3rd INTERNATIONAL Kv7 CHANNELS SYMPOSIUM

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16–18 September 2026
Centro Congressi Federico II
Naples, Italy

Università degli Studi di Napoli Federico II

PROGRAMME & ABSTRACT BOOK

INDEX

ORGANISATION

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SYMPOSIUM SECRETARIAT

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WELCOME

Welcome to the 3rd International Kv7 Channels Symposium!

It is a great pleasure to welcome you to the 3rd International Kv7 Channels Symposium at the Congress Center of the University of Naples Federico II, in the historic city of Naples.

Following the tremendous success of the 2023 meeting, the Kv7 community once again comes together to share the latest developments, foster new collaborations, and celebrate the continued growth of research in this fascinating field. After three years, the time felt right to reconnect, exchange ideas, and strengthen the bonds that make this community so dynamic and forward-looking.

Over the next three days, the meeting will provide an engaging forum for both academic and industry perspectives. With a focus on innovation, collaboration, and translational potential, it offers a unique opportunity to connect with leading scientists and industry partners working at the forefront of Kv7 research.

We are excited to welcome you back to Naples!

Prof. Iain Greenwood
School of Health & Medical Sciences,
City St George's, University of London

Prof. Maurizio Taglialatela
Department of Neuroscience,
University of Naples Federico II , Naples

PRACTICAL INFORMATION

VENUE

Congress Center "Federico II"
University of Naples
Aula Magna Partenope
Via Partenope 36, 80121 – Naples

FIND YOUR WAY

Ground Floor
Registration area / Symposium secretariat
Cloakroom

First Floor
Auditorium: Aula Magna
Exhibition and Catering (Coffee breaks & Lunches)

Third Floor
Poster sessions & Lunch for Poster presenters

CERTIFICATE OF ATTENDANCE

The certificate of attendance will be available for download after the conference through the conference platform Conftool. All participants will receive an email when the certificates are ready.

WIFI CONNECTION

WIFI is available throughout the congress center.

SSID: **guestpartenope**
password: **xs4093001**

GUIDELINES FOR PRESENTERS

Oral Presentations

There is no central speaker upload desk at the conference venue, as all presentations have been submitted in advance of the symposium.

If you have any last-minute updates, please bring your revised presentation on a USB stick to the **Aula Magna** at least **2 hours before the start of your session**. Please note that last-minute uploads may cause delays to the programme and should be kept to a minimum whenever possible.

Posters

Poster sessions will take place during the lunch breaks on **Thursday 17 September** and **Friday 18 September**. Posters will, however, remain on display and be accessible throughout the symposium.

Poster presenters will have a dedicated buffet available on the **3rd floor**, allowing them to collect lunch before the poster sessions begin.

All other delegates are kindly asked to collect lunch from the main catering area on the **1st floor** and then proceed to the 3rd floor for the poster sessions.

NO PUBLICATION POLICY

Each attendee agrees that any information presented at the symposium, whether in a talk, poster session, or discussion, is a private communication from the individual making the contribution and is presented with the restriction that such information is not for public use.

Written approval of the presenting attendee must be obtained prior to the quoting or publishing of any information presented at the symposium, including talks, poster sessions, and discussions; audio or video recording of lectures and photography of slide or poster materials, are prohibited without the written consent of the contributing individual.

These restrictions apply to each participant of the symposium and are intended to cover social networks, blogs, tweets, or any other publication, distribution, communication, or sharing of information presented or discussed at the meeting.

Guests are not permitted to attend the symposium lectures or poster sessions. Each participant of the symposium acknowledges and agrees to these restrictions as a condition of being permitted to attend the conference.

SOCIAL EVENTS

Welcome Reception

Included in the registration fee, everyone is welcome to join.

Date and time: 16th September 2026, 18:15

Place: Symposium Venue (Congress Center "Federico II"), **1st floor**

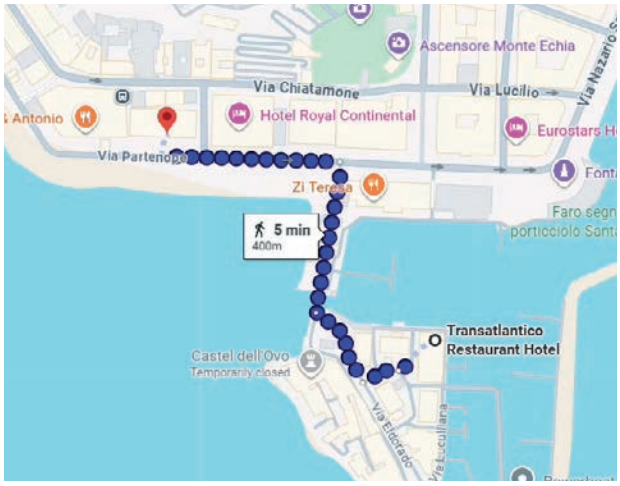
Congress Dinner

Date and time: 17th September 2026, 20:00

Place: Transatlantico, Centro Nautico Santa Lucia, Via Luculliana 15 – 80132 Naples (400m walk from the Symposium venue)

As the sun sets over the Gulf of Naples, we will gather at the waterfront **Transatlantico** restaurant for an evening of traditional Campanian cuisine and excellent networking time!

Please note the symposium dinner is not automatically included in the registration fee and must have been booked separately. Please bring the dinner ticket received at the registration desk with you.



PROGRAMME

WEDNESDAY – 16 SEPTEMBER 2026

12:45 PM – 1:00 PM	**WELCOME AND OPENING CEREMONY** (Aula Magna)
1:00 PM – 1:45 PM	**PLENARY 1 - UTILIZING INSIGHTS FROM ENDOGENOUS MODULATION IN THE PHARMACOLOGICAL TARGETING OF KV7 CHANNELS** - *Sara Liin, Sweden* (Aula Magna) *Chair: Iain Greenwood*
2:00 PM – 3:40 PM	**SESSION 1: KV7.1 STRUCTURE** (Aula Magna) *Chairs: Harley Kurata, Luca Maragliano* • STATE-DEPENDENT KCNQ1 INHIBITOR BREAKS C4 SYMMETRY TO BLOCK ION CONDUCTION – *David Fedida, Canada* • CONFORMATIONS OF KV7.1 PORE MORPH WITH VOLTAGE SENSOR STATES AND REGULATORY SUBUNITS – *Jianmin Cui, United States* • STRUCTURE AND PHOSPHORYLATION MECHANISMS OF THE CARDIAC KCNQ1+KCNE1 CHANNEL UNDER STRESS CONDITION – *Panpan Hou, Macau S.A.R. (China)* • THE PERFECT ∏: ATOM BY ATOM MAPPING OF A KV7 GATING MECHANISM – *Christopher Ahern, United States*
3:40 PM – 4:10 PM	**COFFEE BREAK** (Catering area – 1st floor)
4:10 PM – 6:10 PM	**YOUNG INVESTIGATORS** (Aula Magna) *Chairs: Lidia Carotenuto, Elizabeth Forrester* • FUNCTIONAL CHARACTERIZATION OF KV7.2/7.3, KV7.2/7.5, AND KV7.3/7.5 CHANNELS: IMPACT OF SUBUNIT COMPOSITION AND PHARMACOLOGICAL MODULATION – *Vivian Meiritz, Germany* • AT THE INTERSECTION BETWEEN SODIUM AND POTASSIUM CHANNELS: M-CURRENT DOWNREGULATION DRIVES CORTICAL HYPEREXCITABILITY IN THE SODIUM CHANNELOPATHY DRAVET SYNDROME – *Stefano Iavarone, Germany* • PREDICTING NEURODEVELOPMENTAL OUTCOME IN KCNQ2-RELATED DISORDERS – *Elisabeth Van Boxstael, Belgium* • A GATING NEXUS BETWEEN THE S6-HELIX A AND HELIX C DOMAINS STABILIZES THE ACTIVE CONFORMATION OF KV7 CHANNELS – *Shawn Lamothe, Canada* • RESOLVING CA^{2+}-DEPENDENT CALMODULIN REGULATORY STATES OF KV7.2 USING A FRET-BASED APPROACH – *Emily Walsh, United States* • MOLECULAR DISSECTION OF THE KV7.2-STARGAZIN INTERACTION – *Telmo Leal, Portugal* • ATOMISTIC INSIGHTS INTO KV7.2 VARIANT-INDUCED DYSFUNCTION: A MOLECULAR DYNAMICS STUDY – *Agnese Roscioni, Italy* • MECHANISTIC CHARACTERIZATION AND THERAPEUTIC TARGETING OF KCNQ2 DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY IN A KV7.2 THR274MET/+ MOUSE MODEL – *Shaimaa Haiba, Germany* • EFFECTS OF KV7 CHANNEL ACTIVATORS IN EXPERIMENTAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS – *Rossella Vitale, Italy* • MODELING KCNQ2-ENCEPHALOPATHY USING IPSC-DERIVED EXCITATORY-INHIBITORY NEURONAL NETWORKS – *Noortje Zonnekein, Belgium* • ENDOTHELIAL KV7.5 CHANNELS REGULATE BLOOD–BRAIN BARRIER INTEGRITY AND SEIZURE SUSCEPTIBILITY – *Camilla Celentano, Italy* • REGULATION OF VASCULAR KV7 CHANNELS BY G PROTEIN-COUPLED RECEPTOR KINASES – *Skye Lau, United Kingdom*
6:15 PM – 7:30 PM	**WELCOME RECEPTION** (Symposium venue catering area – 1st floor)

THURSDAY – 17 SEPTEMBER 2026

9:00 AM – 9:45 AM	**PLENARY 2 - UNRAVELLING THE KCNQ K+ CHANNEL FAMILY** - *Thomas Jentsch, Germany* **("D.A. BROWN LECTURE")** (Aula Magna) *Chair: Iain Greenwood*
9:45 AM – 10:15 AM	**COFFEE BREAK** (Catering area – 1st floor)
10:15 M – 12:05 PM	**SESSION 2: KV7.1 PATHOPHYSIOLOGY AND PHARMACOLOGY** (Aula Magna) *Chairs: Guiscard Seebohm, Nina Ottosson* • KV7.1: FROM IX1 TO LQT-1 MOLECULAR PHYSIOLOGY AND DISEASE – *Robert Kass, United States* • PROMISING QT-SHORTENING EFFECT OF THE ENDOCANNABINOID N-ARACHIDONOYL-L-SERINE (ARA-S) IN A TRANSGENIC MODEL OF LONG QT SYNDROME TYPE 1 – *Julien Louradour, Switzerland* • ARRHYTHMIA-MUTANT R231C STABILIZES THE VOLTAGE SENSOR IN CARDIAC POTASSIUM IKS CHANNELS IN THE INTERMEDIATE STATE – *Alicia De la Cruz, Sweden* • LOSS-OF-FUNCTION OF KV7.1 LEADS TO METABOLIC DYSFUNCTION THROUGH AGE-DEPENDENT PANCREATIC BETA-CELL DECLINE – *Anniek Lubberding, Denmark* • DETERMINE THE ANTI-ARRHYTHMIC POTENTIAL OF KV7.1/KCNE1 ACTIVATORS USING LQTS ZEBRAFISH MODEL – *Lucas Dauga, Sweden*
12:05 PM – 1:40 PM	**LUNCH & POSTERS** (Catering area – 1st floor & Poster area – 3rd floor)
1:40 PM – 3:45 PM	**SESSION 3: KV7 MODULATORS AS ANTICONVULSANTS** (Aula Magna) *Chairs: Sarah Weckhuysen, Thanos Tzounopoulos* • TOWARDS A NEW CLASS OF KCNQ CHANNEL MODIFIERS FOR POTENTIAL THERAPEUTIC APPLICATIONS – *Vitya Vardanyan, Armenia* • DEVELOPING ANTIEPILEPTIC DRUG CANDIDATES WITH THE NOVEL KCNQ2 AGONIST EBIO1 AS A LEAD – *Huaiyu Yang, China* • FROM KV7.2 ACTIVATION TO INHIBITION BY CHEMICAL REMODELING – *Carmine Ostacolo, Italy* • AKTIVA7E: A PROOF-OF-CONCEPT PHASE 1B STUDY TO EVALUATE THE SAFETY, TOLERABILITY, EFFICACY, AND PHARMACOKINETICS OF QRL-101, A NOVEL KV7.2/7.3 MODULATOR, IN KCNQ2/KCNQ3 RELATED EPILEPSY – *Manoj Malhorta, United States* • ENHANCED SEIZURE SUSCEPTIBILITY AND COGNITIVE DEFICITS IN MICE EXPRESSING A GAIN-OF-FUNCTION EPILEPSY VARIANT IN PIP2-BINDING RESIDUE OF KV7.2 – *Hee Jung Chung, United States*
3:45 PM – 4:15 PM	**COFFEE BREAK** (Catering area – 1st floor)
4:15 PM – 6:05 PM	**SESSION 4: KV7 IN BRAIN PHYSIOLOGY** (Aula Magna) *Chairs: Hee Jung Chung, Isabella Salzer* • ANGIOTENSIN II REGULATES KV7 CHANNELS IN CENTRAL NEURONS AND BRAIN ENDOTHELIAL CELLS – *Vincenzo Barrese, Italy* • GLIAL KCNQ CHANNELS REGULATE NEURONAL EXCITABILITY VIA GLIAL GABA RELEASE – *Laura Bianchi, United States* • KV7.2 IS THE PRINCIPAL M-TYPE K+ CHANNEL SUBUNIT MODULATING PAIN BEHAVIOURS IN RATS. – *Frederick Jones, United Kingdom* • KV7.2 CHANNELS SHAPE EXCITABILITY AND SALTATORY CONDUCTION AT THE NODE OF RANVIER IN HEAVILY MYELINATED NERVES – *Jianguo Gu, United States* • PHARMACOLOGICAL ACTIVATION OF KCNQ4 SUPPRESSES PERIPHERAL NOCICEPTIVE SIGNALING IN MICE – *Long Wang, China*
6:15 PM – 7:00 PM	**PLENARY 3 - UNRAVELING KCNQ2/3/5 CHANNEL FUNCTION IN THE BRAIN: FROM EPITOPE-TAGGED LINES TO DISEASE MODELS** - *Anastasios Tzingounis, United States* (Aula Magna) *Chair: Maurizio Taglialatela*
8:00 PM – 11:00 PM	**SYMPOSIUM DINNER:** Restaurant Transatlantico , Centro Nautico Santa Lucia, Via Luculliana 15 – 80132 Naples (400m walk from the Symposium venue)

FRIDAY – 18 SEPTEMBER 2026

9:00 AM – 9:45 AM	**PLENARY 4 - STRUCTURAL BASIS FOR LIGAND MODULATION OF HUMAN KCNQ2** - *Jiangtao Guo, China* (Aula Magna) *Chair: Maurizio Taglialatela*
9:45 AM – 10:15 AM	**COFFEE BREAK** (Catering area – 1st floor)
10:15 AM – 12:05 PM	**SESSION 5: KV7 CHANNEL REGULATION** (Aula Magna) *Chairs: Thomas Jepps, Nikita Gamper* • TDP-43-DEPENDENT MIS-SPLICING OF KCNQ2 TRIGGERS INTRINSIC NEURONAL HYPEREXCITABILITY IN ALS/FTD – *Evangelos Kiskinis, United States* • TUNING KV7/KCNQ CHANNEL DENSITY THROUGH ENDOSOMAL 2CL-/H+ EXCHANGE: IMPLICATIONS FOR NEURONAL EXCITABILITY – *Raul E. Guzman, Germany* • GBΓ DUALLY REGULATES THE M-CURRENT VIA INCREASED PIP2 SENSITIVITY AND TRAFFICKING – *Boris Shalomov, Israel* • DRUG:LIPID INTERACTIONS REVEAL AN ENORMOUS DYNAMIC RANGE OF KCNQ POTASSIUM CHANNEL REGULATION – *Harley Kurata, Canada* • AN UPSTREAM OPEN READING FRAME REPRESSES TRANSLATION OF KCNQ2 – *Dalton Huey, United States*
12:05 PM – 1:40 PM	**LUNCH & POSTERS** (Catering area – 1st floor & Poster area – 3rd floor)
1:40 PM – 3:30 PM	**SESSION 6: KV7 IN MUSCLE** (Aula Magna) *Chairs: Fabio Arturo Iannotti, Vincenzo Barrese* • OESTRUS CYCLE REGULATION OF CEREBRAL ARTERY KV7 CHANNELS – *Samuel N Baldwin, Denmark* • NOVEL CRYPTIC SPLICING EVENT IN THE MITOCHONDRIAL KV7.4 CHANNEL – *Thomas Jepps, Denmark* • CONTRIBUTION OF KV7 CHANNELS TO M2 MUSCARINIC RECEPTOR-DEPENDENT CONTRACTIONS OF AIRWAY SMOOTH MUSCLE – *Gerard P Sergeant, Ireland* • THERAPEUTIC EFFECTS OF A KV7.4 ACTIVATOR, URO-K10, ON PULMONARY ARTERIAL HYPERTENSIVE RATS: A NOVEL MODE OF ACTION VIA MITOCHONDRIA – *Sung Joon Kim, South Korea* • IDENTIFICATION OF SODIUM/MYO-INOSITOL TRANSPORTER 1 AS A MAJOR DETERMINANT OF ARTERIAL CONTRACTILITY THROUGH MODULATION OF KV7.4/5 CHANNEL ACTIVITY – *Elizabeth Forrester, United Kingdom*
3:30 PM – 4:00 PM	**COFFEE BREAK** (Catering area – 1st floor)
4:00 PM – 5:20 PM	**SESSION 7: NOVEL THERAPEUTIC APPROACHES** (Aula Magna) *Chairs: Evangelos Kiskinis, Francesco Miceli* • THERAPEUTIC STRATEGIES FOR KCNQ4-ASSOCIATED HEARING LOSS – *Heon Yung Gee, South Korea* • A ROLE OF KCNQ-INDUCED PLASTICITY ON NOISE-INDUCED NEURAL AND PERCEPTUAL HYPERSENSITIVITY TO SOUND – *Thanos Tzounopoulos, United States* • TOWARD SCALABLE PRECISION THERAPEUTICS FOR KCNQ2-DEE – *Aubrie Soucy Verran, United States* • DEVELOPMENT AND EVALUATION OF SYSTEMIC AAV GENE THERAPY TO TREAT NEONATAL KCNQ2 EPILEPTIC ENCEPHALOPATHY – *Rana Eser, United States* • ASO–MEDIATED RESCUE OF ELECTROPHYSIOLOGICAL NETWORK DYSFUNCTION IN IPSC-DERIVED KCNQ2 GAIN-OF-FUNCTION NEURONS – *Lidia Carotenuto, Belgium*
5:20 PM – 5:50 PM	**CLOSING CEREMONY / AWARDS / FAREWELL** (Aula Magna)

The Best Presentation Award is kindly supported by the Journal of General Physiology.

