mele, 2017). However, the potentiating mechanism for acetazolamide on either WT or mutant BK channels is not currently understood.

There are a limited number of reports assessing PNKD treatment and related symptoms in patients harboring *KCNMA1* mutations. Clonazepam was partially effective for two patients with GOF D434G mutations (Du et al., 2005) and reduced the frequency of PNKD attacks in one patient with the N1053S mutation (Wang et al., 2017). Clonazepam was also used to treat dyskinetic tremor, dystonia, and spasticity of the lower extremities in a patient with the R458Ter* mutation (Yesil et al., 2018). A second patient with the GOF N1053S mutation and paroxysmal dystonic postures was unresponsive to oxcarbazepine, sodium valproate, and levetiracetam (Zhang et al., 2015), whereas a patient with the GOF N999S mutation experiencing paroxysmal drop attacks responded positively to DHA and acetazolamide after not responding to clonazepam, amitriptyline, or levetiracetam (Heim et al., 2019). A second patient with the N999S mutation and experiencing the paroxysmal drop attacks did not respond to levetiracetam, carbamazepine, valproic acid, vitamin B complex, CBD oil, DHA, 5-HIAA, or pimozide (Heim et al., 2019) (personal communication). Overall, within the *KCNMA1*-linked channelopathy population, patient responses to therapies treating seizure and movement disorders are not predictable *a priori*.

Precision Medicine and Future Directions

For most genetic channelopathies, pharmacologic treatment does not yet consist of modulating the specific activity of mutant ion channels. For *KCNMA1* and other channelopathies, the first hurdle to precision medicine involves categorizing patient mutations into functional classes, such as GOF or LOF. However, these classes are not trivial to define and can be condition- or cell-type specific, related to the particular splice variants, accessory subunits, post-translational channel modifications, and cellular contexts (intracellular calcium, in particular for the BK channel) in which the mutation is tested. For this reason, it is not yet clear whether strategies employing patient-derived stem cells, which can only be differentiated into a limited set of cell types, would result in BK channel pharmacological modulators that are ultimately clinically-relevant. In addition, GOF, LOF, and potentially benign mutations in BK channel activity are associated with overlapping symptoms (**Figure 4**), raising the question of whether selective agonists or antagonists that restore the 'correct' level of BK channel activity will actually produce the desired outcome on neuronal activity. Without more detailed information about the origination of the changes in excitability underlying the seizure and movement symptoms in *KCNMA1*-linked channelopathy, it is difficult to ascertain which BK channel components to target, and in which neuron or muscle loci.

Selective BK channel pharmacotherapy has been under development for over two decades, mostly intended to treat smooth muscle dysfunction resulting from LOF in BK channel activity (hypertension, bladder incontinence, stroke, and erectile dysfunction) (Christ et al., 2001;Gribkoff et al., 2001;Spektor et al., 2002;Vang et al., 2010;Soder and Petkov, 2011;Liu et al., 2013). Several compounds, including endogenous, naturally occurring, and synthetic, have been identified as BK agonists and trialed as potential therapeutic agents (Hou et al., 2009;Bentzen et al., 2014;Hoshi and Heinemann, 2016). The endogenous class includes heme and heme-breakdown products (Tang et al., 2003;Horrigan et al., 2005), free long-chain poly unsaturated acids (Clarke et al., 2002;2003), metabolites of cytochrome P450, epoxygenase and lipoxygenase (Feletou, 2009;Hou et al., 2009), and 17 beta-estradiol (Valverde et al., 1999). The natural occurring class includes a variety of entities found in herbs, roots, and leaves used in folk medicines for treating asthma and other disorders stemming from smooth muscle dysfunction such as DHS-I (McManus et al., 1993;Nardi et al., 2003). The synthetic class includes benzimidazolone compounds NS004 and NS1619 (Olesen et al., 1994a;Olesen et al., 1994b), the more potent and selective NS11021 (Bentzen et al., 2007), and a newer family of BK channel activators called the GoSlo-SR family (anthra-quinone analogs) (Roy et al., 2012). At least one compound, Cym04 (a

dehydroabietic acid derivative) has been shown to demonstrate some specificity for a particular BK channel splice variant (Cui et al., 2008;Gessner et al., 2012). Despite the ability of these chemical agents to activate BK channels *in vitro* and animal studies, only a single drug with therapeutic action targeting the BK channel is FDA-approved (Cuppoletti et al., 2007). This drug, Rescula (unoprostone isopropyl ophthalmic solution) is a potent BK channel activator used to reduce ocular pressure in glaucoma (Thieme et al., 2001).

