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Genetic Potassium Channel-Associated Epilepsies: Clinical Review of the K_v Family

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Abstract

Next-generation sequencing has enhanced discovery of many disease-associated genes in previously unexplained epilepsies, mainly in developmental and epileptic encephalopathies and familial epilepsies. We now classify these disorders according to the underlying molecular pathways, which encompass a diverse array of cellular and sub-cellular compartments/signalling processes including voltage-gated ion-channel defects. With the aim to develop and increase the use of precision medicine therapies, understanding the pathogenic mechanisms and consequences of disease-causing variants has gained major relevance in clinical care. The super-family of voltage-gated potassium channels is the largest and most diverse family among the ion channels, encompassing approximately 80 genes. Key potassium channelopathies include those affecting the K_V, K_{Ca} and K_{ir} families, a significant proportion of which have been implicated in neurological disease. As for other ion channel disorders, different pathogenic variants within any individual voltage-gated potassium channel gene tend to affect channel protein function differently, causing heterogeneous clinical phenotypes. The focus of this review is to summarise recent clinical developments regarding the key voltage-gated potassium (K_V) family-related epilepsies, which now encompasses approximately 12 established disease-associated genes, from the *KCNA*-, *KCNB*-, *KCNC*-, *KCND*-, *KCNV*-, *KCNQ*- and *KCNH*-subfamilies.

1. INTRODUCTION

Next-generation sequencing (NGS) has enhanced discovery of many disease-associated genes in previously unexplained epilepsies including the familial epilepsies and developmental and epileptic encephalopathies (DEE). We now classify these disorders more precisely depending on the underlying molecular pathways affected[1]. Our ability to identify pathogenic variants in epilepsy-associated genes has potentiated our understanding of gene-specific genotype-phenotype correlations. This is however complicated by the observation that even within the same gene, different pathogenic variants may affect protein function differently, causing heterogeneous clinical phenotypes. The explosion in genomic discovery challenges us to improve our understanding of the underlying molecular mechanisms in an effort to develop meaningful therapies.

One major group of epileptic disorders includes those due to cell-surface ion-channel defects (channelopathies), disrupting a highly diverse array of distinct albeit sometimes overlapping cellular and sub-cellular signalling processes controlling neuronal excitation and inhibition. As the molecular basis of the presumed genetic epilepsies is still being unravelled, the proportion of monogenic ion-channel related epilepsy is unknown, though recently estimated at ~25%[2]. Within the group of ion channels exists a superfamily of sodium, potassium (K^+) and calcium voltage-gated cation channels. The voltage-gated potassium channel superfamily is a large, diverse group of proteins encoded by approximately 80 primary genes and are of major relevance to genetic epilepsy. This article aims to summarise recent clinical developments in the so called 'monogenic' ion channel related epilepsies focusing on the specific voltage-gated K^+ channel (K_v) family. The other K^+ channel families (the K_{Ca}, K_{ir} and K_2P families) are not discussed, though referenced in excellent recent reviews[2-12]. Literature regarding the cellular basis of hyperexcitability in K_v-related disorders and the existing disease models is expansive, and not discussed herein.

2. VOLTAGE-GATED K^+ CHANNELS

K^+ channels are the largest, and most diverse family of the voltage-gated cation super-family[13]. Compared to sodium and calcium channels, whose basic roles are to initiate and conduct action potentials and release calcium in presynaptic nerve terminals, K^+ channels, displaying a myriad of different types and roles, basically 'keep neuronal excitation within limits', 'shape' and function to control action potentials and excitability properties of neurons[14,15]. These roles are achieved through many different types of voltage-gated-like K^+ channels, traditionally divided into 4 larger

families (Figure 1A) broadly based on functional characteristics (i.e. voltage activation across the membrane):

1) K_v family (voltage-gated), the focus of this review,
2) K_{Ca} family (calcium-activated),
3) K_{ir} family (inward rectifier), and
4) K_{2P} (two-pore), not implicated in human epilepsy so far.

Within these families, K^+ channel sub-families are distinguished, based on amino-acid sequence similarities of the protein core (Figure 1A)[13,16].

2.1 Structure & Diversity

K^+ channels are transmembrane (TM) spanning proteins made up of α-subunits[10,16]. Each α-subunit contains a homologous pore (P) segment selective for K^+ ions and gating mechanisms, and other domains that allow responsiveness to diverse stimuli (Figure 1B)[4]. There are approximately 80 human genes encoding different K^+ channel α-subunits. The α-subunit structure of the *K_v family* is 6 TM spanning, and assembly of 4 α-subunits (tetramerisation) leads to the pore-forming K^+ channel. α-subunit combination may be composed of identical (homomeric) or different (heteromeric) α-subunits, the latter of which tends to occur within closely related sub-families[10,17]. Further molecular diversity is brought about by different biophysical and pharmacological properties of α- as well as auxiliary β-subunits (gene encoded cytoplasmic proteins important for co-functions such as assembly, trafficking and inactivation). Each K_v channel has particular cellular (neuronal type) and subcellular (neuronal membrane site) distributions and expression patterns, as well as membrane signalling protein/molecular interactions[10] e.g. Figure 1B, cartoon structure and interacting proteins for a K_v7.2 (*KCNQ2*) α-subunit.

2.2 Ion channel: in-vitro functional screening

Pathogenic gene variants often affect highly conserved regions of the channel subunit, and multiple *in silico* analysis tools help in determining the likelihood of the pathogenic effect of a variant. However, experimental evidence of a deleterious effect is the most powerful criterion in scoring variant pathogenicity[18,19]. For variants in ion-channel genes, this can be well determined by examining the gating properties of mutant versus wildtype (WT)/non-mutant channels, traditionally using *in vitro* electrophysiological studies in non-neuronal heterologous expression systems, such as two-microelectrode voltage-clamping in *Xenopus* Oocytes or patch-clamping in CHO or HEK cells[18]. These models are usually the first step to understanding the molecular

dysfunction of a disease-causing variant without disturbance of many other channel subtypes in a neuronal expression system. A pathogenic variant may exert one or more effects on the assembly, physiology or biophysical properties of the subunit/channel (e.g. affecting voltage-dependence of activation or inactivation, kinetics of channel gating) which ultimately leads to loss-of-function (LOF) or gain-of-function (GOF) effects. A mutant protein (subunit) may also affect WT co-assembled channels, significantly impairing channel function, by a dominant-negative effect (DNE). Ultimately, it is essential to understand the consequences of a variant in functional neuronal and network models, particularly given that many K_v channels are expressed in both inhibitory and excitatory neurons.

3. THE K_v FAMILY AND ASSOCIATED EPILEPSIES

The *K_v family* are the largest K^+ channel family, and unsurprisingly most monogenetic K^+ channel epilepsies are found in this group. Within the K_v family, there are 12 subfamilies (K_v1-12), composed of approximately 40 individual α-subunits (genes), 12 of which are strongly implicated in human monogenic epilepsy. *KCNQ2*-related (K_v7.2) epilepsies represent the longest studied disorders of this group, and most variants have been identified in this gene to date. More recently, pathogenic variants and further disease associated genes in other members of the K_v family have been implicated in human epilepsy from the *KCNA-*, *KCNB-*, *KCNC-*, *KCND-*, other *KCNQ-*, *KCNV* and *KCNH* K_v sub-families (Table 1 & Figure 1). Most pathogenic variants are dominantly inherited and arise *de novo* (heterozygous) in severe phenotypes.