The D.A. Brown Lecture is kindly supported by the Physiological Society.

POSTERS OVERVIEW

1 KCNQ/KV7 CHANNELS LOCALIZE TO DENDRITIC SPINES VIA ANKYRING–190 AND SUPPRESS NMDA RECEPTOR ACTIVITY – *Hongyu Zhao (United States)*

2 FROM CELL CULTURE TO CRYO EM: MEMBRANE PROTEIN PURIFICATION OF KV7.1 CHANNEL FROM MAMMALIAN CELLS – *Minay Mertens (Germany)*

3 A HIGH–THROUGHPUT FUNCTIONAL SCAN OF KCNQ1 S4 VARIANTS – *Eduardo Guadarrama (United States)*

4 CONTRIBUTION OF KV7 CHANNELS TO VASCULAR TONE REGULATION IN HUMAN AND RAT RENAL RESISTANCE VESSELS – *Max Jarchow (Germany)*

5 MELATONIN AND MELATONIN–DERIVED, H2S–CONTAINING COMPOUNDS ACT AS NEW KV7.4 ACTIVATORS – *Dania Ioia (Italy)*

6 KV7.2 CHANNELOPATHY BEYOND THE BRAIN: GAIN–OF–FUNCTION VARIANTS LINKED TO PALATAL MALFORMATIONS – *Kim Marie Thalwitzer (Germany)*

7 NOT JUST BRAINY BUT ALSO BRAWNY: THE ROLE OF KV7 CHANNELS BEYOND THE BRAIN – *Noemi Di Muraglia (Italy)*

8 KV7/KCNQ CHANNEL BLOCKADE ATTENUATES HIPPOCAMPAL BUT NOT RETINAL DEGENERATION IN CLCN3–/– MICE – *Alberto Rafael Diaz Castillo (Germany)*

9 SIX NOVEL VARIANTS IN KV7.2 POTASSIUM CHANNELS CAUSE DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY BY GAIN–OF–FUNCTION EFFECTS – *Antonio Priore (Italy)*

10 A NOVEL ROLE FOR KININOGEN IN THE REGULATION OF KV7 CHANNEL ACTIVITY AND NEURONAL EXCITABILITY IN MULTIPLE SCLEROSIS – *Nicole Rychlik (Germany)*

11 TMPRSS6 CLEAVES KCNE1 AND CAUSES ARRYTHMIAS IN IRON OVERLOAD DISEASE – *Ricarda Zimmermann (Germany)*

12 KCNQ2–DEE: ARE NAV1.1 AND NAV1.6 CHANNELS INVOLVED IN THE SUDEP PHENOTYPE OBSERVED IN THE KCNQ2T274M/+ MOUSE MODEL? – *Manon Saurey (France)*

13 IKS SINGLE CHANNELS: A SURVEY. – *Jodene Eldstrom (Canada)*

14 THE INTERMEDIATE STATE STRUCTURE OF KCNQ1 ILLUMINATES VOLTAGE SENSOR DYNAMICS – *Efthimios Kyriakis (Canada)*

15 MECHANISM OF KCNQ1 GATING REGULATED BY PIP2 AND CALMODULIN – *Jingyi Shi (United States)*

16 17B–OESTRADIOL INHIBITS KV7.4 CHANNELS VIA GPER1–GQ–PLC–PKC SIGNALLING – *Iain A Greenwood (United Kingdom)*

17 KV7.2 PORE STABILITY SHAPES REGULATION BY PHARMA-COLOGICAL ACTIVATORS – *Thomas Hammon (Canada)*

18 KCNE0, AN EARLY DIVERGING KCNE SUBUNIT, CONFERS CONSTITUTIVE ACTIVITY TO KCNQ1 IN LAMPREY – *Koichi Nakajo (Japan)*

19 BINDING SITE AND PUTATIVE MECHANISTIC INSIGHTS INTO THE INHIBITORY EFFECT OF ESTRADIOL (17B–E2) ON THE KV7.1/KCNE1 CHANNEL – *Veronika Linhart (Sweden)*

20 PHARMACOLOGICAL IMPLICATIONS OF ß–ADRENERGIC PHOSPHORYLATION OF KV7.1/KCNE1 – *Mika Jönsson (Sweden)*

21 AN INTEGRATED ASSAY PLATFORM FOR MECHANISTIC AND TRANSLATIONAL PROFILING OF KV7.2/7.3 ACTIVATORS – *Catherine M. Hodgson (United Kingdom)*

22 LOCAL VASCULAR METABOLISM OF PARACETAMOL GENERATES NAPQI TO DRIVE KV7-DEPENDENT VASODILATATION AND INTRAVENOUS PARACETAMOL-INDUCED HYPOTENSION – *Thomas A. Jepps (Denmark)*

23 VOLTAGE CLAMP FLUOROMETRY IN KV7.4 TO DETERMINE PHARMACOLOGICAL MECHANISMS – *Ricardo Pan–Lizcano (Sweden)*

24 EFFECT OF ‘SQUEEZE–TO–INHIBIT‘ AGENT EBIO3 ON SMOOTH MUSCLE RELAXATIONS – *Ugo Ejiegbu (United Kingdom)*

25 BEYOND RETIGABINE: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF AZETUKALNER ACROSS KV7/KCNE COMPLEXES – *Johann W. Schröder (Germany)*

26 THE PARACETAMOL METABOLITE NAPQI PREVENTS GPCR–MEDIATED INHIBITION OF KV7 CHANNELS – *Isabella Salzer (Austria)*

27 CONSERVED BINDING SITE, DIVERGENT MODULATION: RETHINKING MAXIPOST SELECTIVITY ACROSS KV7 CHANNELS – *Andrea Pasquadibisceglie (Italy)*

28 BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF KV7.4 CHANNELS USING AUTOMATED PATCH CLAMP – *Duncan Jarman (Denmark)*

29 GENOTYPE–PHENOTYPE CORRELATIONS IN KV7.2–RELATED EPILEPSIES CAUSED BY DISTINCT MUTATIONS ON THE SAME RESIDUE – *Ilaria Mosca (Italy)*

30 KV7.4 ACTIVATION PROTECTS DOPAMINERGIC NEURONS AGAINST A–SYNUCLEIN/ROTENONE–INDUCED TOXICITY: A NOVEL PHARMACOLOGICAL TARGET IN PARKINSON'S DISEASE? – *Silvia Piccirillo (Italy)*

31 QRL–101–05: A DOUBLE BLIND RANDOMIZED THREE–WAY CROSSOVER PHASE 1 STUDY TO EVALUATE CORTICAL AND MOTOR NERVE EXCITABILITY IN HEALTHY VOLUNTEERS FOLLOWING ADMINISTRATION OF A KV7.2/7.3 MODULATOR; QRL–101 – *Taylor Gray (United States)*

32 ENDOCANNABINOID DERIVATIVES FOR SELECTIVE ACTIVATION OF KV7.1/KCNE1 IN THE CONTEXT OF LONG QT SYNDROME – *Irene Hiniesto–Iñigo (Sweden)*

33 FROM GENE TO FUNCTION: GENERATING STABLE KCNQ ISOFORM CELL LINES FOR SELECTIVE DRUG PROFILING – *Nadine Mengers (Germany)*

34 PREVENTING KV7 DOWNREGULATION BY TARGETING REST UPREGULATION IN CHRONIC PAIN – *Nikita Gamper (United Kingdom)*

35 THE NOVEL KV7 ACTIVATOR C60 REDUCES PAIN IN OXALIPLATIN–INDUCED PERIPHERAL NEUROPATHY – *Flavia De Martino (Italy)*

36 PHYSICOCHEMICAL OPTIMIZATION OF NICOTINAMIDE–BASED KV7.2/3 CHANNEL MODULATORS TO OVERCOME THE POTENCY–SOLUBILITY TRADE–OFF – *Pascal Rosendahl (Germany)*

37 FROM PATIENT NEED TO SHARED STRATEGY: BUILDING A PARTICIPATORY REGISTRY NETWORK FOR KCNQ2–RELATED EPILEPSY – *Pasqualina Cordella (Italy)*

38 HIGH–THROUGHPUT ELECTROPHYSIOLOGICAL CHARACTERIZATION OF KV7.2/KV7.3 CHANNELS USING AUTOMATED PATCH CLAMP – *Will Seibertz (Germany)*

39 MACHINE LEARNING RESOLVES FUNCTIONAL PHENOTYPES AND THERAPEUTIC RESPONSES IN KCNQ2 DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY IPSC MODELS – *Evangelos Kiskinis (United States)*

40 INTEGRATED GENOTYPE-PHENOTYPE FUNCTION ANALYSIS REVEALS DISTINCT PATHOGENIC MECHANISMS FOR COGNITIVE IMPAIRMENT IN KCNQ2-RELATED DISORDERS – *Juan Xiong (China)*

41. IN VIVO EFFICACY OF AMITRIPTYLINE FOR KCNQ GAIN-OF-FUNCTION DISORDERS - *Tristan T Sands (United States)*

42. MACHINE LEARNING FRAMEWORK FOR FUNCTIONAL PREDICTION OF KCNQ2 MISSENSE VARIANTS - *Yuval Oren (Israel)*

43. MODELLING KCNQ2-RELATED EPILEPTIC ENCEPHALOPATHY USING HINEURONS - *Thasneem Musthafa (Ireland)*

44. MOLECULAR BASIS FOR SELECTIVE ACTIVATION OF KV7.3 BY THE ROSEMARY METABOLITE CARNOSIC ACID – *Geoffrey Abbott (United States)*

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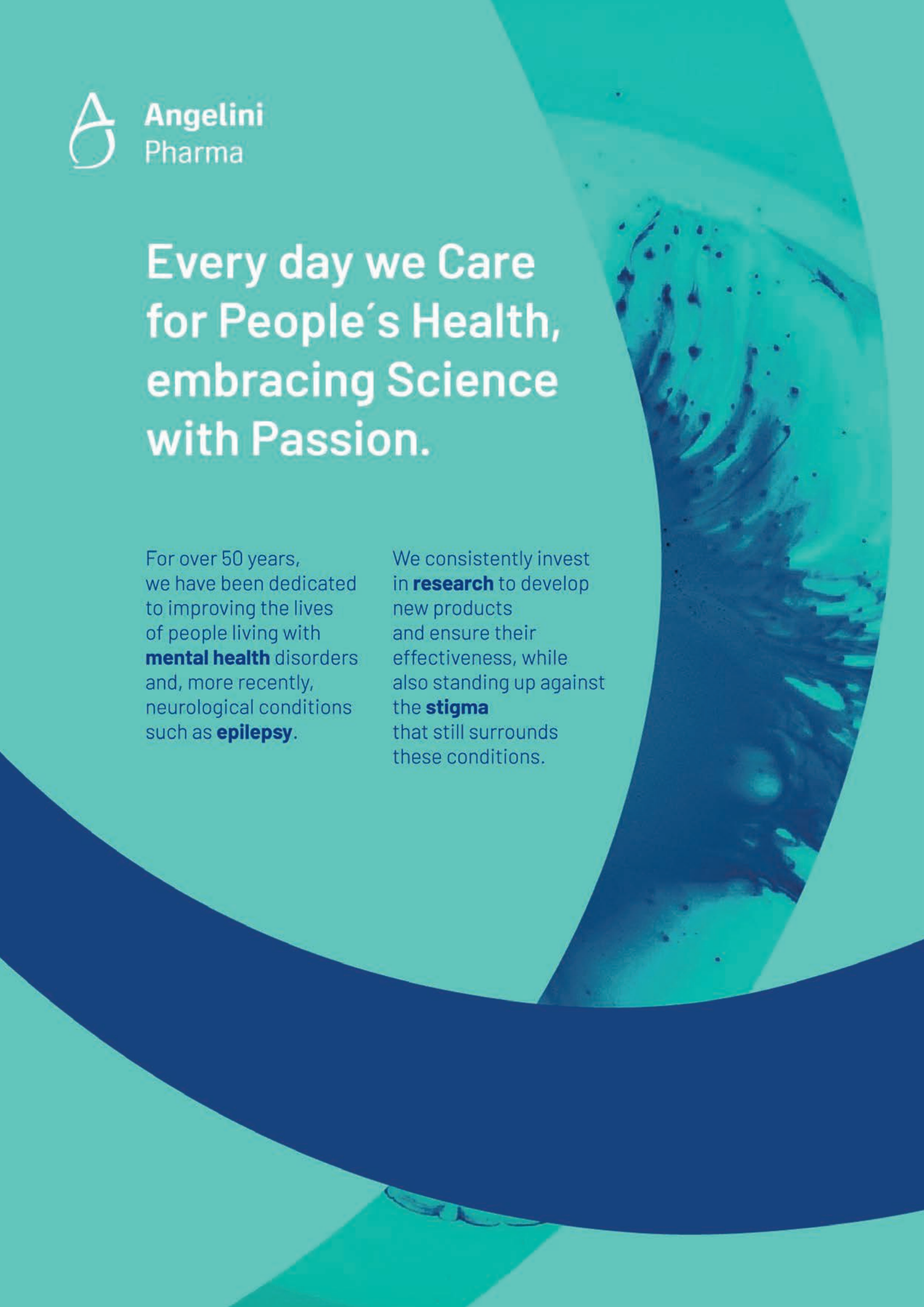

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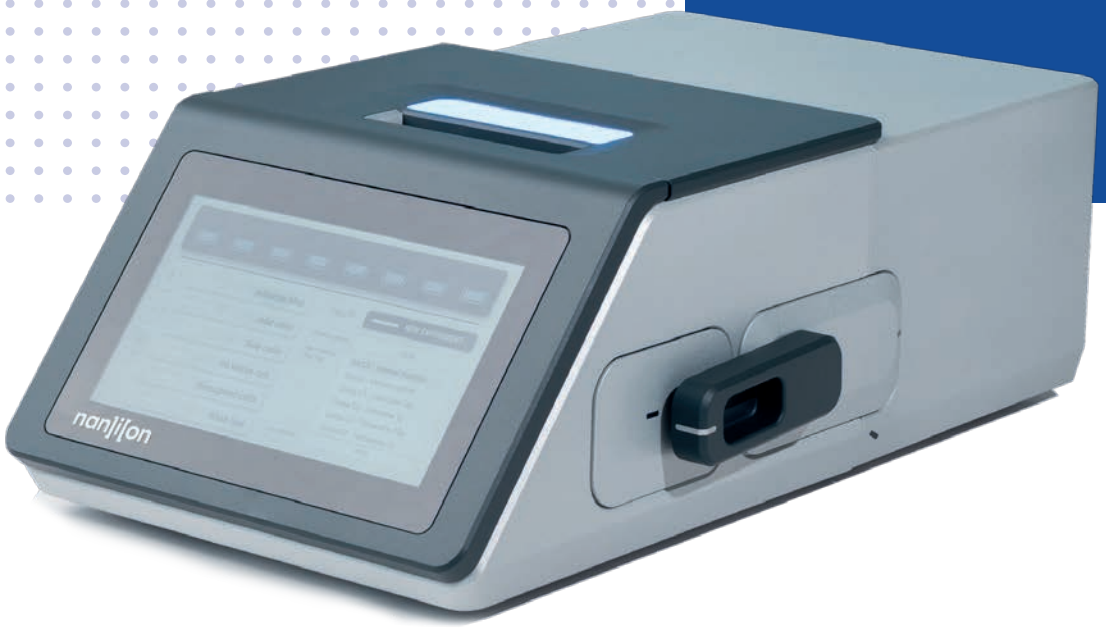

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ABSTRACTS

1.

UTILIZING INSIGHTS FROM ENDOGENOUS MODULATION IN THE PHARMACOLOGICAL TARGETING OF KV7 CHANNELS

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Voltage-gated potassium (Kv) ion channels within the Kv7 family play important roles in regulating cellular excitability. Also, mutations in the genes encoding Kv7 channels are associated with various diseases. Consequently, understanding how Kv7 channels are modulated by endogenous and pharmacological compounds is of broad interest. My research group is fascinated by the modulation of Kv7 channel activity induced by various classes of endogenous compounds. We use a combination of experimental and computational approaches to characterize effects and determine underlying molecular mechanisms. This presentation will highlight past and recent advances in the modulation of Kv7 channels by endocannabinoids, with a special focus on activating effects on the cardiac Kv7.1/KCNE1 channel. In brief, endocannabinoids facilitate activation of Kv7.1/KCNE1 by utilizing two interaction sites responsible for different functional effects. Chemical features of both the endocannabinoid molecule and the residues lining the interaction sites are important for functional effects. This presentation will also provide data on the rescuing capability of endocannabinoids on Kv7.1/KCNE1 channels carrying arrhythmia-associated mutations, the translational potential of endocannabinoid effects across experimental models, and include examples of how insights into the molecular mechanisms of endogenous modulators can be leveraged in drug design. Altogether, my hope is that our research provides new insights into endogenous and pharmacological modulation of Kv7 channels.

2.

UNRAVELLING THE KCNQ K+ CHANNEL FAMILY

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Following our interest in channelopathies, we expanded our research in the mid-1990s from CLC chloride channels to K^+ channels, beginning with KVLQT1 (now known as KCNQ1 or Kv7.1), which had previously been identified by others as the gene mutated in the long QT syndrome, a cardiac arrhythmia disorder. We hypothesized that KCNQ1 might belong to a larger family of K^+ channels. Using primarily homology cloning through wet-lab screening of cDNA libraries, we identified and characterized the four remaining KCNQ family members, KCNQ2–KCNQ5.

Fluorescence in situ hybridization mapped KCNQ2 and KCNQ3 to two distinct chromosomal loci known to harbor genes for benign familial neonatal convulsions (BFNC), strongly suggesting that variants in these genes cause this inherited epilepsy syndrome. In collaboration with human geneticists, we were able to confirm this hypothesis rapidly. We subsequently discovered that KCNQ2 and KCNQ3 form heteromeric channels and went on to characterize their structure–function relationships, pharmacology, and physiological roles using knockout mouse models.