Other potential, but non-selective, BK channel modulators include docosahexaenoic acid (DHA), an omega-3 fatty acid found in oily fish. DHA reversibly binds BKα/β1 expressed primarily in smooth muscle and BKα/β4 channels expressed primarily in the nervous system in mice. DHA increases peak BK current by 20- to 30-fold at certain physiologic voltages (Hoshi et al., 2013a), suggesting potential efficacy for enhancing LOF BK channel activity. While DHA injection in mice lowers blood pressure (Hoshi et al., 2013b), the physiological roles of this fatty acid within the nervous system are not well defined. Injection of DHA in rats increased latency of PTZ-induced seizure implicating its neuroprotective effect (Trepanier, 2014); however, a systemic review assessing protective role of omega-3 supplementation in human seizures reported inconclusive evidence of benefit (Pourmasoumi et al., 2018). Two patients with *KCNMA1*-linked channelopathy have been trialed on DHA (in combination with acetazolamide) (Heim et al., 2019). However, the patients harbor a mutation classified as GOF, suggesting any potential therapeutic effect conferred by DHA might not originate from direct modulation of the BK channel. Thus, the data provide no specific indication on the therapeutic potential for DHA in *KCNMA1*-linked channelopathy patients.

Another potential pharmacotherapy based on FDA-approved drugs to normalize BK channel activity associated with GOF mutations is Ca^{2+} channel inhibitors. For GOF mutations, decreasing the Ca^{2+}-dependent activation could reduce BK current. While this approach could require knowledge of the specific Ca^{2+} source for the channels in tissues where excitability is altered in the patients, one class of inhibitors, dihydropyridines (DHPs), are known to inhibit BK channel activation in a variety of central neurons (Fagni et al., 1994;Zhang and Gold, 2009;Wang et al., 2016;Whitt et al., 2018). The DHPs, nifedipine and nimodipine, and the non-DHP verapamil, are used for some types of dyskinesia (Abad and Ovsiew, 1993). Verapamil has been used for refractory epilepsy and hyperkinetic movement disorders (Ovsiew et al., 1998;Narayanan et al., 2016;Lakshmikanthcharan et al., 2018). In addition, there is at least one report that Verapamil might block BK channels directly, further contributing to a reduction in channel activity through a selective mechanism (Harper et al.,

2001). However, it has not yet been investigated whether doses effective at reducing BK channel activity in the brain would have cardiac side effects. Two *KCNMA1*-linked channelopathy patients with GOF mutations are currently being trialed on verapamil (author A. Meredith, personal observation).

Beyond pharmacology, some therapeutic approaches tailored for individual patients with other monogenetic disorders may be conceivable in the future for *KCNMA1*-linked channelopathy, such as gene editing, gene therapy, or optogenetic restoration of neuronal activity. Yet, substantial challenges are present for their application to any disease or disorder, include target specificity, delivery optimization, elimination of off-target side-effects, timing optimization before or after critical stages of neurological development (Wykes and Lignani, 2018), and creating animal models that best replicate the genetic channelopathy from the molecular level to the clinical phenotype to determine initial safety and efficacy of such therapies (Tanner and Beeton, 2018). While these precision medicines may hold eventual promise, until the challenges are overcome, development of additional pharmacological approaches remains the major path forward clinically.