Pathogenic variants in two genes in the K_v family, *KCNQ1* (K_v7.1) and *KCNH2* (K_v11.1) lead to inherited cardiac ion-channelopathies, the long QT syndromes (LQT1 and LQT2 respectively), often with living dominant pedigrees. A relatively small number of case reports of patients with LQT1 and LQT2, associated with common (focal or generalised) epilepsy phenotypes (non-DEE) have been published. *KCNAB1* (K_vβ1) and *KCNAB2* (K_vβ2) auxiliary β-subunits (which associate with the *KCNA*/K_v1-related subfamily), have also been linked to epilepsy[20,11,21]. These LQT, and K_vβ-subunit genes have not emerged in large epilepsy NGS sequencing studies performed to date. Also until further clinical, genetic and functional (e.g. neuronal) studies demonstrate more concrete association, we do not consider them as established causative/pathogenic K_v associated-epilepsy genes. *KCND1* (K_v4.1) and *KCNH3* (K_v12.2) variants described in a single patient each with epilepsy also require further investigation.

[Insert Figure 1]

3.1 KCNA-related epilepsies

Within the K_v1 subfamily, *KCNA1* ($K_v1.1$) and *KCNA2* ($K_v1.2$) are both implicated in epilepsy and DEE.

3.1.1 KCNA1-related pharmacoresponsive seizures

Pathogenic heterozygous *KCNA1* ($K_v1.1$) variants can cause episodic ataxia type 1 (EA1), other associated neurological manifestations (e.g. myokymia), and less commonly described epilepsy. The epilepsy in initial published cases was often pharmacoresponsive and cognition was normal (though not exclusively)[22]. Zuberi *et al*. (1999) identified EA1 with pharmacoresponsive focal epilepsy in some family members carrying the variant p.Thr226Arg (affecting the S2 segment) exhibiting a LOF with DNE[23]. Three members of a different family with peripheral nerve hyperexcitability and pharmacoresponsive epilepsy (GTC and focal seizures) were found to harbour the LOF variant p.Ala242Pro affecting the S2 segment (but no DNE)[24]. A family with coexistent paroxysmal kinesigenic dyskinesia, pharmacoresponsive (oxcarbazepine) seizures and migraine carrying the p.Leu319Arg variant (affecting the S4-S5 linker) causing LOF (with DNE), has also been reported[25]. Recently, *KCNA1*-DEE phenotypes have also emerged, described in the next paragraph[26].

3.1.2 KCNA1-related DEE

Four patients were recently described with *KCNA1*-related DEE, including infantile-onset epilepsy (generalised and focal seizures, mostly pharmacoresistant), and ataxia in some, harbouring *de novo* variants involving the S6 $K_v1.1$ Pro-Val-Pro (PVP) motif[26]. One patient (p.Pro405Leu variant, affecting the last P of the PVP) had seizures successfully treated with acetazolamide. The variant p.Pro403Ser affecting the initial PVP proline substitution was identified in two twins, corresponding to an identical location of the *KCNA2*-related DEE variant (p.Pro405Leu) which clearly show a LOF with DNE[27] (*see below 3.1.3*) suggesting this variant also causes a LOF. A *KCNA1* variant near the PVP motif (p.Val408Leu) was also associated with cognitive impairment and early-onset epilepsy in three family members[28]. Recently, a *KCNA1* variant affecting amino-acid 408 changed channel opening, closing, and inactivation by altering RNA duplex structure, a novel post-translational modification effect[29]. A neonate of consanguineous parents with profound DEE was found to harbour a

missense homozygous/recessive *KCNA1* variant p.Val368Leu (affecting the pore domain) demonstrating strong LOF effects. Seizures became pharmacoresistant (initially tonic-clonic) despite some benefit from oxcarbazepine. Heterozygous mutant channel studies and parents (heterozygous carriers) were unaffected[30].

3.1.3 KCNA2-related DEE

In 2015, *KCNA2* heterozygous *de novo* variants (mostly missense) were first described causing DEE[27,31,32]. The phenotypic hallmarks of almost all patients thus far described with *KCNA2*-DEE is early onset seizures (first 1-2 years) with and without fever sensitivity, significant neurodevelopmental impairment (cognitive, behavioural) and cerebellar signs, of variable severity[27,32-35]. *KCNA2*-DEE variant functional defects, when initially described,[27,31] were categorized as leading to either (i) pure-LOF (correlating with milder clinical phenotypes with febrile seizures, focal seizures and EEG abnormalities including ESES-like phenomena, no cerebellar atrophy), (ii) pure GOF (more severe clinical phenotypes, generalized seizures and EEG abnormalities, severe ataxia with cerebellar atrophy), or (iii) mixed "GOF+LOF" effects (featuring elements from both other phenotypes and including the most severe clinical phenotypes)(Table 1). Of 23 patients, three recurrent variants represented over 60% of *KCNA2*-related DEE, one occurring in each functional group (Table 1) and affecting a different region of $K_v1.2$ (mostly but not exclusively across the S4-S6 domains)[31]. Some variants within functional groups presented with very similar phenotypes e.g. p.Thr374Ala (GOF+LOF group). For others e.g. the LOF group due to p.Pro405Leu, while broadly homogenous, could have differences in severity (some of this individual variation may be related to depth of phenotyping, and/or age of assessment). Phenotypic variation is likely to expand for some of these variants, but may potentially remain similar for others. Confoglia *et al*. (2019), described a 22 year-old with prominent and worsening action-myoclonus (suggesting progressive myoclonus epilepsy), a variation of the pure-GOF group due to variant p.Arg297Gln[36].

KCNA2-genotype and phenotype continues to expand, recently associated with two new non-DEE, but distinct phenotypes associating with specific LOF variants. One variant occurred in a family with episodic ataxia and pharmacoresponsive epilepsy in 6 of 7 members (p.255_257del affecting S3)[37]. Hereditary spastic paraplegia plus was described due to missense variant p.Arg294His (affecting S4), without epilepsy[38,39].

3.1.4 KCNA2-DEE: Treatments

Treatment with acetazolamide partially rescued motor incoordination in a mutant *kcna2* mouse[40]. Pena *et al.* treated a patient with acetazolamide, leading to clinical improvement in ataxia and seizures, but the effect on neurodevelopment was not described. The improvement was maintained following drug discontinuation[32]. The epilepsy in *KCNA2*-LOF tends to be pharmacoresponsive compared to the GOF group and in most patients, seizures were treated by conventional pharmacological approaches. A tendency toward ESES underscores the importance of diagnosis of this *KCNA2*-DEE variant LOF effect (p.Pro405Leu)[31,41]. K_v1.2 channel blockers may have a clinical benefit in the future, as they have been used already in the GOF group with success (*personal experience of some of the authors in N-of-1 trials in single patients at different sites*).

3.2. KCNB1-related epilepsy

A member of the K_v2 sub-family, *KCNB1* (K_V2.1)-related epilepsy was initially described in children (two with DEE and one with generalised seizures)[42]. To date over 70 patients have been described, all with neurodevelopmental impairment, and most with epilepsy [33,42-45]. Presentation is often in the first years of life, with preceding developmental delay, and pharmacoresistant epilepsy (~85%). Seizures are mainly motor, but also non-motor, and reflex-triggered, and epilepsies have multifocal or generalised patterns, including a broad range of electro-clinical syndromes e.g. infantile spasms, Jeavons syndrome, etc. Epileptiform discharges on EEG vary but are often severely abnormal, ~25% with prominent sleep abnormalities e.g. ESES. Most patients ambulate, but motor problems (some severe, with limited ambulation) and severe language and behavioural problems are common. 50% have autism and some have ataxia, extrapyramidal (15%) or pyramidal (20%) signs[44].