Our work on KCNQ4 followed a similar trajectory. After identifying the gene by homology screening, we mapped it to a chromosomal locus associated with autosomal dominant hearing loss, identified and analyzed pathogenic human variants, localized KCNQ4 in the inner ear by immunostaining, generated several KCNQ4 mouse models, and demonstrated that KCNQ4 also modulates peripheral touch sensation.

The final member of the family that we cloned, KCNQ5, likewise exhibited the properties of M-currents. Using genetic mouse models, we investigated its roles in neuronal physiology.

In addition, we identified KCNE3 as an accessory subunit of KCNQ1. In striking contrast to KCNE1, KCNE3 converts KCNQ1 into a nearly voltage-independent (ohmic) channel, a property that is important for epithelial physiology.

In my talk, I will provide a brief overview of these discoveries and conclude with a short excursion into other ion channels that cooperate with KCNQ4 in the inner ear to enable normal hearing.

3.

UNRAVELING KCNQ2/3/5 CHANNEL FUNCTION IN THE BRAIN: FROM EPITOPE-TAGGED LINES TO DISEASE MODELS

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Neuronal KCNQ (Kv7) potassium channels act as molecular brakes to prevent runaway neuronal excitability. For decades, their function has been framed around a single tenet: KCNQ3 and KCNQ2 assemble to generate the M-current, a voltage-gated non-inactivated potassium channel. Despite its success to explain the role of KCNQ activity in the brain, this tenet reduces KCNQ channel biology into a single current, obscuring how subunit composition and cell type specific regulation might control neuronal function and disease. Here, I will draw on findings from mouse models, including subunit-specific knockouts, knock-ins, and epitope-tagged alleles, to present three emerging ideas. First, KCNQ channels regulate excitability across a broad range of neuronal cell types, beyond those defined by strong spike-frequency adaptation. Second, KCNQ2 acts as an obligatory KCNQ subunit in most neurons. Third, KCNQ3 and KCNQ5 act as regulatory subunits whose contributions vary by cell type. These ideas help explain why gain-of-function variants in KCNQ3 are linked to autism spectrum phenotypes, whereas gain-of-function mutations in KCNQ2 lead to central hypoventilation. Taken together, current work suggest that KCNQ biology and disease are best understood in terms of subunit composition and cell-type context, rather than as a fixed KCNQ2/3 current.

4.

STRUCTURAL BASIS FOR LIGAND MODULATION OF HUMAN KCNQ2

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Voltage-gated potassium channels mediate the efflux of potassium ions upon the depolarization of membrane potential and play essential roles in resetting the resting membrane potential after the action potential firing in excitable cells. The human genome encodes 40 VGKCs that are distributed in 12 families, including the KCNQ family, which consists of five members, KCNQ1-5. In neurons, KCNQ2-KCNQ5 carry the M-current and contribute to the stability of the membrane potential. Due to their important roles neuronal physiology, mutations in KCNQ channels can cause various neurological disorders such as epilepsy. Moreover, the neuronal KCNQs are important therapeutic targets for the treatment of epilepsy and deafness. In this presentation, I will summarize our recent work on ligand activation mechanisms of human KCNQ2. Using cryo-EM, electrophysiology, and computational approaches, we determined structures of KCNQ2 in both closed and open conformations, revealed diverse binding sites of both the endogenous activator PI(4,5)P2 and synthetic agonists including the anti-epileptic drugs retigabine (RTG) and cannabidiol (CBD), and elucidated their activation mechanisms. Furthermore, based on our structures, we designed new KCNQ2 agonists and inhibitor with undiscovered working mechanisms. Our work may guide the development of antiepileptic drugs and analgesics that target KCNQ2.

1.1

STATE-DEPENDENT KCNQ1 INHIBITOR BREAKS C4 SYMMETRY TO BLOCK ION CONDUCTION

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Introduction
KCNQ1 (Kv7.1) is a voltage-gated potassium channel protein with critical roles in membrane ion transport throughout the human body, contributing to physiological processes like cardiac repolarization, epithelial ion transport, and auditory function. In the heart, KCNQ1 assembles with KCNE1 to form the slow delayed rectifier current, known as IKs. Dysregulation of this major repolarizing current, caused by mutations in KCNQ1, is associated with atrial fibrillation, the most common non-lethal cardiac arrhythmia, and less common life-threatening arrythmias that include Long QT syndrome type 1 (LQT1) and Short QT syndrome type 2 (SQT2).

Methods
Here, we use cryo-Electron Microscopy (cryo-EM) to report the structures of the Xenopus KCNQ1 xE1R/R2E (xE1R/R2E) intermediate, and wild-type (xWT) activated states in the presence of UCL2077, a potent state-dependent inhibitor of KCNQ1. Patch clamp electrophysiology of point mutations is used to investigate the importance of putative binding site residues.

Results
The structures reveal that, in contrast to previously studied compounds that bind to KCNQ channels, UCL2077 breaks the four-fold symmetry of the channel and binds only in two diagonal protomers. In the intermediate and activated xKCNQ1 states, the UCL2077 binding site is in a hydrophobic pocket formed by S5-S6 helices and the filter helix from one protomer, and the S6 helix from the adjacent protomer. The binding of UCL2077 in the xE1R/R2E structure triggers voltage sensor domain rotation as well as global and local S6 conformational changes which place residues, including a central phenylalanine, in the inner vestibule which causes an allosteric pore blockade via a regional collapse of the ion permeation pathway. Mutagenesis results from voltage clamp experiments confirm the binding site, and MD simulations suggest that UCL2077 may stabilize a rare conformational state of the channel S6. We further explore UCL2077 preferential binding in the xE1R/R2E intermediate state over the xWT activated state and its selectivity for KCNQ1 over other KCNQ channel isoforms.

Conclusions
Our structures establish an allosteric pore-blocking mechanism for KCNQ1 channels, with potential applicability to neuronal KCNQ family channels. By revealing how UCL2077 binding stabilizes a specific conformational state of the channel, we provide a mechanistic framework for understanding state-dependent inhibition. Furthermore, the UCL2077-bound complexes offer structural templates to guide structure-based drug discovery for the development of new therapeutic agents.

1.2

CONFORMATIONS OF KV7.1 PORE MORPH WITH VOLTAGE SENSOR STATES AND REGULATORY SUBUNITS

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Introduction

In classic models of voltage-dependent channel activation, the pore only opens after the voltage sensor domains (VSD) activate in an "all-or-none" fashion. Whether the VSD alters open pore properties remains unclear. We examine whether the properties of the KCNQ1 channel pore alter with the VSD and regulatory subunits that are located outside of the pore.

Methods

We use Ba2+ block as a probe for this study. External Ba2+ block of KCNQ1 channels is voltage-dependent and the voltage-dependent channel opening in Ba2+ mainly derives from voltage-dependent Ba2+ unblock. We measure the voltage dependence of Ba2+ unblock and block in KCNQ1 channels with various mutations and KCNE coexpression.

Results

Selectivity filter conformational changes indicated by the voltage dependence of Ba2+ unblock vary with the states of the VSD, the alterations of VSD-pore coupling, and the association with regulatory KCNE subunits.

Conclusions

Contrary to the classic view, our results suggest that, instead of being independent of the voltage gating machinery, the selectivity filter of KCNQ1 is flexible and influenced by the subtle changes in the voltage sensor state and the environment outside of the pore.

1.3

STRUCTURE AND PHOSPHORYLATION MECHANISMS OF THE CARDIAC KCNQ1+KCNE1 CHANNEL UNDER STRESS CONDITION

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The cardiac KCNQ1+KCNE1 channel generates the slow delayed rectifier current (IKs), critical for ventricular repolarization and ß-adrenergic adaptation via cAMP/PKA phosphorylation, which increases current and speeds repolarization under stress condition. KCNQ1 loss-of-function mutations cause long QT syndrome type 1 (LQT1), the most common congenital LQTS; variants impairing cAMP response carry the highest risk of sudden cardiac death. Using cryo-EM (2.5–3.4 Å) and electrophysiology, we resolved structures of human KCNQ1+KCNE1 in both closed and open states. KCNE1 association induces six helix-to-loop transitions in transmembrane segments of KCNQ1—three at the S4–S5 linker, and the other three in S6 and helix A. These helix↔loop transitions dramatically remodel the IKs channel gating, ion permeation, PIP2 modulation (dual-PIP2 binding), and pharmacology (including a fenestration for IKs-selective modulators). Analysis of cAMP-insensitive LQT1 variants defined an "IKs PKA phosphorylation axis" spanning the KCNQ1 N-terminus to the pore, where high-penetrance variants cluster in >10,000 clinical cases. The small molecule ML277, binding at the center of the phosphorylation axis, rescues defective cAMP responses in high-risk variants and normalizes beating in patient-derived iPSC cardiomyocytes. These results reveal novel gating mechanism, integrate voltage-, lipid-, and phosphorylation-dependent control, and provide a foundation for targeted therapies to restore IKs function and prevent sudden death in high-risk LQT1.

1.4

THE PERFECT Π: ATOM BY ATOM MAPPING OF A KV7 GATING MECHANISM

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Introduction
Kv7.1 is a cardiac voltage-gated potassium channel that underlies the delayed rectifier current (IKS) in the heart. The slow response to membrane depolarization is a hallmark feature of this channel's physiology, yet the mechanistic basis of how voltage promotes the open potassium conducting state is unknown. To this end, we examined how residues on the S4 voltage-sensor are potentially coupled to the pore domain to form meta-stable intermediate states that support Kv7 channel volte-dependent gating phenomena.

Methods
We focused on previously identified aromatic residues which might couple the pore and voltage sensing domains by using a chemical tuning approach whereby aromatic residues are modified by serial fluorination. This system enable the high fidelity encoding of fluoro=Phe residues at F232,

Results
The data shows that serial fluorination at one site (F232 on the S4 helix, within the voltage sensing domain) resulted in a stepwise voltage gating shift, where each added fluorine atom further biased channel opening. Mutant-cycle analysis of proximal positively charged amino acids indicate that F232 forms a cation-π interaction with K285, a residue at the tip of the S5 segment in the pore domain. Using cryo-electron microscopy, a partial structure of the F232 penta-F-Phe Kv7.1 (Q1-E1) open channel was resolved to 6Å. This study provides the first functional evidence of a cation-π interaction within a eukaryotic membrane protein. These interactions have long been proposed to be central to protein function but have eluded characterization due, in part, to limitations in previous methodologies for their study. The data suggests that the reversible breaking of this non-covalent bond supports channel opening in the Kv7.1 cardiac potassium channel. We also provide the first structural depiction of a recombinant human protein with an encoded unnatural amino acid.

Conclusions
The data supports a gating mechanism whereby the F232-K285 cation-pi interaction represents an intermediate activated state that is broken prior to channel opening. This work also provides a framework for the use of site-specifically encoded fluorinated phenylalanine residues in structural biology of membrane proteins, as a tool to resolve structures in states that are more challenging to resolve with conventional mutagenesis.

2.1

KV7.1: FROM IX1 TO LQT-1 MOLECULAR PHYSIOLOGY AND DISEASE

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Introduction
In 1969 R.W. Tsien and Dennis Noble reported finding a slowly activating outward membrane current that that controlled, in part, the duration of the cardiac ventricular action potential. The discovery of this K+-sensitive current led to unraveling the role of Kv7.1 in cardiac electrophysiology and congenital arrhythmias.

Methods
Single cell and multicellular electrophysiology of cardiac myocytes along with clinical and genetic analysis of patients.

Results
Two components of slowly activating current, were reported and one, named Ix1, because although sensitive to extracellular K+, it wasn't clear that it was K+. Ix1 became the focus of electrophysiologic studies of cardiac cells and a key role of Ix1 in cardiac electrophysiology emerged after Kass and Wiegers showed marked Ix1 enhancement by noradrenaline. Sanguinetti and Jurkiewicz found two K+ channels in guinea pig ventricular myocytes: IKs and IKr. IKs, a slowly activating current was indeed Ix1. Using positional cloning, a new putative K+ channel gene, KVLQT1, was discovered. Coassembly of KvLQT1 with a key beta subunit, mink, formed the the IKs channels previously reported by Sanguinetti and Jurkiewicz. Mutations in the KVLQT1 gene, the gene coding for the, the KCNQ1 or Kv7.1 alpha subunit, were the first channel mutations (variant 1) shown to underlie the Long QT Syndrome (LQTS). A critical study of genotype-phenotype correlations in LQTS revealed gene-specific triggers of serious or fatal cardiac events for LQT1 mutation carriers, occurred when mutation carriers exercised. That IKs was shown to be strongly modulated by adrenergic agonists linked this property of the IKs channel to cardiac events in LQT1 patients. The molecular basis for this link was found to be the assembly of a macromolecular signaling complex on the KCNQ1-KCNE1 K+ channel carboxy terminus which is required for a functional response to beta adrenergic stimulation during exercise. The molecular bridge to adrenergic modulation of the channel is the A kinase-anchoring protein, AKAP-9 or Yotiao, a protein that brings protein kinase A, phosphatase, adenylyl cyclase, and phosphodiesterase to the KCNQ1 carboxy terminus providing key enzymes for rapid phosphorylation and de-phosphorylation of the channel in response to increase sympathetic nerve activity during exercise.

Conclusions
The discovery of K+ sensitive ionic current by Noble and Tsien paved the way to revealing the key physiological roles of the Kv7.1 channel in the heart and its critical contribution to the most prevalent variant of LQTS, LQT-1, which can lead to SCD during exercise.

2.2

PROMISING QT-SHORTENING EFFECT OF THE ENDOCANNABINOID N-ARACHIDONOYL-L-SERINE (ARA-S) IN A TRANSGENIC RABBIT MODEL OF LONG QT SYNDROME TYPE 1

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Introduction
Loss-of-function variants in KCNQ1 (α-subunits of the IKs-conducting cardiac repolarising K+ channels) lead to long QT syndrome type 1 (LQT1), a channelopathy with impaired cardiac repolarization. Patients present with prolonged QT interval on the ECG and a longer action potential duration (APD), which increase the risk of ventricular tachycardia and sudden cardiac death. While beta-blockers are used to reduce subsequent arrhythmias in LQT1 patients, there are no current treatments that target the cause, i.e. the impaired IKs. However, recent work shows that the endocannabinoid N-arachidonoyl-L-Serine (ARA-S) can rescue loss-of-function variants associated with LQT1 by restoring the functional activity of the IKs current in Xenopus oocytes. Hence, we aimed here at testing the ability of ARA-S to shorten the prolonged APD/QT interval in our transgenic LQT1 rabbit models carrying the dominant negative variant KCNQ1-Y315S.

Methods
The effects of 10 µM ARA-S on IKs current densities and APD were assessed in isolated ventricular cardiomyocytes (VCMs) while the QT interval was measured in Langendorff-perfused ex-vivo hearts from LQT1 rabbits. Arrhythmias in ex-vivo hearts were induced by a combination of bradycardia (AV-node ablation), hypokalaemia (2 mM) and isoproterenol (10 nM).

Results
In LQT1 VCMs, preliminary results show an increase in IKs current density with ARA-S compared to baseline (+ 30 mV: baseline: 0.21 ± 0.1 pA/pF, N=2, n=4; ARA-S: 0.57 ± 0.18 pA/pF, N=2, n=5, p=0.14). Furthermore, ARA-S significantly shortened the APD at 90% repolarization compared to baseline (269.5 ± 19.9 to 221.7 ± 14.4 ms; N=2, n=8, p = 0.0078) without affecting the resting membrane potential, the upstroke velocity or the amplitude of the AP. This APD shortening was translated at the organ level to a significant shortening of the QT interval upon perfusion of ARA-S in ex-vivo whole hearts (203.5 ± 3.1 to 187.8 ± 3.4 ms, N=9, p = 0.0048). Finally, episodes of ventricular bigeminy and/or ventricular tachycardia observed in 4/8 LQT1 rabbit hearts at baseline with hypokalaemia and isoproterenol were completely prevented upon perfusion with ARA-S (0/8).

Conclusions
ARA-S shows promising APD/QT shortening effects at the cellular and organ levels in a rabbit model of LQT1 (with impaired IKs). Ongoing experiments investigating antiarrhythmic effects of ARA-S in LQT1 will reveal whether this compound could be considered as a new therapeutic strategy for LQT1 patients.

2.3

ARRHYTHMIA-MUTANT R231C STABILIZES THE VOLTAGE SENSOR IN CARDIAC POTASSIUM IKS CHANNELS IN THE INTERMEDIATE STATE

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Introduction
Voltage-gated cardiac IKs potassium channels, composed of KCNQ1 and KCNE1 subunits, are essential for heart rhythm stability. The arrhythmia-linked R231C mutation in KCNQ1 renders IKs channels voltage-independent, remaining open across physiological voltages, either by immobilizing the voltage sensor or the gate or by destroying the voltage sensor-to-gate coupling.

Methods
We combined the voltage clamp fluorometry technique with the two-electrode voltage clamp technique on Xenopus oocytes expressing human KCNQ1/KCNE1 channels to investigate the R231C mutation. Likewise, cell surface biotinylation and western blot analysis were used to assess R231C-F351A double mutant trafficking to the membrane. Thereafter, different electrophysiology was used to assess these mutants in mammalian cells.

Results
We examined voltage sensor movement and ion permeability in wild-type and R231C-mutated KCNQ1 and KCNQ1/KCNE1 channels. R231C abolished fluorescence signals from voltage sensor-attached fluorophores, indicating disrupted voltage sensor movement. Notably, R231C KCNQ1/KCNE1 channels remained conductive despite the S338F mutation, which blocks the activated open state, but non-conducting in the presence of the F351A mutation, which blocks the intermediate open state.

Conclusions
These findings suggest that the voltage sensor resides in an intermediate state in both R231C KCNQ1 and R231C KCNQ1/KCNE1channels. Additionally, R231 appears critical for KCNE1-mediated inhibition of the intermediate open state in wild-type IKs channels.

2.4

LOSS-OF-FUNCTION OF KV7.1 LEADS TO METABOLIC DYSFUNCTION THROUGH AGE-DEPENDENT PANCREATIC BETA-CELL DECLINE

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Introduction
KCNQ1 is a type 2 diabetes (T2D) susceptibility gene, although the mechanistic link remains unclear. People with Long QT Syndrome 1, carrying loss-of-function (LoF) mutations in KCNQ1, paradoxically present with hyperinsulinemia and an increased burden of T2D. We previously developed a mouse model with a clinically-identified Kcnq1 LoF mutation, which transitioned from initial hyperinsulinemia to age-dependent hypoinsulinemia, possibly explaining the increased T2D risk. We hypothesized that Kcnq1 LoF increases the susceptibility to β-cell decline in response to stressors such as aging and metabolic stress and aimed to investigate the underlying mechanisms.