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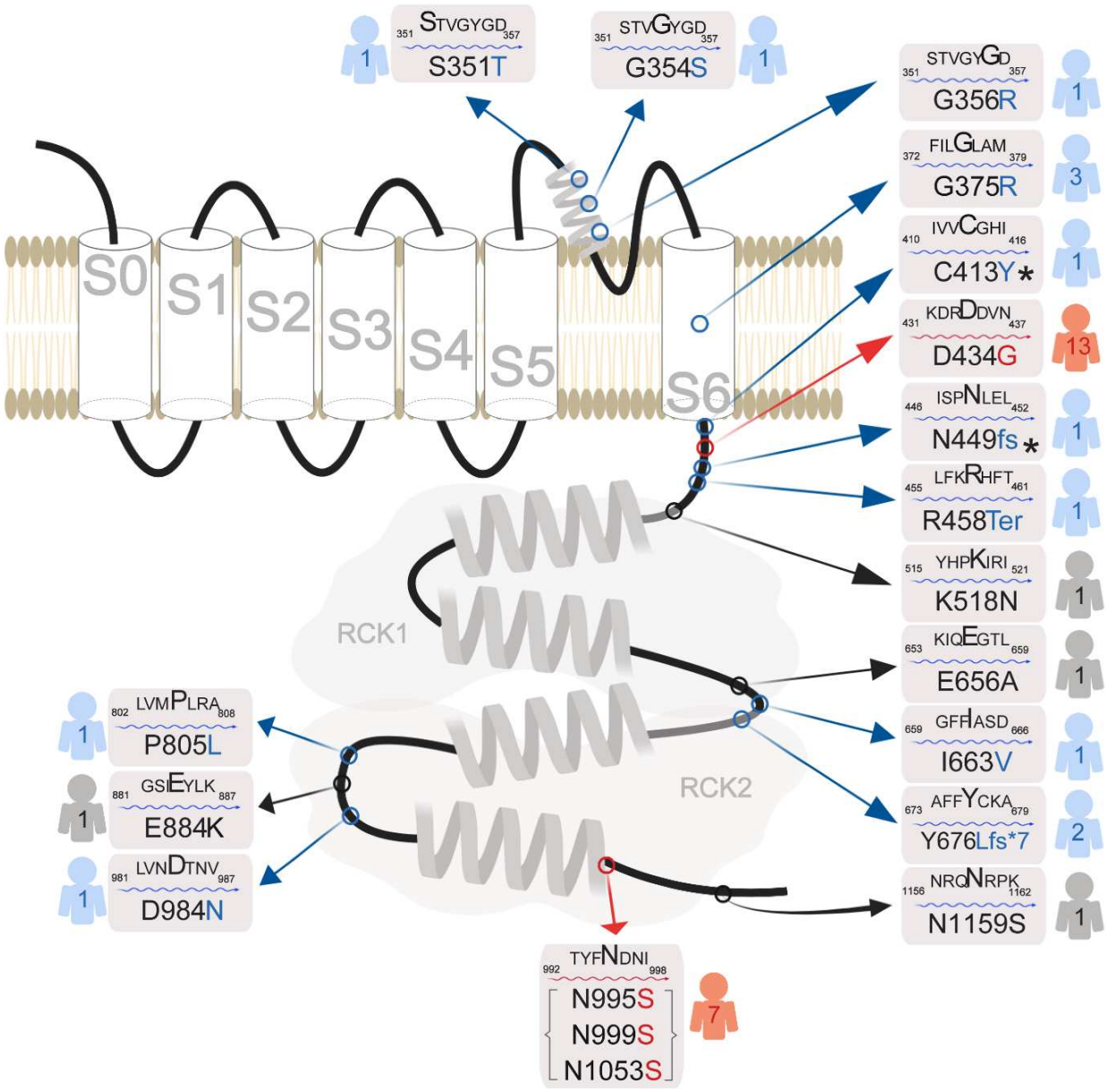

Figure 1 – Human *KCNMA1* mutations. Schematic of the *KCNMA1* gene product, the α-subunit of the BK channel (accession number NM_002247.3). The voltage-sensitive pore-forming region of the BK channel is comprised of transmembrane domains S0-S6, while the intracellular gating ring contains the two Ca^{2+} binding sites in the RCK1 and RCK2 domains. Red indicates GOF mutations (n=2), blue indicates LOF or putative LOF mutations (n=11), and black indicates putative benign mutations (n=3) or variants of unknown significance (VUS) (n=1). *C413Y/N499fs is a double mutation harbored by a single patient. Numbers to the right or left of each mutation indicate the total number of patients carrying each mutation reported in published studies. N995S, N999S, and N1053S are the same amino acid substitution, but are reported in the literature using 3 different reference sequencing number schemes.