KCNB1 genotype-phenotype correlations have been difficult to determine thus far. Most variants are *de novo*, though somatic mosaicism can occur[46]. Variants are mainly missense (75-79%) affecting S4 to S6 regions, but less often truncating which usually (not exclusively) affect the C-terminal *Kv2* region; the latter were less likely to have epilepsy or had pharmaco-responsive infantile spasms[44]. Severity (including seizures and ambulation) may correlate with variants concentrated across the most common hotspots S4, S5 and P-loop[44,45], though not all of these develop epilepsy[44]. Some variants are recurrent (e.g. p.Arg306Cys/p.Arg312Cys/p.Arg312His affecting arginines in the S4 segment) and seizure phenotypes can vary with the same recurrent variant (e.g. p.Arg312His)[44].

KCNB1 pathogenic variants usually lead to complete/partial channel LOF

(including DNEs)[18,42,45]. A recent high-throughput pipeline (electroporation and automated planar patch clamp) demonstrated rapid characterisation of numerous variants. Combined with high-throughput immunocytochemistry-flow-cytometry assays (evaluating cell-surface and total protein expression) delineated LOF mechanisms by a) decreased K^+ conductance, b) altered voltage-dependence, c) reduced protein expression and d) altered cell surface trafficking[18].

3.3 KCNC1-related epilepsies

Of the K_v3 subfamily, *KCNC1* ($K_v3.1$) pathogenic variants cause human epilepsy. *KCNC3*-pathogenic variants cause spinocerebellar ataxia (SCA) type 13 (not epilepsy). *KCNC2* and *KCNC4* have not yet been confirmed in human neurological disorders[47,48]. As discussed below, two recurring *KCNC1* LOF variants p.Arg320His and p.Ala421Val, appear to suggest variant-specific phenotypes.

3.3.1 KCNC1-related phenotypes

In 2015, a recurrent *KCNC1* ($K_v3.1$) heterozygous LOF variant (p.Arg320His) affecting S4 (causing a DNE) was identified in patients (ages 3 to 15 years) with severe progressive myoclonus epilepsy (PME) and ataxia (termed MEAK)[47]. Patients were often wheelchair-bound by teenage years, had mild cognitive difficulties (~50%), generalised EEG abnormalities, occasional tonic-clonic seizures, photosensitivity, pharmacodependence, and progressive cerebellar atrophy[47,49]. Unexpectedly, clinical improvement with fever was observed in 6 patients (and during pregnancy in two)[47,49]. *In vitro*, high temperature experiments showed increasing WT channel availability, explaining some of the fever-induced clinical improvement.

Subsequently, Cameron *et al*. (2019) identified 9 patients with other pathogenic *KCNC1* variants (non-MEAK phenotypes)[50], six with the recurrent *de novo* LOF variant p.Ala421Val (without detecting a DNE) located in the S6 segment, all presenting with infantile seizures (mainly myoclonic) either pharmaco-resistant or pharmaco-responsive (one improved with clobazam, valproate, stiripentol), moderate to severe intellectual disability (ID), ataxia in some and dysmorphism. In a parallel study, Park *et al*. (2019) identified three pathogenic *KCNC1* missense variants in five individuals with different phenotypes including (i) epilepsy with myoclonic-absences, GTCS, ataxia, and developmental delay with the variant p.Ala421Val (three patients)[51] – the phenotype overlaps with that described by Cameron *et al*. (2019) with the same variant but functional analysis demonstrated a DNE, (ii) isolated non-progressive myoclonus-tremor with the

LOF variant p.Cys208Tyr affecting the S1 segment (predicting haploinsufficiency), and (iii) ID with the LOF variant p.Thr399Met (S5-S6 pore loop) showing a DNE.

Finally, a truncating (p.Arg339*) variant (S4-S5 loop) was also found in a family of three individuals with ID (no epilepsy)[52].

3.3.2 KCNC1-related epilepsy: Treatment

Two patients carrying the *KCNC1* p.Ala421Val variant showed seizure improvement with benzodiazepines (e.g. clobazam)[50,51]. Clonazepam with valproate was most effective in MEAK patients with epilepsy[49]. Small molecule K_v3 modulators (e.g. RE01) were recently shown to enhance both WT and mutated $K_v3.1$ channel open-probability with a concomitant shift in the voltage-dependence of activation. Directly activating K_v3 modulators constitute plausible rescue treatments in MEAK and other *KCNC1*-related phenotypes[53].

3.4 KCND-related epilepsies

The K_v4 sub-family consists of 3 members, *KCND1-3* ($K_v4.1$-4.3). *KCND2 and KCND3* are strongly implicated in human epilepsy[54-56].

3.4.1 KCND2

A *de novo* pathogenic *KNCD2* ($K_v4.2$) variant p.Val404Met (showing GOF dominate effect) affecting the S6 segment (immediately after the PVP motif) was identified in twins with infantile-onset refractory myoclonic seizures (hundreds/day), ASD and severe developmental impairment[54]. Non-motor and GTC seizures developed, decreasing by age 10 years. EEG showed generalised polyspike-wave. A truncating LOF variant p.Asn587fsX1 affecting the C-terminal region was identified in a patient with temporal lobe epilepsy, showing attenuated K^+ current density[57].

3.4.2 KCND3

KCND3 ($K_V4.3$) variants inducing a LOF cause SCA 19/22, and rarely occurring GOF-inducing variants were found in Brugada syndrome/atrial fibrillation. Huin *et al.* (2017) described 2 families with SCA 19/22, cognitive difficulties, Parkinsonism (eight individuals), and five individuals with epilepsy (various seizure types) due to an in-frame deletion in *KCND3* (p.Phe227del) affecting the S2 segment[58]. Mean age of epilepsy onset was 5 years (after ataxia) with a mix of GTC, focal, atonic or myoclonic seizures. EEG showed focal discharges. p.Phe227del, functional studies suggest a LOF (defective trafficking and cell surface expression).

Another boy with a *de novo KCND3* duplication (p.Arg293_Phe295dup) displayed an early-onset severe phenotype (cerebellar, motor and attention impairment) and epilepsy[59]. Frequent nocturnal jerks, staring episodes, and mild generalized seizures developed (responding to valproate), with eventual seizure remission. The variant affects the RVF motif (adding a positive charge to the voltage sensor domain) causing a severe shift of the voltage-dependence of activation towards more depolarized voltages.

A *de novo* pathogenic *KCND3* variant (p.Val392Ile) was identified in an 18-month old with seizures (tonic and focal or generalised clonic, often fever-triggered) necessitating polypharmacy, and later developmental regression[60]. The variant was previously implicated in sudden adult death; functional analysis showed increased current density and slowed inactivation.

Thus far, specific therapies have not been identified for *KCND*-related epilepsies.

3.5 KCNQ-related epilepsies

The K_v7 (*KCNQ*) subfamily consists of 5 members *KCNQ1-5* ($K_v7.1$-$K_v7.5$). Pathogenic variants in the genes *KCNQ2*, *KCNQ3* and *KCNQ5* have been identified in a range of epilepsies. $K_v7.2$ α-subunits or $K_v7.5$ α-subunits can form heterotetrameric channels with $K_v7.3$ (Figure 1B).

3.5.1 KCNQ2/3-related BFNE

Pathogenic *KCNQ2* and *KCNQ3* variants exemplify how different variants in the same gene can result in contrasting epilepsy phenotypes, due to differences in the functional properties of mutant $K_v7.2/K_v7.3$ channels[61-64]. In 1998, autosomal dominant inherited *KCNQ2* and *KCNQ3* variants were described in infants with *benign familial neonatal epilepsy* (BFNE)[65]. The neonatal seizures usually remit spontaneously within weeks or months, although later offset and seizure recurrence may occur[64,66]. EEG does not demonstrate severe epileptiform discharges and interictal background is normal, as is neuroimaging and subsequent development. A history of neonatal seizures in a parent should lead to suspicion of this self-limiting disorder. Most BFNE families carry pathogenic *KCNQ2* variants, while *KCNQ3* variants are reported much less commonly. Rarely seizures have a later infantile-onset[64,67-69].