Methods
Mice with Kcnq1 LoF and littermate controls were aged (>70 weeks) or received a high-fat-high-sucrose diet. Glucose homeostasis and β-cell function were assessed using in vivo blood glucose measurements, ex vivo pancreatic islet insulin secretion assays, whole-islet live Ca2+ imaging, and whole-cell patch clamp and Total Internal Reflection Fluorescence (TIRF) microscopy in dispersed β-cells. Additionally, published oral glucose tolerance test data in people with KCNQ1 LoF were reanalysed to investigate age-dependency of insulin secretion in humans.

Results
The initial insulin-hypersecretory phenotype in pancreatic islets from mice with Kcnq1 LoF is accompanied by increased glucose-induced Ca2+ influx, in line with reduced repolarization. However, age and metabolic stress result in β-cell dysfunction and diminished insulin secretion. On a high-fat-high-sucrose diet, wild-type mice preserve blood glucose homeostasis (p=0.55) with a 69±12% increase in insulin secretion compared to a control diet, while in Kcnq1 LoF mice, blood glucose increases (p=0.0003) with a 15±9% reduction in insulin secretion (p=0.001 LoF vs WT). Interestingly, endoplasmic reticulum stress is not involved in this transition to hypoinsulinemia. The dysfunction in Kcnq1 LoF β-cells is characterized by impaired glucose-induced insulin secretion (p=0.0099), reduced exocytosis (p=0.0063), reduced glucose-induced Ca2+ influx (p=0.0002), and reduced insulin secretion in response to the KATP channel blocker tolbutamide (p=0.0378), despite comparable insulin granule count, intact responses to high K+ in exocytosis and Ca2+ influx, intact Na+ current and increased Ca2+ current. Together this indicates impairment of the KATP-dependent trigger for insulin secretion in aged Kcnq1 LoF islets. Oral glucose tolerance test data in people with KCNQ1 LoF were reanalysed and suggest an accelerated age-dependent decline in insulin secretion occurs in humans too.

Conclusions
These findings imply that KCNQ1 LoF reduces β-cell resilience to ageing and metabolic stress, leading to β-cell dysfunction and insufficient insulin secretion, providing a possible mechanistic explanation for the elevated T2D risk.

2.5

DETERMINE THE ANTI-ARRHYTHMIC POTENTIAL OF KV7.1/KCNE1 ACTIVATORS USING LQTS ZEBRAFISH MODEL

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Introduction
The zebrafish (Danio rerio) has emerged as a powerful and relevant model in cardiac electrophysiology. At the embryonic stage, the heart becomes functional within less than 24 hours post-fertilization, and the natural transparency of the embryos allows direct, visualization of cardiac activity. Importantly, the zebrafish heart shares significant electrophysiological similarities with the human heart. In particular, the shape of the ventricular action potential closely resembles to the one observed in humans. This makes zebrafish a valuable model for studying cardiac rhythm disorders such as long QT syndrome (LQTS), as well as for screening disease-causing mutations and potential therapeutic compounds. The aim of this project is to generate zebrafish embryos carrying LQTS phenotype and then test small-molecule KV7.1/KCNE1 (IKs) activators in this arrythmic model.

Methods
To this end, we microinjected human KCNQ1/KCNE1 mRNA—either wild-type (WT) or carrying disease-causing mutations—into 1-cell stage embryos of the transgenic zebrafish line Tg(fli1:EGFP). Tg(fli1:EGFP) line was selected for its specific fluorescent labeling in endothelial cells, enabling direct visualization of cardiac structures and function. High-speed video recordings of the fluorescent heart were acquired to quantify cardiac performence, including heart rhythm and contractility. These parameters were analysed using changes in pixel intensity and body pose estimation artificial intelligence-based softwares. We also evaluated the effects of small-molecule Kv7.1 and IKs activators, including ML277 and polyunsaturated fatty acids (PUFAs), on cardiac function.

Results
Our results demonstrate that knockdown of endogenous Kv7.1 in zebrafish embryos using morpholino induces a significant prolongation of cardiac contraction, consistent with an LQTS-like phenotype. Functional rescue experiments injecting human WT IKs channels successfully restore normal contraction duration. In pharmacological experiments, treatment with ML277 a selective Kv7.1 activator, shortened contraction duration. In contrast, 518-LIN, a synthetic PUFA-based IKs activator, showed minimal effects on cardiac contraction.

Conclusions
In conclusion, zebrafish represent a robust and physiologically relevant model for studying LQTS, as modulation of the endogenous Kv7.1 channel is sufficient to induce a LQTS-like phenotype. Our high-speed video-based phenotyping approach reliably detects LQTS-associated functional abnormalities, such as the prolonged cardiac contraction duration phenotype in zebrafish embryos. Moreover, pharmacological activation of zebrafish Kv7.1 using ML277 resulted in a shortening of contraction duration, supporting its potential anti-arrhythmic properties. In future work, we aim to inject KV7.1/KCNE1 mRNA carrying human LQTS mutations to more accurately recapitulate the human disease phenotype in zebrafish embryos. This model will then be used as a platform for screening small-molecules with potential anti-arrhythmic properties.

3.1

TOWARDS A NEW CLASS OF KCNQ CHANNEL MODIFIERS FOR POTENTIAL THERAPEUTIC APPLICATIONS

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Despite the availability of a wide variety of antiepileptic drugs with diverse molecular targets and mechanisms of action, a significant proportion of patients continue to experience epileptic seizures. Neuronal KCNQ channels, particularly KCNQ2/3 heteromers, are considered key targets of antiseizure medications as the potentiation of these subthreshold channels effectively dampens the hyperexcitability, leading to seizure reduction or seizure freedom for patients. Currently available compounds in the preclinical stage, including retigabine analogs, are KCNQ openers that act by considerably shifting the conductance-voltage relationship of channels toward hyperpolarized potentials. Our recent study on KCNQ1 channels indicated that targeting the constricted cavity region of the channel pore offers a possibility to activate channels without causing substantial changes to their voltage-dependent behavior. Here we demonstrate that this concept is extendable to neuronal KCNQ channels, thereby paving the way for the discovery of a new class of KCNQ modifiers capable of boosting the channel activity via increasing the unitary conductance of channels while maintaining the conductance-voltage relationship largely unchanged.

3.2

DEVELOPING ANTIEPILEPTIC DRUG CANDIDATES WITH THE NOVEL KCNQ2 AGONIST EBIO1 AS A LEAD

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We reported a new scafold compound, Ebio1, serving as subtype-selective activator for the KCNQ2 and KCNQ2/3 channels (Nat Chem Biol, 2024, 20:847). Single-channel patch-clamp, cryogenic-electron microscopy and molecular dynamic simulations, along with chemical derivatives, reveal that Ebio1 engages the KCNQ2 activation by generating an extended channel gate with a larger conductance at the saturating voltage (+50 mV). This mechanism is diferent from the previously observed activation mechanism of ligands on voltage-gated ion channels. Ebio1 caused S6 helices to perform a twist-to-open movement, which was sufcient to open the KCNQ2 gate. Then, Using Ebio1 as a lead compound, we designed and synthesized a series of derivatives and evaluated their antiepileptic activities and drug-like profiles. A drug candidate with a developability profile has been found. The candidate exhibits high potency and favorable subtype selectivity to the KCNQ2 and KCNQ2/3. It has minimal effects on the urinary system. It exhibited high antiepileptic activity in mouse models, particularly with an EC50 value of 0.67 mg/kg in the MES test. In the neurotoxicity assessment with the Rotarod test, the TD50 value of the candidate is 6.7 mg/kg. Thus, compared with investigational drugs, the candidate drug has an advantage in terms of its safety window. Moreover, in a series of pharmacokinetic experiments and toxicology experiments, the candidate molecule also demonstrated a favorable drug-like profile. We will present the above results.

3.3

FROM KV7.2 ACTIVATION TO INHIBITION BY CHEMICAL REMODELING

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Introduction
KCNQ-encoded neuronal Kv7 (Kv7.2–Kv7.5) voltage-gated potassium channels play a key role in regulating neuronal excitability. Dysfunction of these channels is implicated in a wide spectrum of neuropsychiatric disorders, including epilepsy, neurodevelopmental disorders, and neurodegenerative diseases, making them attractive therapeutic targets. Retigabine, the first clinically approved Kv7 channel opener, demonstrated the therapeutic potential of Kv7 activation but was withdrawn mainly due to chemical instability. We have recently performed systematic structure-activity and pharmacological studies to identify and characterize novel series of retigabine analogues with improved pharmacodynamic and pharmacokinetic characteristic as Kv7 activators over the parent compound. These efforts led to the identification of compound 60 (c60), a chemically-stable, highly potent and effective Kv7 channel activator exhibiting stronger in vivo anticonvulsant effects when compared to retigabine. Preliminary results from modelling and mutagenesis experiments performed using the Cryo-electron microscopy (cryo-EM) structures of retigabine-bound Kv7.2 suggested that the c60 side chain accommodates into a large lipophilic cavity positioned at the S5-S6 interface of the pore domain (PD). This region undergoes to critical structural rearrangements during channel opening. Here, we describe a new medicinal chemistry campaign exploring this specific region of the c60 binding pocket: Specifically, we designed and synthesized a novel series of analogues in which bulkier substituents were introduced at the amide nitrogen of retigabine.

Methods
To investigate the molecular basis of Kv7.2 modulation, we combined synthetic and analytical chemistry approaches with in silico modelling and cryo-EM structural studies.

Results
Among the tested compounds we identified compound c106 acting as a potent Kv7 inhibitor. Cryo-EM and in silico analyses revealed that the activator c60 and the blocker c106 bind to the same hydrophobic pocket located at the S5–S6 interface within the pore domain, between adjacent subunits. Despite sharing a common binding site and several conserved interactions, the two compounds stabilize distinct conformational states of the channel. Specifically, c60 binding is associated with an open-state conformation, whereas c106 promotes a closed-state configuration. Comparative structural, computational, and mutagenesis analyses identified residue L307 as a critical determinant of these divergent effects. Substitution of L307 with smaller residues (alanine or valine) abolished the inhibitory action of c106, while leaving c60-induced channel activation largely unaffected.

Conclusions
These results, besides providing structural and functional insights into Kv7 channel gating, expand the arsenal of pharmacological tools available to modulate Kv7 channels, which may prove essential to guide the further design of Kv7 modulators with enhanced selectivity and specificity.

3.4

AKTIVA7E: A PROOF-OF-CONCEPT PHASE 1B STUDY TO EVALUATE THE SAFETY, TOLERABILITY, EFFICACY, AND PHARMACOKINETICS OF QRL-101, A NOVEL KV7.2/7.3 MODULATOR, IN KCNQ2/KCNQ3 RELATED EPILEPSY

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Introduction
QRL-101 is a novel Kv7.2/7.3 modulator small molecule in a unique chemical class from ezogabine. QRL-101 is designed to enhance Kv7.2/7.3 selectivity and to strongly reduce GABA-A activity for the treatment of epilepsy. Enhancement of Kv7.2/7.3 activity, and thus K+ M-current, is a tractable potential mechanism for regulating neuronal hyperexcitability and susceptibility to seizure propagation. KCNQ2 mutation is the leading cause of neonatal epilepsy and may result in other severe disabilities requirung lifelong care. There is currently therapy available for this population that targets enhanching Kv7.2/7.3 activity.

Methods
A proof of concept hybrid randomized, double-blind, placebo controlled Ph1b study will be initated in n=9 12-65yo with a KCNQ2/Q3 pathogenic mutation randomized 2:1 (QRL-101:placebo). A 4-week observational baseline period, to assess seizure frequency, will be followed by enrollment, randomization, 1-month dose adjustment period, 3-month treatment, then a 1-week taper or enrollment into extended dosing for 2 years. KCNQ2/Q3 related epilepsy diagnosis is dependent on a Diagnostic Review Committee in which loss-of-function mutations and a seizure frequency of ≥2 seizures/month is required for enrollment into the study.

Results
Primary endpoints are safety, tolerability, and pharmakokinetics. Key secondary endpoints are efficacy measures determined by reduction in seizures, additional efficacy secondaries include assessment of seizure trends, CDD-SA, CGI and PGI.

Conclusions
Aktiva7e will determine the safety, tolerability, PK, andearly insight on the effiacy of QRL-101 in KCNQ2/Q3 related epilepsy. QRL-101 has the potential to be a disease modifying precision medicine for the KCNQ2/Q3 LoF community.

3.5

ENHANCED SEIZURE SUSCEPTIBILITY AND COGNITIVE DEFICITS IN MICE EXPRESSING A GAIN-OF-FUNCTION EPILEPSY VARIANT IN THE PIP2-BINDING RESIDUE OF KV7.2

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Introduction
Kv7 potassium channels composed of Kv7.2 and Kv7.3 subunits are enriched at the axonal surface, where their current potently suppresses neuronal excitability. Dominant mutations in KCNQ2 and KCNQ3 genes that encode Kv7.2 and Kv7.3 cause benign familial neonatal epilepsy (BFNE) and epileptic encephalopathy (EE). Current treatments for EEs have limited efficacy in alleviating seizures and comorbidities, posing an urgent need to understand the underlying mechanisms. We have previously shown that a dual BFNE/EE variant K526N increases the sensitivity of Kv7.2 channels to the membrane lipid cofactor PIP2. Here, we investigated whether this gain-of-function underlie epileptic seizures and behavioural comorbidities.

Methods
We generated heterozygous Kv7.2 knock-in (KI) mice containing the K555N mutation which corresponds to K526N in human Kv7.2. EEG recording and behaviour tasks were performed in mice to examine seizure susceptibility, cognition, and behaviours. Biochemical experiments and immunohistochemistry were used to assess Kv7 expression and localization, neurodegeneration, and neuroinflammation in vivo.

Results
The heterozygous K555N KI mice showed comparable Kv7.2 expression in their hippocampi and cortices as the wild type (WT) mice. However, the KI mice displayed higher mortality and more generalized tonic clonic seizures and death when challenged by a GABAA receptor antagonist pentylenetetrazole (PTZ). This elevated seizure susceptibility was partially blocked by Kv7 agonist retigabine. Although the K555N KI mice showed normal motor coordination and locomotor activity, they displayed repetitive behavior and increased anxiety compared to the WT mice. Furthermore, the KI mice displayed significant reductions in object location memory, object recognition memory, and fear memory compared to the WT mice. We are currently conducting EEG and histological analyses to identify electrographic and pathological features in the KI mice.

Conclusions
These findings demonstrate that the BFNE/EE K555N mutation enhances seizure susceptibility although this variant is known to increase Kv7 current in the presence of PIP2. This mutation also impairs memory and induces enhanced repetitive and anxious behaviors. These cognitive and behavioral deficits are consistent with the symptoms of patients with the K526N mutation including moderate to drug-resistant neonatal seizures, severe intellectual disability, and behavioral deficits. Future studies will focus on molecular and cellular analysis of excitatory versus inhibitory neurons to identify how a gain-of-function of Kv7 current leads to seizures.

4.1

ANGIOTENSIN II REGULATES KV7 CHANNELS IN CENTRAL NEURONS AND BRAIN ENDOTHELIAL CELLS

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Introduction
The renin-angiotensin system (RAS) plays a pivotal role in the regulation of cardiovascular function, being often over-activated in hypertensive patients. Increased RAS activity and hypertension are also associated with late-onset epilepsy and alteration of blood-brain barrier (BBB) permeability. Angiotensin II (Ang II) regulates Kv7 channels in peripheral neurons and vascular smooth muscle cells, but no evidence is available about the possible modulation of Kv7 channels in the central nervous system (CNS). In the present work, we investigated the effects of Ang II on Kv7 channels expressed in neurons of different brain areas, and in brain endothelial cells (ECs).

Methods
Multi-electrode array (MEA) recordings were performed in brain slices to evaluate neuronal activity. Clonal neuronal (SHSY-5Y) and endothelial (BEND3) cells, ECs derived from induced pluripotent stem cells (hiBECs), as well as brain microvessels (BMVs) and brain samples from mice infused for 28 days with Ang II were used for Western blot and immunofluorescence experiments; permeability in BEND3 and hiBECs was assessed by measuring trans-endothelial electrical resistance (TEER).

Results
Exposure to Ang II (100-500 nM) for 6-24 hours reduced the expression of different Kv7 subunits in both isolated neurons (Kv7.2 and Kv7.3) and ECs (Kv7.5), as well as in brain slices including the cortex, the hippocampus, and the paraventricular nucleus of the hypothalamus, a crucial area involved in the control of sympathetic tone (Kv7.2 an Kv7.3). Slice treatment with Ang II (500 nM for 6 hours) increased neuronal activity, an effect possibly due to Kv7 channel downregulation, as suggested by the reduced sensitivity to the Kv7 blocker XE991 (10 μM). In ECs, Ang II-induced decrease in Kv7.5 levels was associated to enhanced phosphorylation of ZO-1, a critical tight junctional protein, leading to an increased endothelial permeability; all these effects were prevented by the Kv7 activators retigabine and ML-213 (both at 10 μM). In both neurons and ECs, Ang II-induced decrease of Kv7 proteins occurred through enhanced proteasome-mediated degradation. Interestingly, chronic exposure in vivo to Ang II also reduced the expression of distinct Kv7 subunit in neurons and BMVs.

Conclusions
Our data suggest that Ang II decreases Kv7 channel expression and function in multiple cell types, thus causing hyperexcitability both by direct neuronal actions, as well as by impairing BBB function. This dual function further confirms the heterogeneous role of distinct Kv7 channels at various cellular sites as key pharmacological targets for the treatment of CNS diseases characterized by neurovascular alterations.