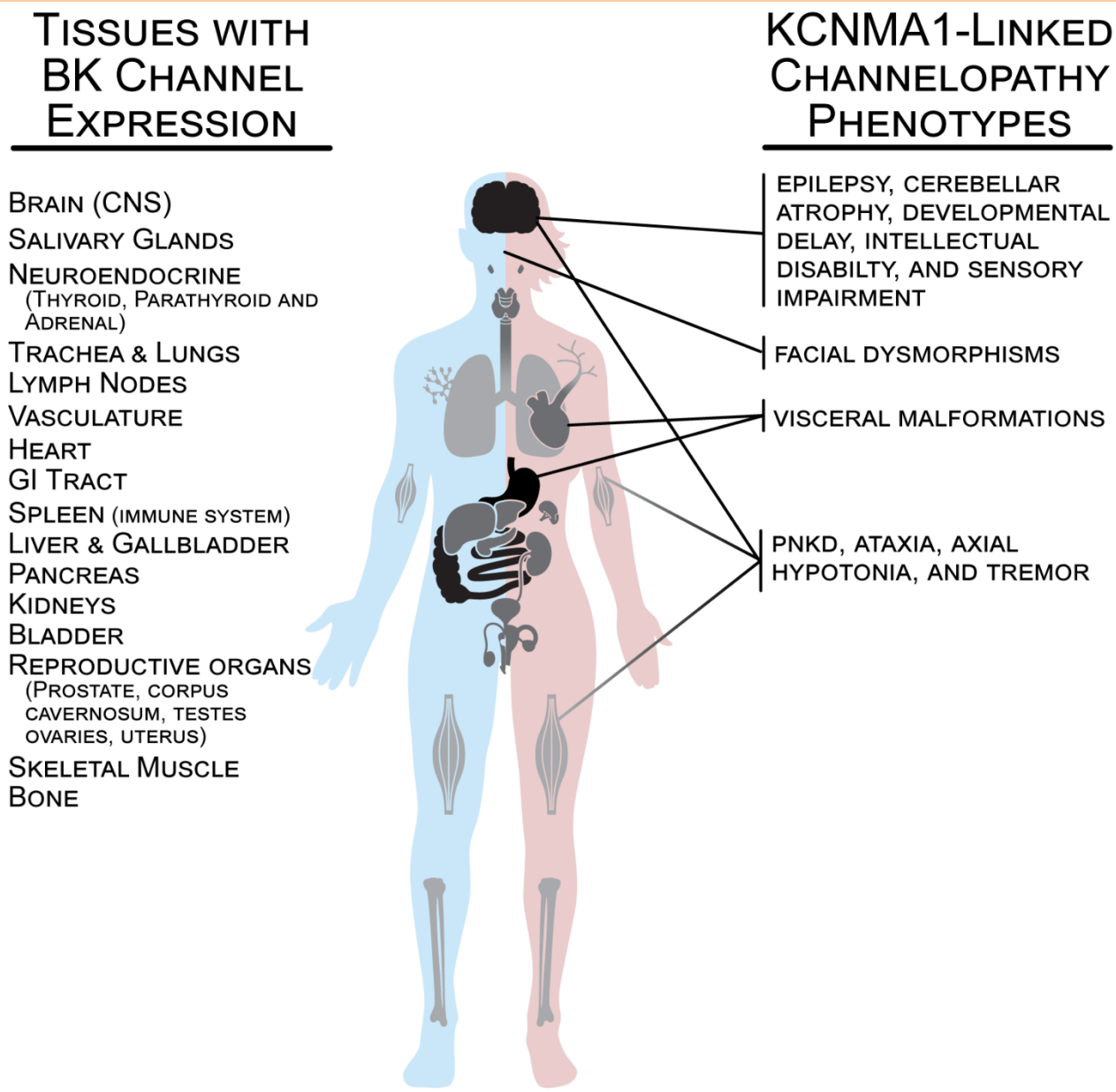

Figure 2 –BK channel expression in human tissues and prominent phenotypes reported in patients with *KCNMA1*-linked channelopathy. Major tissues or systems expressing BK channels are depicted in black (high relative expression), grey (medium), and light grey (low). Organs demonstrating high levels of BK channel expression include the CNS (olfactory system, neocortex, basal ganglia, hippocampus, thalamus, habenula and its tract to the interpeduncular nucleus in the midbrain, cerebellum, vestibular nuclei in the hindbrain, and spinal cord), gastrointestinal (GI) tract (stomach, small intestine and colon), and reproductive organs (corpus cavernosum, prostate, testes, ovaries, and uterus). Organs demonstrating medium BK channel expression include salivary glands, neuroendocrine glands (thyroid, parathyroid, adrenal), heart, urinary bladder, liver and gallbladder, kidneys, and spleen/immune system. Organs demonstrating low levels of BK channel include lungs, lymph nodes, vasculature, skeletal muscle, and bone. Data on expression levels were derived from the Human Protein Atlas v18.1 (https://www.proteinatlas.org) (Uhlén et al., 2015), the NCBI Gene Database (https://www.ncbi.nlm.nih.gov), and published reports (Dworetzky et al., 1994;McCobb et al., 1995;Brenner et al., 2000).