BFNE is known to be caused by *KCNQ2* variants leading to haploinsufficiency, mostly as loss of start codon, stop codon, frameshift and splice variants, deletions, but also certain missense variants tending to localise at the intracellular domain between S2 and S3 segments[70]. Mild to moderate channel LOF (i.e. M-current reduction) occurs in BFNE[61,71].

3.5.2. KCNQ2-related neonatal DEE

Other *KCNQ2* variants can also cause neonatal-onset *KCNQ2*-related encephalopathy or DEE, (likely the commonest cause of genetic neonatal EE)(www.RIKEE.org)[72,73]. This phenotype is characterised by intractable, usually prominent focal tonic, neonatal seizures with onset in the first days of life. EEG shows burst-suppression (>60%)[74] or multifocal epileptiform abnormalities with background attenuation. Acute (neonatal) MRI signal abnormalities of the basal ganglia or thalamus occur in some cases, with later atrophic changes seen. Seizures lessen over time, but may persist or relapse, sometimes in clusters, or as infantile spasms. Adverse neurodevelopmental outcome occurs in all, ranging from moderate to profound ID, despite seizure remission or reduction[61,63,73-77]. Milder phenotypes are seen in probands with mosaicism[78,79]. Most *KCNQ2*-related DEE variants occur *de novo*, but parental mosaicism (sometimes with no or milder BFNE-like phenotypes) can occur[61,70,73].

In contrast to BFNE, *KCNQ2*-DEE mostly results from *de novo* missense variants in particular "hot-spot" zones of $K_v7.2$ including (i) the S4 voltage sensor, (ii) pore, (iii) proximal C-terminus domain that binds phosphatidylinositol 4,5-bisphosphate and calmodulin A, and (iv) the distal domain which binds calmodulin B[74] (Figure 1B), an observation recently confirmed by Goto *et al* (2019) for a large number of *KCNQ2*-DEE missense variants[70]. *KCNQ2*-DEE variants often lead to a DNE and more severe LOF of M-current than seen in BFNE[61-63,73,75,76,80]. However, severe M-current reduction may not always be apparent when studied in non-neuronal *in vitro* models, and other mechanisms also affect channel dysfunction[81].

3.5.3 KCNQ3-biallelic inheritance with LOF

Albeit very rare, biallelic inheritance of *KCNQ3*-LOF variants leading to DEE has been described. One child with neonatal pharmacodependent seizures and non-syndromic ID carried a homozygous frameshift variant (p.Phe534Ilefs*15)[82]. Another child with severe DEE carried a compound heterozygous missense variant (p.Val359Leu/p.Asp542Asn)[83]. In both cases parents were asymptomatic.

3.5.4 KCNQ2-related GOF DEE

While LOF or DNE is the predominant mechanism in *KCNQ2/3*-related BFNE and DEE, certain *de novo* missense variants found in *KCNQ2*-related DEE[63,84] were recently shown to cause a GOF of the M-current[85]. Ten patients with GOF-*KCNQ2* variants (p.Arg201Cys and p.Arg201His)[78] affecting the S4 segment, presented with a novel phenotype; profound newborn encephalopathy without seizures, prominent startle response and

non-epileptic myoclonus, burst-suppression EEG, hypoventilation, later infantile spasms (70%), and often early death. One infant had heterotopia on neuroimaging.

Another *KCNQ2*-GOF variant (p.Arg198Gln) has been identified in 4 patients presenting without neonatal encephalopathy/seizures but developed infantile spasms at 4-6 months with subsequent developmental delay/DEE[86]. Similar findings were reported in a patient with the GOF variant p.Arg144Gln affecting the S2 segment[43,85]. A neonate with myoclonia, burst-suppression EEG, severe delay and early death harboured a further GOF variant (p.Val175Leu) affecting the S3 segment[87].

KCNQ2-GOF variants affect the voltage sensor domain (S2, S3 and S4)(Figure 1B) causing activation shifts to more hyperpolarized potentials (increased M-current), likely due to a series of complex amino acid-based electrostatic interactions affecting channel gating[85,86].The p.Arg201Cys variant with the more profound clinical phenotype displayed more severe GOF effects compared to p.Arg198Gln or p.Arg144Gln (later-onset infantile spasms phenotype).

3.5.5 KCNQ3-related GOF epilepsy

Recently, *de novo KCNQ3*-GOF variants have been described with a phenotype distinctly different and significantly more severe than *KCNQ3*-related BFNE[88]. Children presented with early global delay, severe language and ASD, few seizures, but often prominent EEG sleep-activated multifocal epileptiform discharges[88]. Most variants affect the two outermost arginines of the S4 segment, p.Arg230Cys/His/Ser and p.Arg227Gln, the corresponding location to *KCNQ2*-GOF DEE variants (p.Arg201Cys/His/Ser and p.Arg198Gln)[88].

3.5.6 Treatment

Seizures in *KCNQ2/3*-related BFNE self-remit spontaneously in approximately one third. Though supported by limited evidence (Level D) sodium channel blockers (carbamazepine/oxcarbazepine, phenytoin, lamotrigine) prove effective for the limited time-period of seizures. However initial choices will depend on whether one suspects the *KCNQ*-disorder at outset *vis-a-vis* empiric treatment of unexplained neonatal seizures. Where suspicion is strong or confirmed, carbamazepine/oxcarbazepine has been suggested[89,90]. In the initial acute neonatal presentation of LOF *KCNQ2*-DEE patients, treatment-resistant daily seizures often occur. Where seizure benefit was noted, sodium channel blockers, valproate and levetiracetam had benefits, though often other AEDs are also deployed given the gravity of the unexplained epileptic encephalopathy[74,89].

Retigabine specifically opens KCNQ-channels and has been shown to ameliorate (restore M-current) of particular LOF variants[62]. Limited numbers of infants/children with *KCNQ2*-DEE have been treated. In a series (age 2 months to 6 years), an improvement was noted in seizures and/or initial development in three of four children treated before 6 months, and two of the seven treated later. Not unexpectedly, retigabine possibly worsened one infant with a GOF variant[78,86]. Retigabine was tolerated relatively well in this series but market use has ceased. Recently, however, there has been proposals to clinically develop XEN496 (active ingredient ezogabine/retigabine) for formal efficacy studies in young children specifically with *KCNQ2*-related DEE. Further retigabine/K_v7 channel modulators (e.g. Xen1011) are also being validated in clinical studies. Many pre-clinical studies have identified novel compounds that modulate KCNQ channels, which may be more potent and selective. The hope is to treat *KCNQ2*-related DEE as early as possible, to preserve neurodevelopment, given the importance of $K_v7.2$ channels, and the severe impact that *KCNQ2*-DEE pathogenic variants may have, in the developing fetal-infant brain[91]. However, a strong DNE may pose problematic for KCNQ-channel activators, which may not have the ability to reverse the strong LOF in a clinically sufficient manner, at all or at least without causing side-effects. Antisense oligonucleotide therapies may have the potential to inhibit transcription or translation of an affected *KCNQ2* allele in *KCNQ2*-DEE, hoping to convert it to the haploinsufficiency genotype of the mild BFNE phenotypes[63].