4.2

GLIAL KCNQ CHANNELS REGULATE NEURONAL EXCITABILITY VIA GLIAL GABA RELEASE

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Precise control of neuronal excitability is essential for normal nervous system development and function, and its disruption contributes to epilepsy, learning disabilities, and autism spectrum disorder (ASD). KCNQ (Kv7) potassium channels are key regulators of membrane excitability and are well studied in neurons. In contrast, although KCNQ channels are expressed in glial cells, their functional roles in glia have remained largely unexplored. We show that KCNQ channels expressed in Caenorhabditis elegans glia regulate glial membrane potential and intracellular Ca^{2+}, enabling activitydependent GABA release from glial cells. This glial GABA signaling suppresses neuronal excitability through activation of neuronal GABAA receptors, revealing a previously unrecognized mechanism of excitability control. Expression of human KCNQ channels in nematode glia rescues hyperexcitability phenotypes, demonstrating strong functional conservation across species. Moreover, epilepsy and ASDassociated KCNQ2 loss and gainoffunction variants disrupt gliatoneuron GABA signaling in mutationspecific ways. These defects are partially reversed by the KCNQ channel opener retigabine, highlighting therapeutic relevance. To extend these findings to mammals, we present emerging data from mouse astrocytes demonstrating functional KCNQ expression and a role in regulating astrocytic Ca^{2+} dynamics. Using Ca^{2+} imaging, we find that pharmacological manipulation of KCNQ activity significantly alters astrocytic excitabilityrelated signaling, supporting a conserved glial role for KCNQ channels. These data suggest that astrocytic KCNQ channels shape the ionic and Ca^{2+} landscape necessary for effective glia-neuron communication in mammalian neural circuits. Together, our findings identify glial KCNQ channels as conserved regulators of neuronal excitability that act by controlling glial Ca^{2+} signaling and inhibitory gliotransmission. This work expands the conceptual framework of KCNQ channel function beyond neurons and has important implications for understanding circuit dysfunction and therapeutic targeting in epilepsy and ASD.

4.3

KV7.2 IS THE PRINCIPAL M-TYPE K+ CHANNEL SUBUNIT MODULATING PAIN BEHAVIOURS IN RATS

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Introduction
Non-opioid pain therapeutics are still limited in their use and effectiveness in clinical settings. Kv7 (KCNQ, M-type) potassium channels may represent promising pain targets as these are functionally expressed in peripheral somatosensory neurons and impose strong control over excitability. However, there are five Kv7 subunits expressed in mammalian tissues and attempts to non-selectively target Kv7 channels have met significant barriers due to the side effects associated with non-selective activation of the different subunits in other tissues. As there is currently no consensus as to which subunits are the functionally dominant in nociceptive neurons, identifying principal Kv7 subunit(s) in nociceptive neurons is necessary to develop selective peripheral Kv7 targeting strategies with minimal on-target side effects.

Methods
Here we combine measures of protein expression with functional patch clamp analysis, pharmacological profiling, in vitro and in vivo gene knockdown via intrathecal injection using self-delivering siRNA and behavioural testing to identify the most influential subunits in modulating pain response in rats.

Results
Kv7.2, Kv7.3 and Kv7.5 were most abundantly expressed across the whole dorsal root ganglion (DRG) and in small-diameter nociceptive neurons, with limited expression of Kv7.4 and Kv7.1 in these neurons. We demonstrated that Kv7 activators with different selectivity profiles, such as ICA-272243 (Kv7.2; Kv7.2/7.3) and retigabine (all Kv7s except of Kv7.1) displayed similar augmentation of Kv7 current in TRPV1 positive neurons. Individual subunit knockdown revealed a reduction in Kv7 current and increase in neuronal excitability of TRPV1-positive neurons with kcnq2 knockdown, while manipulations of Kv7.3 or Kv7.5 activity or expression generally failed to produce significant effects. In vivo DRG knockdown of Kcnq2 but not Kcnq5 resulted in an increased thermal and mechanical sensitivity and an increase in nocifensive behaviour in response to capsaicin injection.

Conclusions
Whilst expression profile alone was not sufficient to reveal an obvious targetable subunit, both pharmacological and genetic targeting of Kv7.2 consistently effected the neuronal excitability of TRPV1-positive neurons and the nociceptive behaviours of rats. Therefore, we conclude that Kv7.2 is the most functionally significant Kv7 subunit modulating pain sensitivity in rats.

4.4

KV7.2 CHANNELS SHAPE EXCITABILITY AND SALTATORY CONDUCTION AT THE NODE OF RANVIER IN HEAVILY MYELINATED NERVES

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Introduction
Previous immunohistochemical studies have shown the expression of Kv7.2 channels at nodes of Ranvier (NRs) of myelinated nerves. However, the functions of these channels at NRs remain elusive.

Methods
In the present study, we addressed this issue by directly applying whole-cell patch-clamp recordings at NRs of rat lumbar spinal ventral nerves in ex vivo preparations.

Results
We show that depolarizing voltages evoke large non-inactivating outward currents at NRs, which are partially inhibited by Kv7 channel blocker linopirdine and potentiated by Kv7 channel activator retigabine. Furthermore, linopirdine significantly alters intrinsic electrophysiological properties of NRs to depolarize resting membrane potential, increase input resistance, prolong AP width, reduce AP threshold, and decrease AP amplitude. On the other hand, retigabine significantly decreases input resistance and increases AP rheobase at NRs. Moreover, linopirdine increases excitability at NRs by converting single AP firing into multiple AP firing at many NRs. Saltatory conduction velocity is significantly reduced by retigabine, and AP success rate at high stimulation frequency is significantly increased by linopirdine.

Conclusions
Collectively, Kv7.2 channels play a significant role in regulating intrinsic electrophysiological properties and shaping saltatory conduction at NRs of motor nerve fibers of rats. These findings may provide insights into how loss-of-function mutations in Kv7.2 channels can lead to neuromuscular disorders in patients.

4.5

PHARMACOLOGICAL ACTIVATION OF KCNQ4 SUPPRESSES PERIPHERAL NOCICEPTIVE SIGNALING IN MICE

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Introduction
Peripheral ion channels are important regulators of nociceptive excitability and represent promising targets for non-opioid analgesic development. KCNQ/Kv7 potassium channels provide a stabilizing outward conductance that limits neuronal hyperexcitability; however, the therapeutic value of targeting individual KCNQ subtypes remains incompletely defined. Here, we investigated whether pharmacological activation of KCNQ4 could modulate peripheral nociceptive signaling and produce analgesic benefit.

Methods
We combined molecular profiling, genetic loss-of-function analysis, whole-cell patch-clamp recordings, ex vivo functional assays, and in vivo pharmacology. KCNQ4 expression and functional contribution were assessed in peripheral nociception-related tissues. A newly developed small-molecule activator was evaluated in heterologous expression systems. Primary sensory neuron recordings and animal behavioral studies were used to assess its functional and pharmacological effects.

Results
KCNQ4 was detected in peripheral compartments associated with nociceptive regulation, and genetic deletion of Kcnq4 enhanced pain-related sensitivity, supporting an endogenous inhibitory role for this channel. A newly developed small-molecule activator potentiated KCNQ4 currents and was further evaluated in peripheral preparations. In primary sensory neurons, the compound enhanced KCNQ-mediated M-current and suppressed action potential firing; these effects were markedly reduced in Kcnq4-deficient neurons. In vivo, the compound produced significant analgesic efficacy in multiple preclinical assays, with effects substantially attenuated in Kcnq4-deficient mice. Behavioral safety assays did not reveal obvious locomotor suppression or motor impairment under the tested conditions.

Conclusions
These findings identify KCNQ4 as a peripheral inhibitory regulator of nociceptive signaling and support pharmacological activation of KCNQ4 as a promising non-opioid strategy for analgesic development. The newly developed small-molecule activator provides a useful chemical probe for dissecting KCNQ4 function in peripheral nociceptive regulation.

5.1

TDP-43-DEPENDENT MIS-SPLICING OF KCNQ2 TRIGGERS INTRINSIC NEURONAL HYPEREXCITABILITY IN ALS/FTD

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Motor neuron hyperexcitability is a broadly observed yet poorly understood feature of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Nuclear depletion and cytoplasmic aggregation of the RNA splicing protein TAR DNA-binding protein 43 (TDP-43) are observed in most ALS and FTD patients. Here we show that TDP-43 dysfunction causes mis-splicing of KCNQ2, which encodes a voltage-gated potassium channel (Kv7.2) that regulates neuronal excitability. Using iPSC-derived neurons and postmortem ALS/FTD brain and spinal cord tissue we find widespread, disease-specific and TDP-43-specific skipping of an exon encoding the KCNQ2 pore domain. The mis-spliced mRNA escapes degradation and is translated into a nonfunctional protein with severely reduced ion conductance that aggregates in the endoplasmic reticulum and causes intrinsic hyperexcitability in ALS neuronal models. This event, which correlates with higher phosphorylated TDP-43 levels and earlier age of disease onset in patients, can be rescued by splice-modulating antisense oligonucleotides that dampen hyperexcitability in induced pluripotent stem cell cortical neurons and spinal motor neurons with TDP-43 depletion. Our work reveals that nuclear TDP-43 maintains the fidelity of KCNQ2 expression and function and provides a mechanistic link between established excitability disruption in ALS/FTD patients and TDP-43 dysfunction.

5.2

TUNING KV7/KCNQ CHANNEL DENSITY THROUGH ENDOSOMAL 2CL-/H+ EXCHANGE: IMPLICATIONS FOR NEURONAL EXCITABILITY

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Introduction
CLCN3 and CLCN4 encode the endosomal 2Cl-/H+ exchangers ClC-3 and ClC-4, which are highly expressed within the CNS, including hippocampal formation. Pathogenic variants in these genes give rise to the rare CLCN3- and CLCN4-neurodevelopmental conditions (CLCN3/4-NDCs), characterised by a range of neurological and neuropsychiatric complications, including global developmental delay, intellectual disability, seizures, behavioural issues, and brain abnormalities. The mechanisms by which ClC-3 and ClC-4 regulate neuronal function and viability, as well as the molecular pathways affected in CLCN3- and CLCN4-NDCs, remain unknown. Here, we investigate how deficits in ClC-3 or ClC-4 alter neuronal excitability and morphology in hippocampal neurons.

Methods
We combined patch-clamp electrophysiology with simultaneous intracellular biocytin filling in acute hippocampal slice preparations from Clcn3-/- and Clcn4-/- mouse models. We characterised the passive and active membrane properties of CA2 neurons, followed by detailed 3D morphological reconstructions using Neurolucida software.

Results
Two firing patterns were found in the hippocampus's Cornu Ammonis 2 (CA2) region: regular and burst firing. At post-natal day 13, 62% of the assessed CA2 wild-type neurons showed a rhythmic bursting behaviour; this was reduced to 19% in Clcn4-/- and completely absent in the Clcn3-/- condition. Changes in the firing patterns were accompanied by a depolarising shift in the action potential threshold and an increase in the afterhyperpolarizing phase of the action potentials. Blockade of Kv7/KCNQ and, to a lesser extent, Kv1, but not BK, SK, or Kv2 channels, recapitulates the wild-type firing pattern phenotype in the Clcn3-/- neurons. Consistent with an increase in the Kv7/KCNQ density, total KCNQ-mediated deactivating currents were larger in Clcn3-/- CA2 than in the WT CA2 neurons. Application of 1 mM TEA, a concentration that preferentially blocks KCNQ2 channels over KCNQ3 or KCNQ2/3 heterotetramers, resulted in a greater block in Clcn3-/- CA2 neurons, indicating an increased contribution of KCNQ2 channels to the total deactivating currents. Moreover, we detected abnormalities in the neuronal complexity. Branching and lengths of apical and basal domains were significantly reduced in the Clcn3-/- and moderately altered in the Clcn4-/- neurons.

Conclusions
Our study establishes that 2Cl−/H+ exchangers are critical regulators of neuronal intrinsic excitability, acting by precisely modulating Kv7/KCNQ channel density. We further show that disruption of this exchange function contributes to significant changes in dendritic structure. Our findings provide new insights into the underlying molecular mechanisms of 2Cl-/H+ exchangers in neurons and identify Kv7/KCNQ channels as a potential therapeutic target for patients with CLCN3- and CLCN4-related neurodevelopmental disorders.

5.3

GBΓ DUALLY REGULATES THE M-CURRENT VIA INCREASED PIP2 SENSITIVITY AND TRAFFICKING

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Introduction
Heterotrimeric G protein βγ subunits (Gβγ) are responsible for a variety of physiological processes, including associated signaling pathways downstream of G protein-coupled receptors (GPCRs) and direct modulation of ion channels, thereby regulating neuronal excitability and diverse cellular functions. The neuronal M-current, carried by Kv7.2/7.3 channels, is a key determinant in regulating the excitability of neurons and seizure susceptibility, and its disruption contributes to epileptic disorders and altered network excitability. Pathogenic variants in GNB1, which encodes the Gβ1 isoform, cause a neurodevelopmental encephalopathy characterized by developmental delay and epileptic-like seizures, further underscoring the importance of Gβγ-dependent regulation of neuronal excitability. Previous work on Kv7.4 channels demonstrated that Gβγ interacts with and positively regulates channel activity, prompting us to determine whether, and how, Gβγ interacts with M-current channels.

Methods
Twoelectrode voltage clamp (TEVC) recordings were performed in Xenopus laevis oocytes to assess Kv7.2/7.3 currents under different Gβγ conditions. Giant membrane patches (GMPs) were immunostained to quantify plasmamembrane (PM) Gβ and Kv7.2/7.3 channel abundance. Proximity ligation assay (PLA) was used to assess protein–protein interactions in HEK293 cells, and peptide array mapping was employed to identify Kv7.2/7.3 regions interacting with Gβγ. A voltagesensing phosphatase (VSP) was used to deplete PIP2 and probe Gβγ effects on PIP2dependent channel regulation.

Results
Coexpression of Kv7.2/7.3 with Gβγ produced an approximately 2fold increase in current amplitude without significant changes in standard biophysical parameters. Gβ isoforms differentially enhanced Kv7.2/7.3 current, with Gβ1 producing the largest increase, followed by Gβ2 and then Gβ3. Diseaseassociated Gβ1 mutants K78R and I80T increased Kv7.2/7.3 similarly to wildtype (WT), whereas I80N was lossoffunction. Sequestration of Gβγ from the plasma membrane (PM) by myristoylated phosducin (myrphosducin) reduced Kv7.2/7.3 current and induced a depolarizing shift of ~4 mV in the voltage dependence compared with channels coexpressed with Gβγ. Coexpression of Gβγ with Kv7.2/7.3 significantly increased channel abundance at the PM, whereas coexpression of myrphosducin reduced PM abundance, indicating that membraneassociated Gβγ is critical for efficient Kv7.2/7.3 trafficking and surface stability. Using peptide array mapping and PLA, we found that Kv7.2/7.3 interacts directly with Gβγ. Furthermore, VSPmediated PIP2 depletion revealed that Gβγ slowed the rate of Kv7.2/7.3 current rundown compared with control, consistent with stabilization of channel-PIP2 interactions.

Conclusions
Our results show that Kv7.2/7.3 channels are dually regulated by Gβγ: Gβγ increases current by enhancing channel abundance at the plasma membrane and by stabilizing Kv7.2/7.3-PIP2 interactions, indicating that Gβγ acts as a trafficking regulator and direct physiological gating modulator of Kv7.2/7.3.

5.4

DRUG:LIPID INTERACTIONS REVEAL AN ENORMOUS DYNAMIC RANGE OF KCNQ POTASSIUM CHANNEL REGULATION

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Introduction
Kv7 voltage-gated potassium channels exhibit polymodal regulation by factors including voltage, PIP2 phospholipids, and calmodulin. Due to the powerful neuromodulatory role of Kv7 channels, there are ongoing efforts to develop Kv7-targeted activator drugs, primarily as therapeutics for seizures and pain. However, the functional interplay between therapeutic drugs and physiological regulators like PIP2 is poorly understood.

Methods
We used whole-cell patch clamp electrophysiology, rapid drug application, and PIP2 modulation by PI-5-kinase co-expression or DrVSP (voltage-sensitive phosphatase). Kinetic models were constructed to describe the functional relationship between PIP2 and drug association with channels.

Results
We characterized the effects of an experimental Kv7.2 activator/potentiator, ML213, in altered cellular PIP2 levels. When PIP2 levels are ambient/low, Kv7.2 exhibits activation V1/2s in the range of -20 mV in drug-free conditions, along with prominent ML213-mediated current augmentation. However, when PIP2 levels are elevated, Kv7.2 V1/2 is shifted to very hyperpolarized voltages, and the nature of the ML213 response changes, exhibiting minimal augmentation but powerful gating shifts that strongly stabilize channel opening. Critical features of the functional interplay between ML213, PIP2, and voltage, can be reproduced by a simple model based on mutual stabilization of ML213 and PIP2 when channels are open. The model and data highlight that in the presence of the ML213, PIP2 is tightly bound by Kv7.2 and strongly resistant to hydrolysis by a voltage-sensitive phosphatase. Also, as PIP2 levels are decreased, channels approach a characteristic 'limiting V1/2' that reflects the intrinsic voltage sensitivity of the channels. This fundamental channel property is useful to disentangle influences of voltage vs. PIP2 on gating properties, along with mechanistic effects of mutations.

Conclusions
Our study aligns empirical features with underlying principles of how PIP2 shapes pharmacological responses of Kv7 channels, and highlights how conventional biophysical measurements of ion channel gating may not fully reveal the effects of disease-linked mutations.

5.5

AN UPSTREAM OPEN READING FRAME REPRESSES TRANSLATION OF KCNQ2

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Introduction
Upstream open reading frames (uORFs) within the 5'-untranslated region (5'-UTR) of mRNA transcripts can regulate protein translation. Despite widespread prevalence within the human genome, they remain unidentified for many clinically relevant genes, including the neuronal voltage-gated potassium channel subunit KCNQ2. Heterozygous loss-of-function KCNQ2 variants are known to cause a range of neurodevelopmental disorders and epileptic encephalopathies, but there remains an unmet clinical need for patients harboring these variants.

Methods
We analyzed ribosome profiling datasets to assess the KCNQ2 5'-UTR for actively translated uORF motifs. Putative motifs were disabled using site-directed mutagenesis in plasmid constructs and overexpressed in mammalian cells. Translation efficiency of KCNQ2 was determined using reporter gene assays, western blotting, quantitative PCR, and whole-cell patch clamp electrophysiology. Finally, adenine base editing was used to endogenously disable the uORF motif in a neuron-like cell line natively expressing KCNQ2, validated with ribosome profiling.

Results
We identified a single uORF in KCNQ2 that is highly repressive of protein translation and demonstrated that mutations disabling the uORF start codon enhance translation of encoded potassium channels. Additionally, we show that adenine base editing of the uORF start codon can weaken ribosome engagement at the uORF and enhance translation of the protein endogenously.