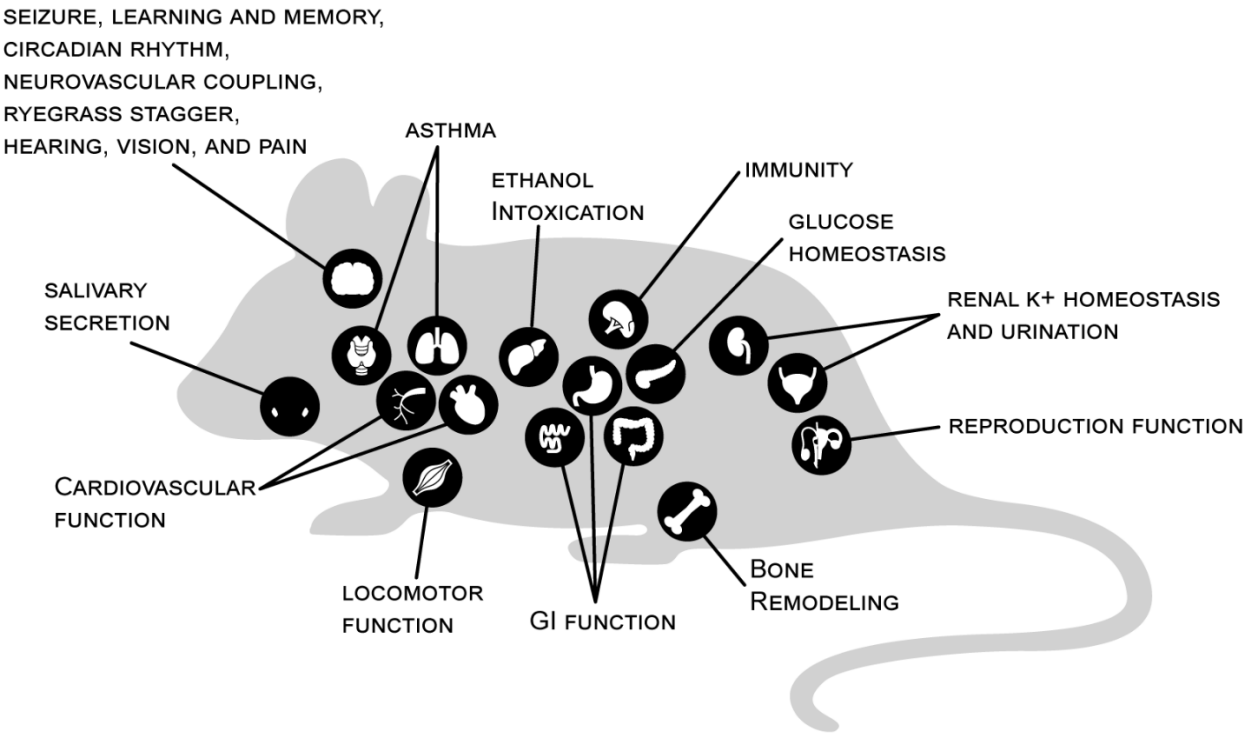

Figure 3 – BK channel dysfunction by organ and functional system in rodent models. BK channel knockout mice (*KCNMA1*$^{-/-}$) show a wider range of pathophysiology compared to human *KCNMA1* patients with LOF mutations. Such findings are consistent with the extensive distribution of BK channels across tissues. Key organ and functional systems disrupted by BK channel dysfunction in mouse models are indicated. BK channel activity was required for several aspects of neurological operations in rodent models, such as regulation of neuronal excitability (Jin et al., 2000;Faber and Sah, 2003;Brenner et al., 2005;Shruti et al., 2008;Sheehan et al., 2009), locomotor function (Meredith et al., 2004;Sausbier et al., 2004;Chen et al., 2010), circadian rhythm (Meredith et al., 2006;Kent and Meredith, 2008;Montgomery et al., 2013;White et al., 2014;Whitt et al., 2016), learning and memory (Typlt et al., 2013b), vision (Henne and Jeserich, 2004;Grimes et al., 2009;Tanimoto et al., 2012), hearing and vestibular reflexes (Pyott et al., 2007;Maison et al., 2013;Rohmann et al., 2015;Pyott and Duncan, 2016;Nelson et al., 2017), and neurovascular coupling (Filosa et al., 2006;Girouard et al., 2010). In addition to neurological roles, rodent models further revealed that BK channels are required for regulation of cardiovascular function (Sausbier et al., 2005;Imlach et al., 2010;Lai et al., 2014;Nagaraj et al., 2016), airway control (Sausbier et al., 2007;Goldklang et al., 2013;Manzanares et al., 2014), urination (Meredith et al., 2004;Thorneloe et al., 2005;Brown et al., 2008;Sprossmann et al., 2009), glucose homeostasis (Houamed et al., 2010;Dufer et al., 2011), renal K^+ homeostasis (Liu et al., 2007;Rieg et al., 2007), reproductive function (Werner et al., 2005;Werner et al., 2008;Li et al., 2014), ethanol intoxication (Martin et al., 2004;Pietrzykowski et al., 2008), gastrointestinal function (Sausbier et al., 2006a;Sorensen et al., 2008), body weight (Halm et al., 2017), pain (Hayashi et al., 2016), immunity (Essin et al., 2009), bone remodeling (Sausbier et al., 2011;Hei et al., 2016), and salivary secretion (Maruyama et al., 1983;Stummann et al., 2003).