3.5.7 KCNQ5-related epilepsy and DEE

Recently *KCNQ5 de novo* heterozygous missense variants have been found in 4 children with ID, two with treatment-resistant epilepsy (*KCNQ5*-related DEE)[92]. The variants in the S1 segment, the S6 segment and the cytoplasmic C-domain (p.Ser448Ile) showed LOF effects. One GOF variant (p.Pro369Arg) in the proximal C-terminus had a dramatic effect on the stability of $K_v7.5$ opening, and led to infantile spasms. In 2019, two studies presented in abstract form identified 4 missense C-terminus LOF variants associated with genetic generalised epilepsy in families (mainly childhood absences, juvenile myoclonic or adult-onset tonic-clonic seizures)[93], and also as a GOF variant in a child with early-onset epilepsy and ID (affecting the distal S6 segment)[94]. A *KCNQ5* intragenic duplication was identified in a patient with ID and adolescent absence epilepsy[95].

[Insert Table 1]

3.6 KCNH-related epilepsies

KCNH-genes (originally named *ether-a-go-go* or eag genes from a mutant voltage-dependent K^+ channel in Drosophila) include three subfamilies of two eag genes (*KCNH1* and *KCNH5*), three erg (i.e. eag-related) genes (*KCNH2*, *KCNH6*, *KCNH7*) and three elk (eag-like) genes (*KCNH8*, *KCNH3* and *KCNH4*). All channel subtypes are expressed in brain,[3] however, human epilepsies described most convincingly so far, occur due to *KCNH1* and *KCNH5* pathogenic variants (See section *3. The k_v Family and Associated Epilepsies,* paragraph 2).

3.6.1 KCNH1-related DEE

KCNH1 (eag1/hEAG1) encodes K_v10.1, in which *de novo* GOF variants lead to DEE, shown recently to cause Temple-Baraister syndrome [96] and Zimmermann-Laband syndrome[97], two clinically overlapping dysmorphic phenotypes characterised by seizures, nail hypoplasia, skeletal anomalies and ID (severe to profound). Subsequent reports have identified *KCNH1*-related DEE in patients with overlapping features[98-101], with now over 20 cases published. Development is often impaired prior to seizure-onset, which childhood-onset (including neonatal-infantile) of focal or generalised seizures.[100] Half required polytherapy and half monotherapy, and one worsened with rufinamide. No clear electro-clinical syndromes or genotype-phenotype correlations have emerged. Interestingly, two mosaic parents had epilepsy without DEE.

Some *KCNH1* variants are recurrent and most are distributed across K_v10.1 domains S4 to S6. The recurrent variant p.Ile494Val affecting the S6 domain highlights the functional importance of these domains. Functional characterisation so far shows GOF (due to a decreased threshold of activation and delayed deactivation)[96,97].

There has been a lack of specific inhibitors to study the eag subfamily[102]. Inadvertent *KCNH2* (HerG)(K_v11.1) blocking in cardiac tissue could lead to LQT (fatal arrhythmias). However, highly stable spider-venom peptides that potently and selectively inhibit *KCNH1* (eag/hEAG1)(e.g. Ap1a) over hERG/*KCNH2* were recently identified[102]. High throughput screening methods identifying compounds with such selectivity could enhance knowledge of neuronal/brain eag (*KCNH1*) function[3] and drug therapies (e.g. in *KCHN1*-GOF variants).

3.6.2 KCNH5-related DEE

A *de novo* pathogenic *KCNH5* (*eag2*/K_v10.2) variant (p.Arg327His) affecting the voltage sensor domain (S4) Arginine residue leading to GOF[103,104] was identified in infantile onset DEE. Seizures began at 6 months (tonic-clonic and hemiclonic, often prolonged/clusters), as well as an ESES-like disorder. Seizures became partially controlled. Severe

developmental impairment and regression occurred. *KCNH5* (K_v10.2) is expressed in excitatory (interneurons) and inhibitory neurons[105]. Like *KCNH1*, a role in synaptic and neuronal hyperexcitability through secondary effects is possible[8].

3.7 KCNV2-related epilepsies

Some subunits have been classified in the K_v sub-families possessing α-subunit characteristics, but behave *silently* as homomeric channels[6,106]. When co-assembling with other K_v subunits they can act as modifiers. In particular K_v8.2 (*KCNV2*) co-assembles with K_v2.1 as a heterotetramer, suppressing K_v2.1 current and surface expression. Homozygous *KCNV2* variants are associated with retinal cone dystrophy (OMIM *607604). Pathogenic variants identified in two unrelated children associated with epilepsy, one with focal seizures (p.Arg7Lys) and another with DEE (p.Met285Arg) showed a severe GOF effect[12,107].

4. SUMMARY AND OUTLOOK

4.1 K_v disease diversity

The K_v channel family and K_v channelopathies harbour vast physiological complexities and disease-spectra, respectively. In addition to previous work deciphering much of the known K_v physiology and confirmation of K_v7.2/K_v7.3 *KCNQ2*-BFNE in 1998, in recent years pathogenic variants in further K_v genes have been identified in association with a spectrum of human heritable epilepsies and neurodevelopmental phenotypes. We have focused on the clinical and genetic landscape of 12 disease-associated genes in the specific K_v family, in which pathogenic variants have highly plausible functional mechanisms.

When genetic testing identifies a predicted causative variant, careful consideration of variant (channel) functional characterisation has gained increased clinical relevance[74]. The pathogenic variants identified in K_v-associated epilepsies concentrate in highly conserved regions across the channel protein, and for some K_v epilepsies in particular hotspot zones, often spanning the voltage sensor or pore domains. For some K_v channelopathies, specific variants are associated with consistent electrophysiological and clinical phenotypes. For other variants, including the same gene or even same amino acid residue, different functional and clinical phenotypes may arise[73].

While the immense diversity described poses management challenges, technological advances in the functional characterisation of variants (e.g. electrophysiological, immunohistochemical, multi-state structural modelling of ion channel gating, etc), are increasingly establishing more precise and robust

mechanisms underlying K_v-channelopathy[108]. Recently deployed high-throughput techniques for a series of newly diagnosed *KCNB1*-affected patients, demonstrates the benefits of reference datasets for genetic test interpretation, and re-classification of variants of uncertain significance[18].

4.2 Therapeutic challenges and 'theranostics'

Given the rarity, but more so, individual patient-specific disease mechanisms for any particular K_v-associated epilepsy/DEE, it is not surprising that conventional anti-epileptic drug treatments appear to have a limited impact on developmental outcomes, as well as the overall evidence-base for seizures. Empiric treatment approaches to the epilepsies however, are highly important, and pharmacoresponsiveness (even if partial) may be achieved by also considering the underlying electro-clinical syndromes, as well as the molecular/cellular and pathway defects (e.g. corticosteroids/vigabatrin for infantile spasms, use of benzodiazepines in *KCNC1*-epilepsies, screening for and treating the presence of ESES in some disorders associated with some K_v epilepsies, sodium channel blockers in *KCNQ2*-LOF variants). While in some K_v-associated DEEs, seizure type and severity may be determined by developmental age, early diagnosis and treatment and appropriate treatment is required to have an impact on the overall outcome.