Conclusions
This study establishes a previously underexplored regulatory feature for KCNQ2 and highlights the importance of understanding uORFs for clinically relevant genes, both for assessing disease risk and therapeutic potential.

6.1

OESTRUS CYCLE REGULATION OF CEREBRAL ARTERY KV7 CHANNELS

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Introduction
Non-genomic, fast acting oestrogenic signalling negatively regulates the membrane abundance and function of KV7 channels within gastrointestinal epithelia. Natural fluctuations of oestrogen across the rodent oestrous cycle similarly negatively regulates KV7.4 in mesenteric and renal arteries (Baldwin et al., 2023).

It remains to be discovered whether this mechanism also occurs within the cerebrovasculature, where KV7 channels are essential components of tone and receptor mediated signalling cascades

In the present study, we investigated the properties of KV7.4/5 across the rodent oestrus cycle in middle cerebral and basilar arteries (MCAs/BAs) from female Wistar Kyoto rats. We report for the first time, the contribution of microtubule/dynein dependent KV7 subcellular transport to oestradiol (E2) mediated KV7 channel inhibition and trafficking.

Methods
Animals were separated into Dioestrus/Met-oestrus (D/M) and Pro-oestrus/Oestrus (P/E) by changes in cervical cytology. MCA and BAs were mounted in a wire myograph for isometric tension recording of arterial tone. The relative abundance of Kcnq1-5/Kcne1-5 transcripts was measured in whole lysates of cerebral arteries via RT-qPCR. The membrane abundance of KV7.4/5 was determined in single XY cross sections in whole mounted middle cerebral arteries by confocal microscopy.

Results
The sensitivity to the Kv7.2-7.5 activator ML213 and the membrane abundance of KV7.4 & 5 was significantly increased in arteries from D/M, where serum E2 is low, independent of a change in transcript abundance. The sensitivity to CGRP was also enhanced in BAs from these animals, which coincided with a greater sensitivity to KV7 channel inhibition. Supplemental E2 inhibited ML213 sensitivity and decreased the membrane abundance of KV7.4. The effects of E2 were inhibited by both colchicine, which destabilises the microtubule network preventing its dynamic reorganisation, and ciliobrevin, the dynein motor protein inhibitor.

Conclusions
The present study reinforces the growing body of literature which suggests that physiological fluctuations of E2 are a key regulator of KV7 channel function within the female rodent. Further, we demonstrate for the first time, the contribution of the microtubule network and the motor protein dynein to the E2-KV7 signalling axis.

6.2

NOVEL CRYPTIC SPLICING EVENT IN THE MITOCHONDRIAL KV7.4 CHANNEL

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Introduction
Recent work has identified voltage-gated potassium channel subunits of the Kv7 (KCNQ) family, particularly Kv7.4 (KCNQ4), in mitochondrial fractions from cardiac and neuronal tissue. The mechanisms governing mitochondrial targeting and functional diversity of ion channels such as Kv7.4 remain poorly understood. One emerging determinant of mitochondrial protein heterogeneity is alternative pre-mRNA splicing, including cryptic splicing events that generate non-canonical transcript variants. In the present study, we report the discovery and characterization of cryptic splicing events in the mitoKv7.4 channel.

Methods
Skeletal muscle RNA was isolated from rats and humans and reverse transcribed for PCR analysis using primers spanning exons 7 to 10 of KCNQ4. Sequencing of the PCR product determined the nucleotide sequence and mass spectrometry was used to determine changes in the protein sequence. Mitochondrial oxygen consumption was measured by high-resolution respirometry. Two-electrode voltage clamp was performed on Xenopus oocytes injected with specific Kv7.4 isoforms.

Results
In rat and human skeletal muscle samples, we identified a cryptic splicing event in which the wild-type exon 9 is skipped and replaced by the insertion of a segment of intron 9. The inserted sequence was in frame and did not result in a frameshift or alter amino acid sequence of downstream exons.

Oxygen consumption in human skeletal muscle decreased in a concentration-dependent manner with the Kv7.2-7.5 activator ML213.

Heterologous expression of the wild-type, exon 9-deleted, and intron 9-inserted constructs in oocytes enabled us to record changes in membrane currents. Deletion of exon 9 increased the current amplitude 3.3 ± 0.4-fold and shifted the V0.5 by +11.3 ± 0.7 mV. However, insertion of the partial intron 9 sequence identified in rat and human skeletal muscle produced current characteristics similar to those of wild typeKv7.4 currents.

Conclusions
We identify a novel cryptic splice event in KCNQ4, which likely underlies the mitoKv7.4 channel. The physiological impact associated with mitochondrial function of this cryptic splice event remains to be determined.

6.3

CONTRIBUTION OF KV7 CHANNELS TO M2 MUSCARINIC RECEPTOR-DEPENDENT CONTRACTIONS OF AIRWAY SMOOTH MUSCLE

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Introduction
Cholinergic nerve-evoked contractions of airway smooth muscle (ASM) were traditionally thought to be exclusively mediated by activation of M3 muscarinic receptors (M3Rs) via pathways involving Ca2+ release from intracellular Ca2+ stores. However, there is increasing recognition that activation of postjunctional M2Rs also play an important role1. The M2R-dependent responses involve Ca2+ entry via voltage-dependent Ca2+ channels (VDCC)2, but the mechanisms linking M2Rs to activation of VDCC are unclear. ASMC possess Kv7 channels, which are known to be regulated by activation of muscarinic receptors3. Therefore, the purpose of the present study was to investigate if M2R-dependent contractions of ASM involved inhibition of Kv7 channels.

Methods
Contractions of mouse ASM were recorded using isometric tension recording. Nerve-evoked contractions were induced by electric field stimulation (EFS) at 100 and 10 second intervals. Real-time quantitative PCR and immunocytochemistry were used to investigate the expression of KCNQ and Kv7 subtypes in mouse ASM cells. The whole cell patch clamp technique was employed to examine the effects of M2R activation on Kv7 currents recorded from HEK293 cells co-expressing M2Rs with Kv7.4 or Kv7.5.

Results
Kv7.5 was the most highly expressed Kv7 subtype in mouse ASM, followed by Kv7.4. Reducing the EFS stimulus interval from 100 to 10s increased the amplitude of neurogenic contractions of murine bronchial rings, consistent with activation of M2Rs4. Inhibition of Kv7 channels with XE991 increased the amplitude of EFS responses recorded at 100 s intervals, but not at 10 s. The Kv7 channel openers ICA069673 and ML213 inhibited M2R-dependent contractions and similar effects were obtained using the adenylate cyclase activator forskolin and the PKA activator 6MB-cAMP. The effects of M2R activation on Kv7 currents was determined by examining the effect of carbachol on HEK293 cells co-expressing M2Rs with Kv7.4 or Kv7.5. Activation of M2Rs reduced the amplitude of both Kv7.4 and Kv7.5 currents. ICA069673 reversed the inhibitory effects of M2R activation on Kv7.4, while ML213 reversed the effects on both Kv7.4 and Kv7.5. Inclusion of PIP2diC8 in the pipette prevented the inhibitory effects of carbachol on Kv7.4, but not Kv7.5, whereas 6MB-cAMP potentiated Kv7.5 currents but not Kv7.4.

Conclusions
M2Rs exert their contractile effects on ASM via inhibition of Kv7.4 and Kv7.5 channels by pathways that deplete PIP2 and reduce PKA activity.

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6.4

THERAPEUTIC EFFECTS OF A KV7.4 ACTIVATOR, URO-K10, ON PULMONARY ARTERIAL HYPERTENSIVE RATS: A NOVEL MODE OF ACTION VIA MITOCHONDRIA

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Introduction
The prognosis of pulmonary arterial hypertension (PAH) is still poor. In the rat PAH model induced by monocrotaline injection (PAH-MCT), our previous myography study showed that the pulmonary arteries (PAs) are effectively relaxed by a novel Kv7.4 activator, URO-K10 (UK10).

Methods
Dual-wire myography for PA contractility measurement. Whole-cell patch clamp to record K+ currents. TMRM fluorescence measurement to record mitochondrial membrane potential (ΔΨm). Mitochondrial respiration was assessed using the Seahorse XFp Cell Mito Stress Test.

Results
In vivo application of URO-K10 using osmotic mini-pump to the PAH rat (PAH-MCT/UK10) significantly improved the survival period as well as reducing the PA wall thickening. Electron microscopy and immunoblot studies indicated increased mitochondrial fission and impaired mitophagy in PAH-MCT, which were reversed in PAH-MCT/UK10. Interestingly, the expression of Kv7.4 was found in the mitochondria enriched fraction of PASMCs, and the direct application of UK10 partly depolarized the ΔΨm. The ΔΨm was more hyperpolarized in PAH-MCT than control, and was reversed in PAH-MCT/UK10. Oxidative phosphorylation analysis revealed decreased levels of maximal respiration, ATP production, and OCR:ECAR ratio in PAH-MCT PASMCs, which were restored in PAH-MCT/UK10. The immunoblot analysis of electron transport chain proteins revealed decreased complex V and UCP2 while increased complex I and II in PAH-MCT, consistent with the hyperpolarized ΔΨm, and these changes were restored in PAH-MCT/UK10. MitoSOX analysis showed increased mitochondrial ROS and recovery in PAH-MCT and PAH-MCT/UK10, respectively. Also, in the right ventricular cardiomyocytes from PAH rats, URO-K10 reversed aberrant mitochondrial dynamics and mitophagy markers.

Conclusions
We suggest that URO-K10 not only reduces the resistance of pulmonary circulation but also restores mitochondrial homeostasis and PA remodelling, offering a novel therapeutic strategy for the treatment of PAH.

6.5

IDENTIFICATION OF SODIUM/MYO-INOSITOL TRANSPORTER 1 AS A MAJOR DETERMINANT OF ARTERIAL CONTRACTILITY THROUGH MODULATION OF KV7.4/5 CHANNEL ACTIVITY

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Introduction
As the sodium/ myoinositol transporter (SMIT1) is a positive regulator of Kv7.4/7.5 channels in arterial smooth muscle we postulated that altering SMIT1 expression could have a major impact upon vascular reactivity. Consequently, this study aimed to characterise the effects of changes of SMIT1 membrane abundance on vascular tone and the molecular mechanisms involved.

Methods
Isometric tension recordings on 2nd order mesenteric arteries and left anterior coronary arteries from male and female Wistar Han rats were performed with morpholino based protein knockdown of SMIT1. Whole artery membrane potential recording alongside electrophysiology patch-clamp recordings of Kv7.4 channels. Proximity ligation assay and whole artery antibody-based imaging.

Results
Morpholino-mediated knockdown of SMIT1 enhanced U46619- and methoxamine-mediated contractions, while attenuating relaxations to the Kv7 activator ML213, isoprenaline and CGRP in mesenteric arteries. Incubating HEK cells expressing Kv7.4 with raffinose for 1 h increased current amplitude and left shifted voltage-dependence by ~8mV when cells were co-expressed with SMIT-1 only. Similar treatments of arteries increased SMIT1 membrane abundance in smooth muscle cells, impaired receptor-mediated contractions of mesenteric and coronary arteries, augmented relaxations to ML213 and adenosine and induced membrane potential hyperpolarisation. The SGK1 inhibitor EMD638683, involved in regulating SMIT1 membrane expression, prevented the acute raffinose-induced increase in SMIT1 and the associated anti-contractile effects. Proximity ligation assays revealed that raffinose incubation increased the association of SMIT-Kv7.4/7.5 as well as Kv7.4 and Gβγ subunits.

Conclusions
Altering the membrane abundance of SMIT1 had a dramatic effect on the arterial response to vasonstrictors and vasodilators mediated by greater coordination of Kv7 channels and Gβγ subunits. This work identified SMT1-Kv7 channel complexes and SGK1 regulation as key modulators of arterial responsiveness.

7.1

THERAPEUTIC STRATEGIES FOR KCNQ4-ASSOCIATED HEARING LOSS

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Introduction
KCNQ4 encodes a voltage-gated potassium channel (Kv7.4) expressed in the basolateral membrane of cochlear outer hair cells (OHCs), mediating potassium efflux essential for OHC repolarization and survival. Pathogenic variants cause DFNA2, an autosomal dominant nonsyndromic hearing loss characterized by late-onset, progressive, high-frequency loss spanning all frequencies with age. Most variants cluster around the pore region, exerting dominant-negative effects on wild-type Kv7.4 and disrupting potassium recycling, leading to progressive OHC degeneration. KCNQ4 is the most frequently mutated gene in autosomal dominant nonsyndromic hearing loss in our cohort, yet no curative treatment currently exists.

Methods
We systematically analyzed gnomAD and the Yonsei University Hearing Loss (YUHL) cohort to identify overlooked KCNQ4 variants, characterizing their functional impact by patch-clamp electrophysiology, surface biotinylation, and AlphaFold2 structural modeling. For therapeutic development, we generated Kcnq4 p.W276S knock-in mice and applied two allele-targeting strategies: (1) in vivo CRISPR/Cas9 gene editing to disrupt the dominant-negative allele in OHCs; and (2) allele-preferential antisense oligonucleotides (ASOs) delivered via round window membrane injection at postnatal days 1–3. Auditory function was assessed by ABR and DPOAE, and OHC function by patch-clamp and RNA sequencing.

Results
Numerous rare KCNQ4 pore-region variants in public databases and the Korean general population significantly impaired K+ channel activity via dominant-negative mechanisms, suggesting they are underappreciated contributors to adult-onset hearing loss. KCNQ activators rescued variants with residual channel activity, while pore-disrupting variants were non-responsive. In vivo CRISPR/Cas9-mediated allele disruption, even at ~0.6% editing efficiency, significantly improved ABR and DPOAE thresholds and restored hyperpolarized OHC membrane potential, confirmed by a novel ex vivo thallium flux assay. The lead ASO candidate (ASO-123) preferentially suppressed mutant over wild-type Kcnq4 transcripts, attenuated progressive hearing loss across all tested frequencies for up to 7 weeks, improved OHC survival in apical and mid-cochlear turns, and partially restored IK,n current and resting membrane potential. Transcriptomic analysis confirmed restoration of potassium transport and hearing maintenance programs without immune activation.

Conclusions
KCNQ4 variants contribute more broadly to progressive hearing loss than currently recognized. Both CRISPR/Cas9-based allele disruption and ASO-mediated allele silencing restored auditory function in the DFNA2 mouse model, establishing promising therapeutic platforms for dominant-negative KCNQ4-associated hearing loss.

7.2

A ROLE OF KCNQ-INDUCED PLASTICITY ON NOISE-INDUCED NEURAL AND PERCEPTUAL HYPERSENSITIVITY TO SOUND

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In all sensory systems, damage to peripheral organs reduces input to the brain, triggering brain plasticity to compensate for this loss. In the auditory system, cochlear damage induces compensatory changes in both cortical and subcortical regions, with the auditory cortex (AC) showing particularly robust plasticity. This plasticity results in increased neuronal responsiveness to the spared inputs, allowing neuronal sound-evoked responses to recover or even increase despite peripheral loss. While this plasticity supports recovery of sound detection, it can also lead to disorders like tinnitus and hyperacusis, characterized by neural hyperactivity and pathological perceptual sound hypersensitivity. Despite the impact of cortical plasticity on the recovery of auditory perception and associated disorders, the circuit, cellular, and synaptic mechanisms underlying this plasticity remain poorly understood, limiting the development of effective treatments.

Our recent studies in the AC unmasked cell-type-specific plasticity that is associated with restored, albeit maladaptive at times, neural and perceptual responses after noise-induced hearing loss (NIHL). This plasticity involves changes in the intrinsic (channel) properties of parvalbumin-expressing cortical interneurons (PVs), partly driven by biophysical changes in KCNQ potassium channels. Here, we report that transient post-NIHL activation of KCNQ channels with a highly specific small molecule induces a novel form of corrective plasticity that counteracts maladaptive changes, including neural and perceptual hypersensitivity to sound.

7.3

TOWARDS SCALABLE PRECISION THERAPEUTICS FOR KCNQ2-DEE

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Introduction
KCNQ2 encodes the Kv7.2 voltage-gated potassium channel subunit, which plays a critical role in stabilizing neuronal membrane potential and limiting hyperexcitability. Pathogenic variants in KCNQ2 give rise to two distinct clinical phenotypes. Benign familial neonatal epilepsy (BFNE), caused by haploinsufficiency of KCNQ2, presents with infantile seizures and normal neurodevelopmental outcomes. In contrast, KCNQ2 developmental epileptic encephalopathy (DEE) results from missense mutations with dominant-negative effects, whereby dysfunctional subunits disrupt KCNQ2/KCNQ3 channel complexes, reducing effective gene dosage well below 50%, leading to severe epilepsy and profound cognitive impairment. This genotype-phenotype dichotomy presents a therapeutic opportunity: allele-specific knockdown of the dominant-negative allele could potentially shift the phenotype toward a milder, BFNE-like state.

Methods
We designed and screened over 1,000 RNase H-activating antisense oligonucleotides (ASOs) to achieve allele-selective knockdown of mutant KCNQ2. Long read whole genome sequencing was performed in a cohort of 10 patients with KCNQ2-DEE to identify benign single nucleotide polymorphisms (SNPs) in phase with pathogenic variants, enabling allele-specific targeting. From an index patient, we generated patient-derived induced pluripotent stem cell (iPSC) neurons for high-throughput screening of ASOs, assessing potency and selectivity. Lead candidates were further evaluated for immunogenicity in BJAB cytokine release assays and for safety in vivo following intracerebroventricular (ICV) injections in wild-type mice, with assessment of acute and delayed neurotoxicity and histopathology.

Results
Multiple candidate ASOs demonstrated robust potency and allele selectivity. Lead compounds achieved up to 85% knockdown of the mutant KCNQ2 allele in patient-derived neurons, with minimal impact on the wild-type allele. Top candidates were well tolerated in cytokine release assays and showed no concerning findings in preliminary mouse toxicity studies.

Conclusions
These findings provide proof-of-concept for allele-selective knockdown as a therapeutic strategy for KCNQ2-DEE. Our lead ASO targets a SNP with an allele frequency of approximately 30%, potentially enabling treatment of ~21% of the KCNQ2-DEE population. Development of a portfolio of ASOs targeting common haplotypes, rather than private mutations, may enable N-of-1 therapeutic strategies to scale. Successful clinical translation will require robust natural history data, validated biomarkers, and patient-reported outcomes to ensure meaningful and safe evaluation. Supported by the FDA's emerging plausible mechanism pathway, this portfolio-based strategy offers a path to broader therapeutic access for patients with rare diseases such as KCNQ2-DEE.