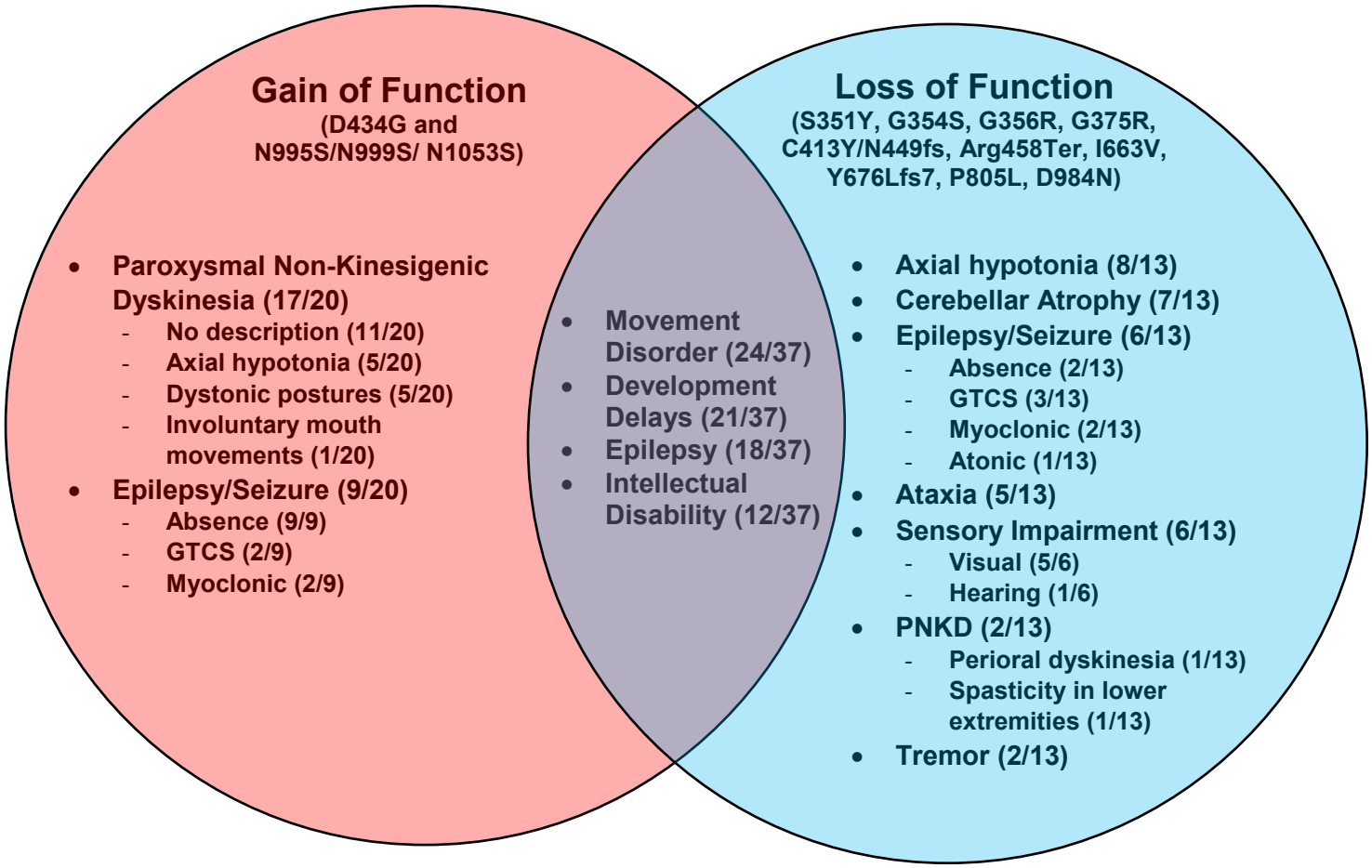

Figure 4 – Patient Phenotypes for Gain-of-Function versus Loss-of-Function *KCNMA1* Alleles. Summary of the main phenotypes exhibited by KCNMA1-linked channelopathy patients: seizure, movement disorder, neurodevelopment, and intellectual disability. The denominator is the total number of patients included in each mutation group, and the numerator is the number of patients reported with the particular phenotype. The intersection includes shared symptoms among all patients (n=37) identified with any *KCNMA1* mutation (n=16) reported in this review. Additional subtype descriptors for PNKD, epilepsy, and sensory impairment are annotated. 11/ 20 of GOF patients had no additional description for their PNKD symptoms in the published reports.

Supplemental Table 1. Missense SNPs and Mutations in the *KCNMA1* gene reported by population sequencing databases.

Database	Missense SNPs	Mutations
ClinVar	46	**7/16** - G354S, G375R (rs1554829003), D434G (rs137853333), Y676Lfs*7 (rs762705295), E884K (rs1554966197), N995S/N999S/N1053S (rs886039469), N1159S (rs563967757)
ExAC	253	**2/16** - Y676Lfs*7 (rs762705295), N1159S (rs563967757)
gnomAD	311	**7/16** - D434G (rs137853333), K518N (rs770007121), E656A (rs149000684), Y676Lfs*7 (rs762705295), E884K (rs1554966197), N995S/N999S/N1053S (rs886039469), N1159S (rs563967757)

The number of missense SNPs and mutations in the *KCNMA1* gene reported by 3 population sequencing databases differs (Lawson, 2000;Lek et al., 2016;Landrum et al., 2018). The denominator of 16 in the mutation column reflects the total number of mutations identified in symptomatic patients reported in this review. No Reference SNP cluster ID was reported for G354S. *ClinVar* (https://www.ncbi.nlm.nih.gov/clinvar/); *ExAC* Exome Aggregation Consortium (http://exac.broadinstitute.org); *gnomAD* Genome Aggregation Database (https://gnomad.broadinstitute.org). The authors acknowledge the Genome Aggregation Database (gnomAD), Exome Aggregation Consortium, and the groups that provided exome and genome variant data to these resources. A full list of contributing groups can be found at: https://gnomad.broadinstitute.org/about and http://exac.broadinstitute.org/about.