Much excitement, and sometimes "hype", has arisen with the realisation of genomic diagnostics and functional mechanisms underpinning K_v-associated genetic epilepsies, with potential for 'theranostic' approaches, including novel specific/selective ion-channel modulators, known or new drug library screening, drug repurposing, antisense oligonucleotides, and the potential of gene therapies, though meaningful therapies are largely not yet realised. However, when viewed through the lens of known genotype-phenotype correlations, and mechanism of disease, therapeutic options are beginning to emerge. The consideration of a K^+ channel activator such as a retigabine/retigabine-derivative (e.g XEN496) in the LOF *KCNQ2*-DEE group, or even avoiding it in the GOF group, may become more clearly defined strategies in the future. Likewise using a $K_v1.2$ blocker in the GOF-*KCNA2* DEE group, may be added to the armamentarium of pharmacotherapies for this sub-group of *KCNA2*-related epilepsies. Furthermore, parents or patients may wish to be aware of experimental trials for emerging therapies. This may be part of a multi-centre study design, or at local level, may require consideration of *N-of-1* trials.

4.3 Limitations and Future Goals

Despite advances in K_v physiology and recent K_v channelopathy identification, there remains the task of understanding them in complex neuronal networks, and in

mammalian, not at least the human brain. Mechanisms of neuronal excitability are better understood for some K_v members than others (e.g *KCNQ2* versus *KCNH5*), though not discussed in this review. Straightforward GOF versus LOF effects expressed in a single cell non-neuronal model, the current gold standard initial approach, may not necessarily reflect the neuronal levels. Many K_v channels demonstrate roles in both inhibitory and excitatory neurons, with potential to produce opposing effects. In neurons, other non-ion conducting functions/components of K_v channels may be altered by pathogenic variants which may be absent in non-neuronal cell models[18]. Genetic, epigenetic or environmental modifiers, may influence the mechanisms observed. Though not emphasised in this review, major approaches utilising recent advances in all experimental paradigms including *in-vitro* electrophysiological, animal (e.g. knock-in or knock-out mice, Zebrafish, Drosophilia, etc), and evolving human cell/tissue models (e.g. human induced pluripotent stem cell-derived neurons/organoids), collectively will help to further understand the complex molecular diversity of K_v-related channelopathies in neuronal networks[7].

While major therapeutic challenges remain, the community of health care professionals and scientists are now striving to achieve translational breakthroughs for K_v-related and other genetic DEEs. The importance of transnational networks, multidisciplinary gene-specific databases, and registries to hasten progress in phenotypic characterization, patient stratification, and individual tailoring of therapeutic approaches, as patient cohorts and gene variants increase, has recently been emphasised for *KCNQ2*-related DEE, with a GOF mechanism[86]. Given the importance of K_v channels in neuronal function, therapeutic breakthroughs will have translational effects for other epilepsies not just those considered monogenic.

Figure 1 Legend:
A) Voltage-gated ion-channel superfamily (phylogenetic tree reconstructions): channel relationships are based on structurally related ion-channel genes, amino-acid sequence relations of the minimal pore regions, and membrane topologies arranged in groups/families. Distinct branches are K^+ channels (red), Calcium (Ca_v) and sodium (Na_v) channels (blue), CNG and HCN channels (pink), and TRP and related channels (green)[modified with permission from Yu & Catterall (2004) and publishers [13]. The *K_v family* and subfamily members are expanded.
B) K_v7.2/7.3 hetero-tetrameric channel and K_v7.2 α-subunit cartoon: showing structure, interacting proteins and hot-spot regions for *KCNQ2*-DEE variants (red pink circles)[74]. α-subunits are composed of 6 transmembrane segments connected by five loops. S1-S4 is the voltage sensing domain (VSD) and S5-S6 plus a connecting loop (highly conserved sequence) forms the ion-selective pore. S4 has a series of positively charged (arginine) residues allowing K_v7.2 to change its opening probability in response to membrane potential changes. K_v channels share a similar core structure, but differences in connecting loops, length (N and C-terminals) and interacting proteins/molecules confer individual properties. K_v7 channels exhibit a large C-terminus [109], important for channel gating, assembly and trafficking through interaction with regulatory molecules. It contains 4 α-helices; helices A and B (*KCNQ2*-DEE variant 'hot-spots') contain calmodulin (CaM) binding and other sequences; helices D and C act as subunit interaction and assembly domains (sid)[9]. Other important regulatory

sites include phosphatidylinositol 4,5-bisphosphate (PIP2)[83], syntaxin, A-kinase-anchoring proteins (AKAPs), protein kinase C and Ankyrin-G. Physical and functional interaction between other non-channel molecules occur e.g. sodium-dependent myo-inositol transporters (SMITs)[109,110].

Table 1. Members of the K_v family (of the voltage-gated K^+ channel superfamily) implicated in heritable human epilepsies

K_v gene (channel subunit) Basic neuronal functions	**Epilepsy (and related) phenotypes**	**Examples of key functional defects and pathogenic variants:** amino-acid abbreviation[$] (subunit location)	**Individual therapeutic considerations** (excluding standard/empiric epilepsy treatment)
***KCNA1* (Kv1.1)** Functions: delayed rectifier current; delayed activation after the AP. Efficient neuronal repolarization. Limits firing frequency, reduces amplitude of the AP.[5]	Focal szs (pharmacoresponsive), EA1 (one cognitive impairment)	LOF (DNE): T226R (S2)[23]	Carbamazepine
	Pharmacoresponsive focal and generalised szs (with myokymia but not EA1)	LOF (no DNE): A242P (S2)[24]	Not specified
	Szs (including infancy), cognitive difficulty, myokymia	Increased rate and extent of inactivation: P408L (S6)[28]	Carbamazepine (one on phenytoin had cerebellar atrophy)
	PKD, some had responsive szs (impaired awareness), and migraine	LOF (DNE): L319R (S4-S5 link)[25]	Oxcarbazepine
	DEE	No functional studies: PVP motif (S6) P405L & P405S. P403S (S6)[26]	Acetazolamide: one (P405L) patient
	DEE (pharmacoresistant, movement disorder)	Homozygous LOF: V368L (S5-S6 linker)[30]	Oxcarbazepine, one patient seizure benefit
***KCNA2* (Kv1.2)** Functions: similar to Kv1.1	*Least severe DEE:* Szs: mainly focal, episodes of SE, some ESES/ESES-like, resolve mainly by 5-15y. Milder ataxia, no cerebellar atrophy in majority. Developmental impairment: milder than "pure GOF group"; some can have severe impairment	*"Pure LOF group"* (DNE) e.g. P405L (S6)[27,31,41]	The most pharmacoresposive *KCNA2*-DEE group. Consider testing for ESES, consider drugs also used in EA type 1
	Severe DEE: Szs generalised, but some also focal/ 23% become sz free/ pharmacodependence. Ataxia often debilitating. Marked cerebellar atrophy. Hypotonia, tremor, cognitive dysfunction severe, some psychiatric/ behavioural features.	*"Pure GOF group"* hyperpolarizing shift of voltage-dependent activation, and increased amplitude (channels permanently open) e.g. recurrent R297Q (S4)[27,31]	Consider Kv1.2 blockers, own unpublished experience by the authors who can be contacted
	Markedly severe DEE: Earliest onset e.g. uncontrolled neonatal szs. Focal> generalised szs/none sz free. Cerebellar atrophy earlier (childhood). Development: most severely impaired (e.g quadriplegia)	*"GOF & LOF" group:* an overall GOF effect a) smaller hyper-polarizing shifts of activation curve & ↓ current (T374A)(pore region) OR b) inactivation curves shifting to more hyperpolarized/negative e.g. L290R (S4)[31]	Consider Kv1.2 blocker, own unpublished experience by the authors who can be contacted
	Ataxia and myoclonic epilepsy (PME-like)	Pure-GOF: R297Q (S4)[36]	Consider Kv1.2 blocker, as above
	HSP (and ataxia, tremor, cognitive involvement) variable developmental outcomes. No szs.	LOF (DNE): recurrent variant R294H (S4)->proton current through gate pore, LOF less than DEE[38,39]	No szs
	EA, infantile and later pharmacoresponsive generalized (IGE) and focal szs. Normal development	LOF (DNE): one variant p.255_257del (S3)[37]	Pharmacoresponsive (sodium channel blockers and valproate mainly used but also other AEDs)
***KCNB1* (Kv2.1)** Functions: delayed rectifier current; delayed activation after the AP; repolarisation[5]	DEE, multifocal /generalised epilepsies/electro-clinical syndromes, 25% prominent sleep abnormalities	LOF: +/-DNEs (mainly S4-S6) some recurrent e.g. R306C (S4)[42,44]	Consider testing for ESES.
	Some less likely with epilepsy or had pharmacoresponsive infantile spasms	LOF: truncating: such variants (so far described) tend to affect the C-terminal *Kv2* region[42,44]	
***KCNC1* (Kv3.1)** Functions: A-type/transient current. Determinants of high-frequency AP firing via fast inactivation and activation	Progressive myoclonus epilepsy, myoclonic tonic-clonic, ataxia(MEAK), pharmacodependent	LOF (DNE): R320H recurrent (S4), variant specific phenotype[47,49]	Benzodiazepines (e.g. clonazepam). In MEAK, clinical improvement occurred with fever.
	DEE, early onset epilepsy (e.g. myoclonic absences)	LOF: A421V recurrent (S6) DNE in Park et al (2019)[51]; no DNE in Cameron et al (2019)[50]:	
	Non-epilepsy (DE)	LOF: DNEs R317H (S4), Q492X (C-terminus), T399M (S5-S6 pore loop)[50,51]; haploinsufficiency R339X (S4-S5 loop)[52]	
	Myoclonic tremor (normal otherwise)	LOF: C208Y (S1)[51]	
***KCND2* (Kv4.2)**	ASD with intractable epilepsy	GOF: V404M (S6 near PVP motif)[54]	-