7.4

DEVELOPMENT AND EVALUATION OF SYSTEMIC AAV GENE THERAPY TO TREAT NEONATAL KCNQ2 EPILEPTIC ENCEPHALOPATHY

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Introduction
KCNQ2 developmental-and-epileptic-encephalopathy (KCNQ2-DEE) is the most common genetic neonatal epilepsy and is frequently refractory to pharmacologic treatment. KCNQ2-DEE cause severe seizures early in life and long-term cognitive impairment. Despite strong genetic causality, therapeutic options remain limited, and it is unclear whether systemic gene replacement can effectively rescue disease phenotypes, particularly given the narrow therapeutic window associated with both loss- and gain-of-function mechanisms. Here, we evaluate the feasibility of systemic adeno-associated virus (AAV)-mediated KCNQ2 gene supplementation as a treatment strategy for KCNQ2-DEE. In parallel, we incorporate a miRNA-based regulatory circuit to explore whether moderated expression improves safety or efficacy.

Methods
AAV transgene cassettes expressing murine wild-type KCNQ2 under a neuronal-specific hSyn promoter were engineered in both unregulated and regulated formats. Regulated constructs incorporated miRNA-based elements to attenuate expression strength. All constructs contained an N-terminal HA tag to distinguish exogenous KCNQ2. Vectors were packaged into neuronal-tropic AAV.CAP-B10 capsid and delivered systemically via temporal vein injection at postnatal day 2 (P2) at a dose of 1×10^{12} vg/mL. For therapeutic evaluation, heterozygous KCNQ2-T274M mice were injected neonatally and assessed for seizure susceptibility using pentylenetetrazole (PTZ) challenge with behavioral scoring (modified Racine scale). Cognitive performance was evaluated using the Barnes maze. Ongoing studies are extending this approach to adult time points to determine whether delayed intervention can rescue established seizure and cognitive phenotypes. Brains were analyzed for expression post-mortem, utilizing quantitative immunohistochemistry (IHC), with single-cell segmentation performed using Cellpose.

Results
Systemic neonatal delivery of KCNQ2 resulted in widespread neuronal expression across the brain. Both regulated and unregulated constructs achieved robust transgene expression, with regulated constructs exhibiting reduced overall expression levels and less cell-to-cell variability. In pilot cohorts, neonatal gene supplementation produced preliminary seizure rescue following PTZ challenge. Barnes maze testing revealed learning deficits in untreated heterozygotes, while treated animals showed performance trends toward wild-type levels. Notably, preliminary comparisons suggest that unregulated KCNQ2 delivery may provide stronger seizure rescue than moderately regulated constructs.

Conclusions
These findings provide proof-of-concept that systemic AAV-mediated KCNQ2 gene replacement can rescue key seizure and behavioral phenotypes in a mouse model of KCNQ2-DEE. Early-life intervention appears particularly effective, supporting the potential for neonatal gene therapy in severe genetic epilepsies. While regulatory elements can modulate expression profiles, initial results suggest that robust transgene expression may be critical for therapeutic benefit. Ongoing studies will determine whether treatment at later developmental stages can reverse established disease phenotypes, informing the therapeutic window for clinical translation.

7.5

ASO-MEDIATED RESCUE OF ELECTROPHYSIOLOGICAL NETWORK DYSFUNCTION IN IPSC-DERIVED KCNQ2 GAIN-OF-FUNCTION NEURONS

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Introduction
Pathogenic gain-of-function (GOF) KCNQ2 variants cause severe developmental disorders characterized by cognitive deficits, behavioral issues, language difficulties, motor impairments and, in some cases, epileptic seizures. KCNQ2-GOFs remain poorly studied in a neurodevelopmental context, and no targeted therapies are currently available. Antisense oligonucleotides (ASOs) are an emerging therapeutic modality, particularly for rare genetic neurological disorders. Pan-allelic knockdown ASOs represent a promising strategy for KCNQ2-GOF, where haploinsufficiency is generally well tolerated. In this study, we characterized the electrophysiological phenotype of iPSC-derived excitatory neurons (iNeurons) generated from two patient-derived GOF lines using longitudinal high-density microelectrode array (HD-MEA) recordings. We then evaluated a small library of LNA gapmer ASOs for their ability to reduce KCNQ2 expression in iNeurons over time and to restore neuronal network electrophysiological activity.

Methods
Doxycycline-inducible NGN expression was used to generate iNeurons from two patient-derived iPSC lines carrying the gain-of-function KCNQ2 variants KCNQ2R201C/+ and KCNQ2R201H/+, alongside a CRISPR-corrected isogenic control line and one commercial healthy line. Longitudinal electrophysiological network development was assessed using HD-MEAs. To explore therapeutic strategies, a library of ASOs targeting KCNQ2 was designed for pan-allelic knockdown. KCNQ2-targeting ASOs were applied to neuronal cultures 1 day after plating, then potency, efficacy, on-target selectivity of KCNQ2 expression reduction, and preliminary neuronal safety were evaluated following a single exposure in iNeurons. Effects on neuronal network activity were monitored over time across multiple concentrations.

Results
Both KCNQ2R201C/+ and KCNQ2R201H/+ iNeurons exhibited delayed network maturation and an overall hypoexcitable phenotype compared to controls. This was characterized by reduced spike frequency and impaired synchronized network bursting, including decreased burst frequency, duration, and intra-burst activity. In our library, three lead KCNQ2-targeting ASOs demonstrated strong potency (IC50<100 nM), high knockdown efficiency (>85%), and on-target selectivity. Among these, ASO3 showed the best efficacy and safety profile, with no apparent safety concerns following a single exposure in iNeurons. ASO3 treatment normalized KCNQ2R201C/+ and KCNQ2R201H/+ network development toward wild-type activity across major electrophysiological metrics, with a clear concentration-dependent effect (1 μM vs 50 nM).

Conclusions
In conclusion, in this study we identified a distinct KCNQ2 GOF neuronal network phenotype and demonstrated that HD-MEA recordings in iNeurons provide a suitable platform for the in vitro evaluation of therapeutic strategies. Our findings further support the potential of LNA gapmer ASOs as a means to modulate KCNQ2 expression and ameliorate disease-associated network dysfunction.

YI 1.

FUNCTIONAL CHARACTERIZATION OF KV7.2/7.3, KV7.2/7.5, AND KV7.3/7.5 CHANNELS: IMPACT OF SUBUNIT COMPOSITION AND PHARMACOLOGICAL MODULATION

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The Kv7 channel family consists of 5 different subtypes and is encoded by the KCNQ1-5 genes. Kv7.2, 7.3, and 7.5 are the basis for the so-called M-current and are predominantly expressed in skeletal muscle and the brain, where they stabilize the resting membrane potential, contribute to neuronal afterhyperpolarization, regulate the firing frequency of tonically active neurons, and support neuroplasticity by maintaining theta resonance. Mutations in the KCNQ2, KCNQ3 or KCNQ5 genes have been linked to epilepsy.

To enable the characterization of distinct Kv7 heteromers, we designed three vectors containing either the KCNQ2-P2A-KCNQ3, KCNQ2-P2A-KCNQ5, or KCNQ3-P2A-KCNQ5 sequence. An additionally GFP tag at the end of the vector was used to visualize transfected cells, which were analyzed using whole-cell voltage clamp recordings. Obtained currents were evaluated for current density (CD) evoked at a test potential of +40 mV, potential of half maximal activation (Vh), and their activation and deactivation kinetics.

We found significantly larger CD and more hyperpolarized Vh when compared between Kv7.2/7.3 (CD = 550.36 pA/pF, Vh = -45 mV) and Kv7.2/7.5 (CD = 87.82 pA/pF, Vh = -41 mV) heteromers. Furthermore, Vh of Kv7.2/7.3 was more depolarized compared to Kv7.3/7.5 (-57 mV) heteromers. Finally, there was a significant difference in Vh of 16 mV between Kv7.2/7.5 and Kv7.3/7.5 heteromers with the latter being more hyperpolarized. Overall activation kinetics of Kv7.2/7.5 are significantly slower at depolarizing test potentials > 20 mV compared to Kv7.2/7.3. Preliminary experiments with a derivative of the neurosteroid pregnenolone show an increasing effect of this compound on the M-current.

Thus, the different subunit compositions appear to be distinguishable based on their activation kinetics, current amplitude and Vh. The upregulation of Kv7 channels by a pregnenolone derivate implies a regulatory role of this neurosteroid in excitability and thereby support its role as a potential therapeutic target as previously suggested by Vallée [1]. Future experiments will focus on further neurosteroid derivatives and their effects on the firing rate of native neurons from mice.

[1] M. Vallée, Neurosteroids and potential therapeutics: Focus on pregnenolone, The Journal of Steroid Biochemistry and Molecular Biology, 2016, Volume 160, from page 78-87.

YI 2.

AT THE INTERSECTION BETWEEN SODIUM AND POTASSIUM CHANNELS: M-CURRENT DOWNREGULATION DRIVES CORTICAL HYPEREXCITABILITY IN THE SODIUM CHANNELOPATHY DRAVET SYNDROME

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Introduction
Dravet Syndrome (DS) is a severe developmental and epileptic encephalopathy caused by loss-of-function variants in the SCN1A gene. Traditionally, its pathogenesis has been attributed to impaired inhibition from parvalbumin-positive fast-spiking interneurons. Recent studies, however, suggest that this mechanism alone cannot fully explain lifelong seizures and comorbidities. Growing evidence implicates that secondary, maladaptive changes following the primary genetic defect can significantly contribute to diseases progression.

Methods
To explore these mechanisms, we examined a clinically relevant mouse model carrying the recurrent Scn1a p.(Ala1783Val) variant, focusing on the somatosensory cortex (SSC) at the pre-epileptic (P13-15) and epileptic (P20-23) stages. We combined patch-clamp recordings from acute mouse brain slices, with single-nuclei RNA-sequencing, and subsequently validated the transcriptomic findings with in silico modeling, pharmacological assays, and direct M-current recordings.

Results
We analyzed inhibitory function in the SSC and the properties of layer 5 pyramidal neurons (L5PN). While inhibitory function showed only very subtle alterations, we observed pronounced hyperexcitability in L5PN during the epileptic stage — a newly identified hallmark of pathological remodeling in DS. To uncover the molecular drivers of this phenotype, we performed single-nuclei RNA-sequencing of SSC tissue and identified widespread transcriptional dysregulation, much of which preceded seizure onset. Integrated analyses—including differential gene expression, and pseudodisease trajectory analysis—highlighted aberrant developmental expression of Kcnq3 and Kcnq5 as key contributors. Replicating the transcriptomic alterations in an in silico model, sustained that reduction of M-current is one of the most likely culprit explaining the newly observed electrophysiological phenotype. Performing direct measurements of the M-current using the patch-clamp technique, we demonstrated that it was significantly reduced during the epileptic stage. We therefore performed pharmacological experiments and observed that (i) reducing the M-current in wild-type mice was sufficient to elicit the L5PN hyperexcitability phenotype, and (ii) restoring the M-current with the retigabine derivative c60 was sufficient to normalize excitability in DS mice.

Conclusions
We demonstrate that the M-current reduction is necessary and sufficient to the newly observed L5PN hyperexcitability in DS. Taken together, these findings identify a novel, experimentally validated disease mechanism in DS and highlight a promising, druggable target for therapeutic intervention.

YI 3.

PREDICTING NEURODEVELOPMENTAL OUTCOME IN KCNQ2-RELATED DISORDERS

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Introduction
KCNQ2 pathogenic variants give rise to a broad phenotypic continuum, from self-limited (familial) neonatal epilepsy to developmental and epileptic encephalopathy (DEE), with substantial outcome variability despite a shared neonatal seizure onset, complicating early prognostication.

Methods
We conducted a multicentric retrospective cohort study including individuals carrying a (likely) pathogenic variant in the KCNQ2 gene, with a minimum follow-up age of three years. Mosaic variants were excluded. Two hundred and forty-two individuals were included. Ten neonatal predictors significantly associated with developmental outcomes ($P \leq 0.001$) were identified. The cohort was randomly split into training/validation sets. The ten predictors were used to train random forest models to predict (i) dichotomous outcomes and (ii) three-category outcomes for cognition, language, and gross motor milestones.

Results
Six clinical predictors (neonatal hypotonia, family history of neonatal seizures, electroencephalogram (EEG) characteristics, age at seizure onset, seizure type at onset, and seizure frequency at onset) and four genetic predictors (de novo status, exon localisation, variant class, and position within known KCNQ2-DEE hotspot regions were retained. Dichotomous models showed the highest predictive performance, with macro F1 scores of 89% for normal vs. mild-profound intellecual disability (ID), 85% for achievement of first words, and 92% for achievement of independent walking. Three-category models remained clinically informative: macro F1 scores were 88% for normal vs. mild vs. moderate-profound ID, 83% for first words ≤16 months vs. >16 months vs. never, and 89% for independent walking ≤18 months vs. >18 months vs. never.

Conclusions
These prediction models provide a scalable, practical tool for early prognostication in KCNQ2-RD.

YI 4.

A GATING NEXUS BETWEEN THE S6-HELIX A AND HELIX C DOMAINS STABILIZES THE ACTIVE CONFORMATION OF KV7 CHANNELS

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Introduction

Kv7 (KCNQ1-5) channels exhibit polymodal regulation by factors such as voltage, calmodulin and PIP2. PIP2 binding to the S4-S5 linker and S6-Helix A (HA) stabilizes channel opening. Kv7 channels also possess long intracellular C-termini involved in calmodulin and PIP2 regulation. The most conserved regions of the long C-terminus are Helix A and the 30 amino acid Helix C (HC), which contains a pair of aspartate residues that are absolutely conserved among all Kv7s.

Methods

We used whole-cell patch clamp electrophysiology of candidate mutant sites identified by analysis of various Kv7 channel cryo-EM structures. Rapid drug application and PIP2 modulation by DrVSP enabled time-resolved manipulation of drug and PIP2 levels.

Results

We demonstrate that arginine residues (Kv7.2 R325 and R332) in the S6-helix A domain interact with conserved aspartates (Kv7.2 D563 and D566) in helix C to form an intrasubunit gating nexus. These interactions do not directly involve PIP2, but appear to stabilize the open pore conformation, which is essential to form a stable complex with PIP2. Individually, Kv7.2[R325A] and Kv7.2[R332A] mutants cause profound loss of activity under ambient PIP2 conditions, but are enhanced by PIP-5-kinase overexpression (to elevate PIP2 levels) and/or the Kv7 activator, ML213. Identical results are observed with mutations at predicted partner sites, Kv7.2[D563A] and Kv7.2[D566A]. Moreover, disruption of these interactions causes channels to become highly sensitive to DrVSP-mediated rundown in ML213, suggesting weakened association with PIP2. Combinatorial mutants [R325A][R332A] or [D563A][D566A], which disrupt both interaction pairs are not rescued by PIP-5-kinase overexpression, and poorly activated by ML213.

Conclusions

In summary, we demonstrate that a conserved Kv7 gating nexus between the S6-Helix A and Helix C is essential for stabilization of open channels by either PIP2 or activator drugs.

YI 5.

RESOLVING CA^{2+}-DEPENDENT CALMODULIN REGULATORY STATES OF KV7.2 USING A FRET-BASED APPROACH

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Introduction

Kv7.2 potassium channels are key determinants of neuronal excitability, and their dysfunction leads to severe epileptic disorders. Calmodulin (CaM) binds to helices A and B within the C-terminus of Kv7 channels and is required for proper channel function. Specifically, the conserved polycationic cluster of residues on the distal B helix of Kv7 has been shown to be a crucial binding and functional site for CaM and PIP2. Pathogenic mutations of these residues on Kv7.2 reduce channel activities and are linked to neuronal diseases such as epileptic encephalopathy. How these pathogenic mutations regulate CaM-Kv7.2 binding, and whether they can cause CaM to dissociate or partially disengage, remain unresolved. We hypothesized that mutations of the distal B helix that are associated with epileptic encephalopathy induce distinct structural conformations of Kv7.2-CaM that have not been captured by current structural models.

Methods

To probe these novel CaM-dependent regulatory states in epileptic encephalopathies, we utilized a Förster Resonance Energy Transfer (FRET)-based biosensor using the Kv7.2 A-B regulatory domain flanked by donor (CFP) and acceptor fluorophores (YFP), called FRET-Q2RD. This construct enables real-time detection of conformational changes and CaM binding through nanometer-scale distance measurements. Pathogenic mutants of FRET-Q2RD localized to the distal B helix were introduced to assess their effects on CaM binding. Proteins were expressed in E. coli and purified using affinity chromatography for in vitro FRET measurements. FRET efficiency was quantified using a multi-mode plate reader across defined Ca^{2+} concentrations and pathogenic mutations to assess changes in CaM binding state and complex stability.

Results

We designed a FRET-based biosensor system to probe novel binding configurations between CaM and the Kv7.2 AB domain utilizing pathogenic mutations in a critical overlapping CaM-PIP2 binding domain. Using the FRET-Q2RD we aimed to assess the Ca^{2+}-dependent changes in energy transfer efficiency, which are expected to reflect shifts in conformation of the complex between CaM and Kv7.2 A-B regulatory domain. We proposed that pathogenic mutations on FRET-Q2RD would alter FRET efficiency patterns within the CaM-AB complex, providing insight into how these mutations contribute to CaM binding and configuration changes and leading to pathogenic epileptic encephalopathy.

Conclusions

This work establishes a protein-FRET based framework for understanding diverse modes of CaM-dependent regulatory states of Kv7.2. By linking pathogenic mutations of the conserved KRK region to structural outcomes, it provides a dynamic view of channel regulation and a foundation to understand the structure-function relationship of Kv7.2-associated epileptic encephalopathy.

YI 6.

MOLECULAR DISSECTION OF THE KV7.2-STARGAZIN INTERACTION

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Introduction
Neuronal excitability dysfunction is a key feature of neurodevelopmental disorders like epilepsy. Understanding the underlying mechanisms associated with this dysfunction is crucial for developing therapeutic strategies. M-type (Kv7) channels, which are low-threshold, slowly activating potassium channels, modulate neuronal excitability and network function. Due to their role in seizure suppression, M-channels are an important drug target, however, available M-channel activators have very adverse side effects and have been withdrawn from clinical use. An alternative approach is to target channel interactors, with restricted brain region expression, to modulate channel activity indirectly and bypass side effects.