Supplemental Table 2. Neurological gene panels which include the *KCNMA1* gene.

Gene Panel Name and Lab	Genes	Methods
All Neuro panel Centogene AG - the Rare Disease Company Germany	1205	Sequence analysis of the entire coding region
Generalized epilepsy and paroxysmal dyskinesia Centogene AG - the Rare Disease Company Germany	1	Deletion/duplication analysis Sequence analysis of the entire coding region
Childhood Epilepsy Amplexa Genetics, Amplexa Genetics A/S Denmark	125	Mutation scanning of the entire coding region
Epilepsy, Intellectual Disability, and Autism Spectrum Disorder Amplexa Genetics, Amplexa Genetics A/S Denmark	569	Mutation scanning of the entire coding region
Epilepsy and Seizure Plus Sequencing Panel with CNV Detection Prevention Genetics United States	222	Deletion/duplication analysis Sequence analysis of the entire coding region Targeted variant analysis
Childhood Epilepsy NGS Panel Fulgent Genetics United States	209	Deletion/duplication analysis Sequence analysis of the entire coding region
Neonatal Epilepsy NGS Panel Fulgent Genetics United States	275	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy Advanced Sequencing and CNV Evaluation Athena Diagnostics Inc United States	234	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy Advanced Sequencing and CNV Evaluation - Generalized, Absence, Focal, Febrile and Myoclonic Epilepsies Athena Diagnostics Inc United States	84	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy and Seizure Disorders: Deletion/Duplication Panel EGL Genetic Diagnostics Eurofins Clinical Diagnostics United States	107	Deletion/duplication analysis
Neurogenetic Disorders - panels MGZ Medical Genetics Center Germany	597	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy/Seizure Knight Diagnostic Laboratories - Molecular Diagnostic Center Oregon Health & Science University United States	98	Sequence analysis of the entire coding region
Epilepsy Asper Biogene Asper Biogene LLC Estonia	175	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy Comprehensive NGS Panel Fulgent Genetics United States	398	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy Hereditary Panel GENETAQ Molecular Genetics Centre and Diagnosis of Rare Diseases Spain	37	Sequence analysis of the entire coding region

Dystonia Asper Biogene Asper Biogene LLC Estonia	38	Deletion/duplication analysis Sequence analysis of the entire coding region
Autism Spectrum Disorders 53-Gene Panel Center for Human Genetics, Inc United States	53	Sequence analysis of the entire coding region
Dystonia (NGS panel for 43 genes) CGC Genetics Portugal	43	Sequence analysis of the entire coding region
Single gene testing KCNMA1 CeGaT GmbH Germany	1	Sequence analysis of the entire coding region
Generalized epilepsy and paroxysmal dyskinesia (sequence analysis of KCNMA1 gene) CGC Genetics Portugal	1	Sequence analysis of the entire coding region
Dystonia All Panel CeGaT GmbH Germany	54	Sequence analysis of the entire coding region
Paroxysmal Movement Disorders Panel CeGaT GmbH Germany	4	Sequence analysis of the entire coding region
Paroxysmal Dyskinesia Panel CeGaT GmbH Germany	6	Sequence analysis of the entire coding region
Idiopathic Generalized and Focal Epilepsy Panel CeGaT GmbH Germany	40	Sequence analysis of the entire coding region
KCNMA1 Single Gene Fulgent Genetics United States	1	Deletion/duplication analysis Sequence analysis of the entire coding region
Neurology: Sequencing Panel EGL Genetic Diagnostics Eurofins Clinical Diagnostics United States	164	Sequence analysis of the entire coding region
Epilepsy and Seizure Disorders: Sequencing Panel EGL Genetic Diagnostics Eurofins Clinical Diagnostics United States	110	Sequence analysis of the entire coding region
Clinical Exome Fulgent Genetics United States	4673	Deletion/duplication analysis Sequence analysis of the entire coding region
Epilepsy with paroxysmal disorders panel Genome Diagnostics Laboratory University Medical Center Utrecht Netherlands	5	Sequence analysis of select exons Sequence analysis of the entire coding region

The 29 gene panels were identified using NCBI Genetic Testing Registry (GTR) database (https://www.ncbi.nlm.nih.gov/gtr/).