Functions: A-type/transient current. Limits low-frequency firing/backpropagation of AP[111]	TLE	LOF: truncated N587fsX1 (C-terminal)[57]	-
***KCND3* (Kv4.3)** Function: similar for Kv4-subfamily	Epilepsy (mild) childhood, early onset cerebellar ataxia, intellectual disability (DEE)	LOF: R293_F295dup (RVF motif S4) severe shift voltage-dependence gating more depolarized[59]	During course valproate helped szs, but not development
	SCA19/22 + childhood epilepsy (5 individuals from 2 families), cognitive impairment, ataxia	LOF: F227del (defect trafficking, cell surface expression)[58]	-
	Severe epilepsy, developmental regression (DEE)	GOF: V392I (S6)[60,112]	-
***KCNQ2* (Kv7.2)** M current: activated by depolarisation, slowly activating and slowly deactivating. Opposes sustained depolarisations & repetitive AP firing; determines excitably threshold firing/ responsiveness to synaptic inputs[4,113]	Self-limiting neonatal and infantile seizure phenotypes (BFNE) with generally normal neurodevelopment.	LOF (usually haplo-insufficiency), less severe loss of M-current	Sodium channel blockers
	Neonatal onset (developmental) epileptic encephalopathy (often with burst-suppression EEG)	LOF: many missense variants along 4 "hot-spot" Kv7.2 zones (Fig 1B); often DNE (severe loss of M current)	Sodium channel blockers, Retigabine (related Kv7 openers emerging)
	PNH (e.g. myokymia), with or without BFNS	LOF[72]; DNE [114]	As per BFNE
	Profound newborn encephalopathy *without szs,* startle response, non-epileptic myoclonus, hypoventilation, infantile spasms (70%), early death	GOF: R201C/R201H[78], affect S4 Arg residue/ V175L[87]	Consider avoiding K+ channel openers (e.g. retigabine)
	Infantile spasms at 4-6 months (normal appearing neonate)	GOF: e.g. R198Q (S4)[86]	Consider avoiding K+ channel openers (e.g. retigabine)
***KCNQ3* (Kv7.3)** Function: see M-current above.	BFNE (much rarer than *KCNQ2*-related BFNE)	LOF: missense majority[115]	Sodium channel blockers
	Severe DEE (neonatal onset)	LOF bi-allelic (rare) homozygous e.g. F534Ifs*15[82], compound heterozygous missense V359L/D542N[83]	Consider K+ channel openers (e.g. retigabine)
	Few szs but developmental disability: verbal, with ASD/ASD features and sleep-activated spikes	GOF missense: R227Q (S4)[88]	Consider checking for ESES
	Neurodevelopmental disability: nonverbal, with ASD/autistic features and ESES-like	GOF missense: R230C, R230H, R230S (S4 Arg)[88]	
***KCNQ5* (Kv7.5)** Function: see M-current above	DEE (e.g. infantile spasms), Focal szs/absences	GOF: P369R C-terminal,[92] LOF: S448I C-terminal[92]	Treat empirically e.g. (if IGE/GGE phenotype)
	ID alone	LOF[92]	
	GGE: absences/juvenile myoclonic/adult onset GTC,[93] Focal hypertonic szs with ID[94]	LOF: C-terminal,[93] GOF: G347S (S6)[94]	
KCNV2 (Kv8.2) Coassembles & supresses K_v2.1	DEE / FS, Afebrile focal szs	GOF: M285R/R7K[107]	-
***KCHN1*/**eag1 (Kv10.1) Function: similar to erg. Neuronal currents not well known (lack of blockers)[3]	Overlapping phenotypes with DEE including TBS and ZLS, epilepsy focal/generalised (mild to severe), infant-childhood onset	GOF: mainly distributed from S4 to S6 e.g. p.I494V[96] and I467V[97] (both recurrent)	No specific therapies, some pharmacoresponsive, beware with rufinamide[100]
***KCNH5*/**eag2 (Kv10.2) Function: similar to *KCNH1*	GTCS/hemi-clonic, ESES-like, ASD (single case)	GOF (one variant to date): R327H[103]	Some response to valproate. Consider checking for ESES/ESES-like

Abbreviations: AED, anti-epileptic drug; BFNE, benign familial neonatal epilepsy; GTCS, generalised tonic clonic seizures; ESES, electrical status (epilepticus) during slow wave sleep; RMP, resting membrane potential; AP, action potential; I/GGE, idiopathic/genetically generalised epilepsy; TBS, Temple-Baraitser Syndrome; ZLS, Zimmermann-Laband Syndrome; EA1 (episodic ataxia type 1); GOF: gain-of-function; LOF, loss of function; sz, seizure; ASD, autistic spectrum disorder; DEE, developmental/epileptic encephalopathy; DE, developmental encephalopathy; SCA, spinocerebellar ataxia; FS, febrile seizures; MEAK, myoclonus epilepsy and ataxia due to K^+ channel variant; PKD, paroxysmal kinesigenic dyskinesia; TLE, temporal lobe epilepsy. **Symbols/legend:** ↓; reduced; $^{\$}$heterozygous gene variants unless otherwise stated, not all known associated variants described.

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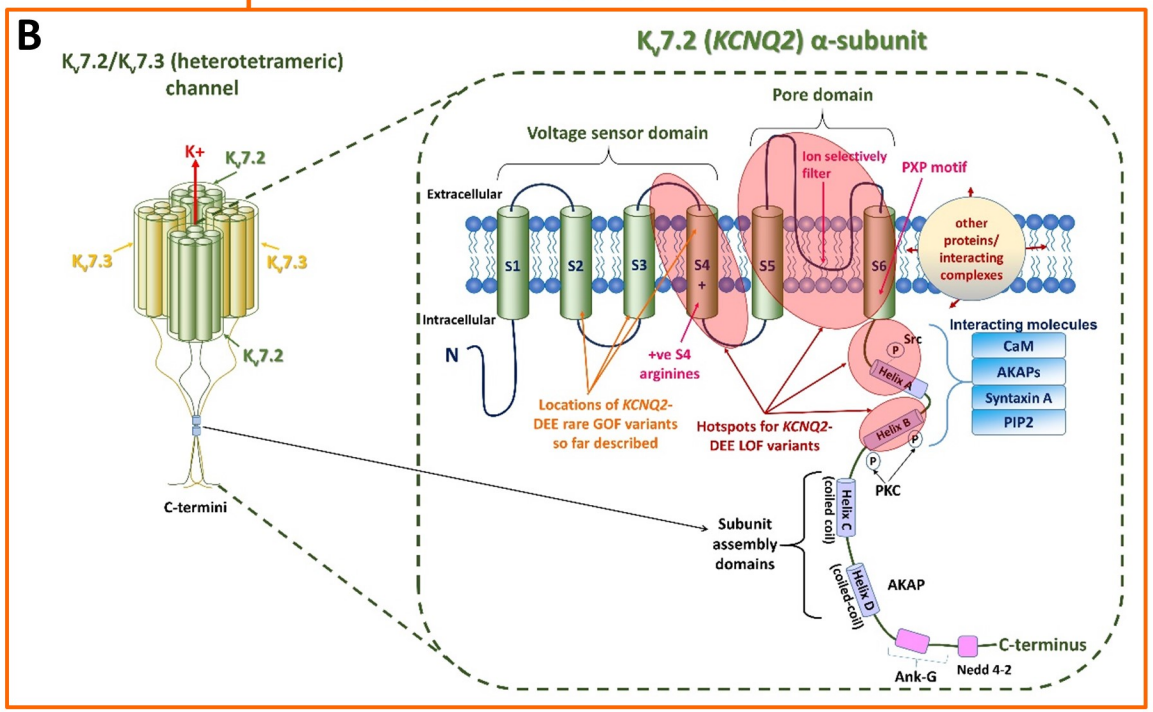
K_V Family

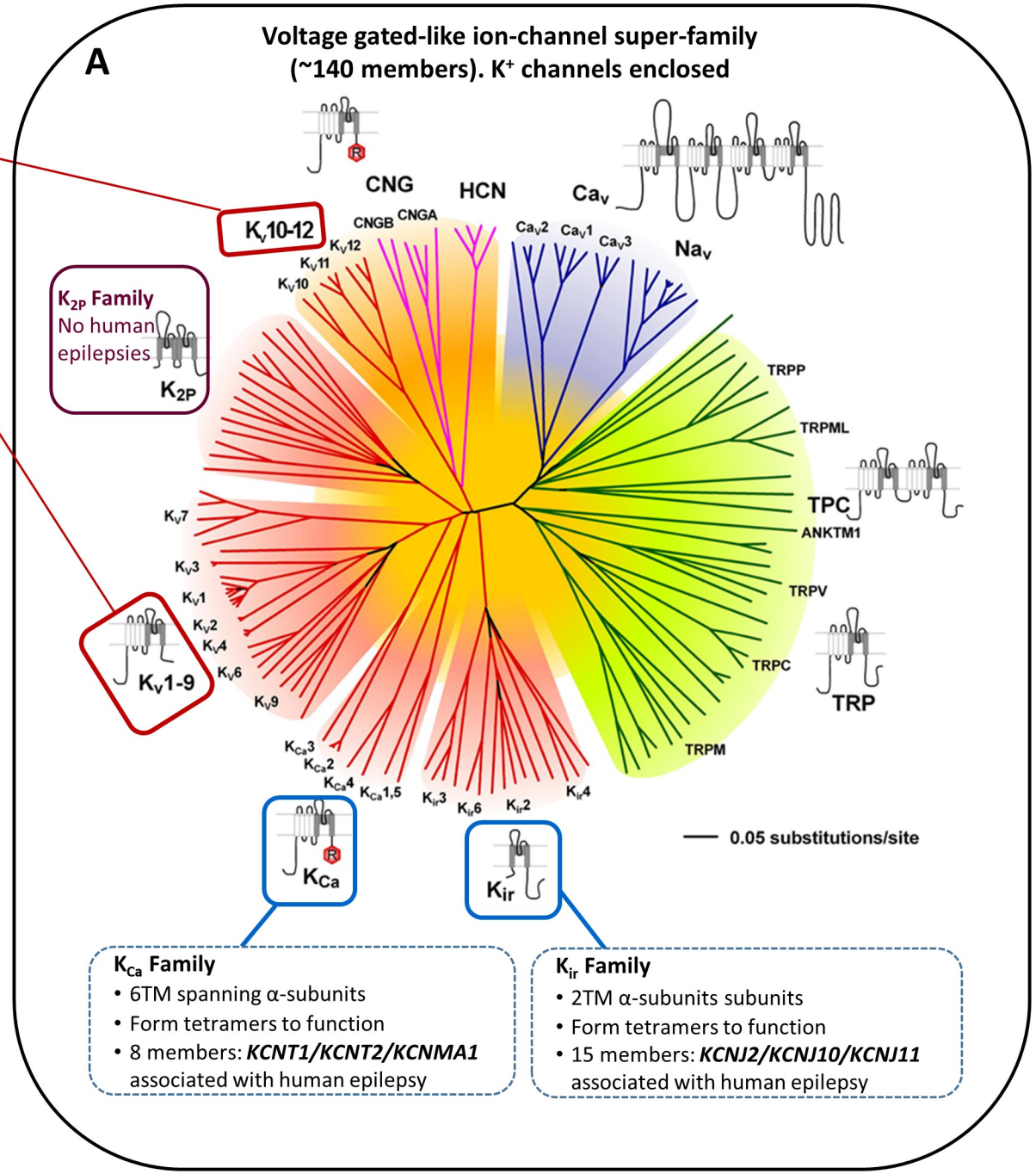
- K_V1-12 sub-families (41 members: α-subunits/genes)
- 6TM spanning α-subunits/assemble into tetramers
- Most ***(bold)*** sub-families linked to human epilepsy

Channels (α-subunits)	Gene names	Other names
K_V1.1-K_V1.8	***KCNA****1-7, 10*	*Shaker*
K_V2.1-K_V2.2	***KCNB****1-2*	*Shab-related*
K_V3.1-K_V3.4	***KCNC****1-4*	*Shaw-related*
K_V4.1-K_V4.3	***KCND****1-3*	*Shal-related*
K_V5.1	*KCNF1*	*Modifier*
K_V6.1, K_V6.4	*KCNG1,4*	*Modifier*
K_V7.1-K_V7.5	***KCNQ****1-5*	
K_V8.2-K_V6.2	***KCNV****2,3*	*Modifier*
K_V9.1-K_V9.3	*KCNS1-3*	*Modifiers*
K_V10.1-K_V10.2	***KCNH****1,5*	*Eag1-3*
K_V11.1-K_V11.3	*KCNH2,6,7*	*Erg1,2*
K_V12.1-K_V12.3	*KCNH8,3,4*	*Elk1-3*

B



A



K_{Ca} Family

- 6TM spanning α-subunits
- Form tetramers to function
- 8 members: ***KCNT1/KCNT2/KCNMA1*** associated with human epilepsy

K_{ir} Family

- 2TM α-subunits subunits
- Form tetramers to function
- 15 members: ***KCNJ2/KCNJ10/KCNJ11*** associated with human epilepsy

AUTHOR DECLARATION TEMPLATE, EJPN

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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