We identified a novel Kv7.2 interactor, Stargazin, a Transmembrane AMPA receptor regulatory protein (TARP) family member essential for AMPA receptor regulation and fast excitatory neurotransmission. Our previous studies demonstrated that co-expression of Stargazin with Kv7.2 increased channel surface expression and Kv7.2-mediated currents but also leading to channel activation at less depolarized potentials. Here, we have developed new tools to investigate this interaction and characterize it at the molecular level.

Methods
We used SplitFAST, a fluorescence complementation assay that allows the visualization of protein-protein interactions in live cells, to confirm the Stargazin-Kv7.2 interaction and investigate its regulation upon cellular depolarization and PKC activity. We generated a three-dimensional computational model of the complex, utilizing Rosetta Dock and leveraging on previous experience with other membrane proteins. Using this model, we developed Stargazin mutants with specific impairment in their interaction with Kv7.2 by utilizing co-immunoprecipitation assays, SplitFAST, electrophysiology and cell surface labeling, we probed the interaction between the different Stargazin mutants and Kv7.2, as well as the functional consequences of disrupting the Stargazin/Kv7.2 interaction.

Results
Our results revealed the direct Stargazin-Kv7.2 interaction and identified Stargazin mutant(s) that do(es) not bind to Kv7.2 while maintaining interaction with AMPAR. These mutant forms of Stargazin will be instrumental in the non-ambiguous characterization of the functional role of Stargazin-Kv7.2 interaction in input integration, and to distinguish the role of Stargazin in regulating intrinsic excitability and AMPAR-mediated transmission.

Conclusions
Overall, this study provides strong evidence of Stargazin as an interactor and as a bona-fide auxiliary subunit of Kv7.2. The SplitFAST assay enabled real-time analysis, offering spatial-temporal insights, while the biochemistry assays provided a comprehensive molecular characterization of the interaction. This study lays the foundation for exploring modulators targeting newly characterized Kv7.2 regulatory mechanisms.

YI 7.

ATOMISTIC INSIGHTS INTO KV7.2 VARIANT-INDUCED DYSFUNCTION: A MOLECULAR DYNAMICS STUDY

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Kv7.2/3 channels, encoded by members of the KCNQ gene family, are responsible for generating the M-current, a slowly activating and deactivating potassium conductance that plays a critical role in neuronal excitability [1]. Variants in the KCNQ2 gene have been linked to phenotypically heterogeneous neurological diseases, ranging from self-limited familial neonatal epilepsy (SLFNE) at the mild end to neonatal-onset developmental and epileptic encephalopathy (NEO-DEE) at the severe end, making this protein a prime candidate for drug development [2]. While most mutations in the Kv7.2 pore reduce the M-current, a new class of Gain-of-Function (GoF) variants, affecting residues near the intracellular side of the pore's inner gate (IG), have been recently identified [3,4]. In addition, a Loss-of-Function (LoF) variant, located in the helices behind the selectivity filter, has been reported to induce an inactivated-like state of the channel, a conformation physiologically absent in Kv7.2, which does not undergo inactivation under normal conditions [5].

Here, we first investigate the impact of three IG GoF mutations (G313S, A317T, L318V) on the structure of the wild-type (WT) Kv7.2 protein using all-atom molecular dynamics (MD) simulations on the microsecond timescale at different voltages, analyzing both closed and open-channel configurations. All the examined GoF mutations induced a widening of the gate of the closed configuration with respect to the WT protein, consistent with a GoF effect revealed by electrophysiological experiments, while they showed no effect on the open conformation [3].

We then investigated the LoF variant [A265V] through standard MD simulations on the microsecond timescale of the open-channel configuration. The mutation, located in the helices behind the selectivity filter, promoted a stable inactivated-like state of the channel pore. Based on our simulations, we propose a non-canonical inactivation mechanism driven by the formation of a hydrophobic interaction cluster that destabilizes the selectivity filter, revealing a pathological mechanism distinct from that of the GoF variants. In conclusion, we provide an atom-detailed structural characterization of different Kv7.2 variants, which is crucial to understanding the molecular mechanisms underlying the different phenotypes.

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YI 8.

MECHANISTIC CHARACTERIZATION AND THERAPEUTIC TARGETING OF KCNQ2 DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY IN A KV7.2 THR274MET/+ MOUSE MODEL

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Introduction

Developmental and epileptic encephalopathies (DEEs) are severe neurodevelopmental disorders characterized by early-onset, treatment-resistant seizures and persistent cognitive impairment. KCNQ2-DEE is frequently caused by dominant-negative loss-of-function variants in KCNQ2, which encodes the Kv7.2 potassium channel, a key regulator of the neuronal M-current and intrinsic excitability. The recurrent variant p.Thr274Met reduces M-current density and leads to neuronal hyperexcitability with a developmental trajectory that appears to differ across brain regions. While cortical pyramidal neurons show sustained hyperexcitability from neonatal stages onwards, hippocampal neurons may exhibit a more transient phenotype. This raises the possibility that disease mechanisms and therapeutic windows are region- and age-dependent. Here, we examined the spatiotemporal profile of Kv7.2 dysfunction and assessed the efficacy of a novel Kv7 channel modulator (C60).

Methods

Whole-cell patch-clamp recordings were performed in acute hippocampal and cortical slices from a knock-in mouse model (Kcnq2WT/T274M) across defined developmental stages. Intrinsic excitability and M-current were assessed in hippocampal pyramidal neurons and compared to cortical pyramidal neurons. Furthermore, modulation of neuronal excitability by the retigabine analogue C60 was assessed by patch-clamp recordings. For translational validation, C60 was then evaluated in human cortical tissue obtained from neurosurgical resections using microelectrode array (MEA) recordings in organotypic slices and whole-cell patch-clamp recordings in acute slices.

Results

Hippocampal pyramidal neurons displayed increased excitability during the first postnatal week, which diminished at later stages. In contrast, cortical pyramidal neurons exhibited persistent hyperexcitability across developmental stages of Kcnq2WT/T274M mice. Modulation by C60 was evaluated at two developmental time windows, and was found to reduce neuronal firing in hyperexcitable cortical neurons. In human cortical tissue, C60 similarly decreased neuronal firing in both pediatric and adult samples. Notably, this effect was observed at approximately ten-fold lower concentration compared to retigabine, indicating enhanced potency. These findings suggest that cortical hyperexcitability is sustained despite partial normalization of hippocampal function.

Conclusions

Kv7.2 dysfunction leads to region-specific and developmentally dynamic alterations in neuronal excitability, with transient hippocampal and sustained cortical phenotypes. These findings point to an area-dependent, early developmentally restricted window for intervention and support C60 as a potent Kv7 channel modulator with translational potential.

YI 9.

EFFECTS OF KV7 CHANNEL ACTIVATORS IN EXPERIMENTAL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

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Introduction
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration of motor neurons (MNs), leading to muscle weakness and eventual respiratory failure. Multiple pathological mechanisms, including excitotoxicity, oxidative stress, protein aggregation, and disruption of ionic homeostasis have been identified in ALS. A reduction in potassium current mediated by voltage-gated Kv7 channels have been found in ALS-MNs, contributing to neuronal hyperexcitability, a hallmark and early event in ALS pathogenesis. Moreover, recent evidence indicates that mutant forms of TDP-43 (TARDBP), identified in ALS patients, can alter KCNQ2 splicing, leading to loss function of the channel. Furthermore, the Kv7 activator retigabine exerted neuroprotective effects in ALS-MNs. Unfortunately, retigabine is no longer available for clinical use, due to toxicity unrelated to its mechanism of action. Therefore, the aim of this study was to evaluate the possible neuroprotective effects of two novel Kv7 activators identified by our research group, namely compound 60 (C60) and JNJ-37822681 (JNJ), in cellular models of ALS.

Methods
We generated stable NSC-34 motor neuron-like cell lines overexpressing either C9orf72 sequence or a mutated form of the superoxide dismutase-1 (SOD1-G93A), two gene-variants associated to ALS. Cell proliferation and viability were evaluated using Water Soluble Tetrazole (WST) assays; reactive oxygen species (ROS) were measured with fluorometric intracellular ROS detection kit; endoplasmic reticulum (ER) stress was assessed by measuring the levels of C/EBP-homologous protein (CHOP) by quantitative PCR.

Results
C9orf72 overexpression reduced NSC-34 cell viability by approximately 40%, whereas SOD1-G93A clones exhibited a reduction in proliferation rate by 40%. In both cell lines, C60, JNJ, or retigabine (1-10 μM) did not increase cell survival of proliferation. However, Kv7 activators (10 μM) markedly prevented the increase in intracellular ROS levels induced by acute exposure (30 min) to H_2O_2 (0.1 mM) in both C9orf72 and SOD1-G93A models. Furthermore, C60, JNJ, and retigabine attenuated CHOP upregulation in NSC-34-C9orf72 cells, indicating a reduction in ER stress.

Conclusions
Overall, these findings suggest that the Kv7 channel openers C60 and JNJ, by reducing ER stress and oxidative damage, may represent new potential therapeutic tools in ALS. Experiments on human iPSC-derived motor neurons carrying ALS-causing mutation (TARDBP) and in vivo models of ALS (SOD1-G93A mice) are ongoing to further support our findings and validate the therapeutic potential of these Kv7 channel activators.

YI 10.

MODELING KCNQ2 ENCEPHALOPATHY USING IPSC-DERIVED EXCITATORY–INHIBITORY NEURONAL NETWORKS

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Introduction
Pathogenic KCNQ2 gain-of-function (GOF) variants enhance neuronal M-current, and cause severe developmental disorders characterized by cognitive, behavioral, language, and motor impairments, with potential seizures. These variants enhance neuronal M-current, however, their downstream effects on neuronal function and network behavior remain poorly understood, limiting the development of effective treatments. To address this, we characterized excitatory–inhibitory neuronal co-cultures derived from human iPSCs carrying two GOF variants and used this platform to evaluate two pharmacological approaches explored as potential precision medicine strategies.

Methods
Doxycycline-inducible expression of the transcription factors NGN and ASCL1 was used to generate cortical-like excitatory and inhibitory iPSC-derived neurons, respectively, from two human iPSC lines carrying the GOF KCNQ2 variants R201C and G239S, along with their isogenic controls. Excitatory and inhibitory neurons were co-cultured at a 70:30 ratio with primary murine astrocytes and maintained for up to 70 days in vitro (DIV). Culture composition, synaptic marker expression, and occurrence of the GABA switch were characterized through immunocytochemistry (ICC). Longitudinal electrophysiological network development was evaluated using high-density microelectrode arrays (HD-MEAs).

Results
ICC for GABA and MAP2 confirmed the successful establishment of excitatory–inhibitory neuronal co-cultures, which maintained the expected 70:30 ratio up to DIV70 and showed progressive maturation over time. Across this period, the KCC2/NKCC1 expression ratio increased from 0.4 to 0.6, reflecting the developmental shift in GABAergic signaling from depolarizing to inhibitory. Functionally, pharmacological blockade of GABAA receptors with 100 µM picrotoxin (PTX) induced an approximately fivefold increase in network activity in DIV70 co-cultures, whereas no effect was observed in glutamatergic monocultures, supporting the presence of functional inhibitory transmission. Furthermore, ICC of the presynaptic markers vGlut and VGAT confirmed the presence of glutamatergic and GABAergic synapses. KCNQ2-GOF lines maintained overall excitatory–inhibitory co-culture composition but showed reduced survival. ICC indicated a delayed shift in GABAergic signaling (DIV56 in GOF vs DIV42 in controls) and reduced glutamatergic presynapses. HD-MEA recordings revealed delayed activity onset and a hypoexcitable phenotype, with decreased firing rate, burst frequency, and spikes per burst. Finally, amitriptyline and donepezil, proposed precision therapies for GOF patients, were tested in KCNQ2-G239S co-cultures under acute and chronic conditions; unexpectedly, both further suppressed overall and network-synchronized activity, exacerbating the hypoexcitable phenotype.

Conclusions
These results validate a robust excitatory–inhibitory co-culture model and demonstrate that KCNQ2-GOF variants disrupt neuronal maturation and network function. They provide new insight into KCNQ2-GOF encephalopathy and may inform the development of effective therapeutic strategies.

YI 11.

ENDOTHELIAL KV7.5 CHANNELS REGULATE BLOOD–BRAIN BARRIER INTEGRITY AND SEIZURE SUSCEPTIBILITY

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Introduction
Blood-brain barrier (BBB) disruption and consequent alterations of ion transport play a pivotal role in the onset and progression of many neurological diseases. Notably, BBB breakdown represents a risk factor for epilepsy, and seizures can damage brain endothelial cells (BECs), thus further exacerbating the disease. However, the molecular mechanisms linking endothelial dysfunction to seizure susceptibility remain poorly understood. We recently demonstrated that KCNQ-encoded voltage-gated potassium Kv7 channels (specifically, Kv7.1, Kv7.4 and Kv7.5) are expressed in BECs and regulate BBB permeability. Moreover, we showed that Kv7.5 was selectively reduced in brain microvessels (BMVs) from epileptic rats, and that pharmacological activation of Kv7 channels reduced BECs damage induced by kainic acid (KA). Building on these evidence, in this study we investigated if KCNQ5 deletion affected BBB function and seizure susceptibility in vivo, and endothelial permeability in vitro; moreover, we evaluated the possible effects of carnosic acid (CA), a rosemary-derived Kv7.5 opener, on BMVs integrity and seizure protection.

Methods
BBB permeability was assessed by measuring fluorescein isothiocyanate (FITC)–dextran uptake in freshly isolated BMVs from 6–10 week old wild type (wt) and kcnq5-/- male rats. Status epilepticus (SE) was induced by repeated KA injections until the appearance of stage ≥3 seizures, scored using the modified Racine scale. CA was administered at 10 mg/kg 30 min prior to induction of seizures. Animals were culled 24 h after reaching SE and brains were collected to isolate BMVs. Permeability in BECs derived from induced pluripotent stem cells (hiBECs) was evaluated by trans-endothelial electrical resistance (TEER) measurements.

Results
BMVs from kcnq5-/- rats displayed increased basal permeability by about 45% compared to wt controls, accompanied by a selective reduced expression of the tight junction protein Zonula Occludens 1. When exposed to epileptogenic stimuli, kcnq5/ rats required lower KA doses to reach SE and exhibited shorter seizure latency compared to wt animals. Interestingly, while KA markedly increased BBB permeability in wt rats, FITC-dextran uptake in Kv7.5deficient animals remained unchanged, suggesting preexisting maximal damage. Consistently, KCNQ5-/- hiBECs displayed reduced TEER values by about 40% compared to isogenic controls, suggesting impaired barrier function. Finally, CA treatment reduced both seizure severity and BBB disruption in wt rats, whilst did not ameliorate seizure outcome in kcnq5/ rats.

Conclusions
Together, these findings identify Kv7.5 as a critical determinant of BBB integrity and seizure susceptibility, highlighting its potential as a therapeutic target in epilepsy and other disorders characterized by impaired BBB.

YI 12.

REGULATION OF VASCULAR KV7 CHANNELS BY G PROTEIN-COUPLED RECEPTOR KINASES

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Introduction

Kv7 channels are key regulators of vascular tone, with Kv7.4 and Kv7.5 prominently expressed in vascular smooth muscle cells. Although they regulate vascular contractility, the mechanisms governing Kv7 channel trafficking within the vasculature remain poorly understood. G-protein-coupled receptor kinases (GRKs) are traditionally implicated in G-protein-coupled receptor (GPCR) desensitisation through phosphorylation. However, GRKs also target non-GPCR substrates and influence multiple signalling pathways by directly interacting with other proteins, often in a phosphorylation-independent manner. To our knowledge, this is the first report demonstrating a link between GRKs and Kv7 channels, showing that inhibition of GRK2/3 attenuates ML213-induced relaxation within rat cerebral arteries.

Methods

Expression of KCNQ1-5, GRK2–6, and β-arrestin 1 and 2 genes was quantified by RT-qPCR in rat cerebral arteries from male Wistar-Han rats. The effects of ML213 (Kv7.2 and Kv7.4 channel opener) were assessed via wire myography on pre-contracted basilar from male Wistar rats, in the presence of several GRK blockers (GRK2/3 inhibitor - compound 101 and GRK5 inhibitor - compound 707). Concentration-response curves were plotted logarithmically, with values expressed as mean ± SEM. Kv7.4 currents were recorded using whole-cell patch-clamp electrophysiology in HEK293 cells transiently transfected to express KCNQ4.

Results

ML213 produced a concentration-dependent relaxation in rat basilar arteries, with an IC50 of ~3 μM. In the presence of compound 707, the efficacy of ML213 was improved, with 80% relaxation observed in arteries treated with compound 707. In contrast, in basilar arteries treated with compound 101, ML213 was weakly effective, with 3 μM producing ~ 20% relaxation. Gene expression of KCNQ1-5, GRK2–6, and β-arrestin 1 and 2 was confirmed in cerebral arteries, with greater relative transcript abundance of GRK2 and GRK5 (n=3). Kv7.4, but not Kv7.1, currents were abolished by compound 101 within 10 minutes (n=3). Compound 707 had no effect.

Conclusions

This study shows that the GRK2/3 inhibitor Compound 101 suppresses Kv7 activity in rat cerebral arteries and identifies a novel link between GRKs and vascular Kv7 channels.

1.

KCNQ/KV7 CHANNELS LOCALIZE TO DENDRITIC SPINES VIA ANKYRING-190 AND SUPPRESS NMDA RECEPTOR ACTIVITY

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Introduction
KCNQ/Kv7 channels composed of Kv7.2 and Kv7.3 subunits are well established as key regulators of neuronal excitability through their axonal localization and mediation of the M-current (IM). However, emerging evidence suggests additional dendritic roles. Here, we investigate whether Kv7 channels are present at excitatory synapses and define their function and targeting mechanisms in dendritic spines.

Methods
We combined immunocytochemistry, structured illumination microscopy (SIM), and photoactivation localization microscopy (PALM) to determine subcellular localization of Kv7 channels in cultured rat hippocampal neurons. Biochemical fractionation and immunoelectron microscopy were used to confirm postsynaptic localization in rodent brain tissue. Protein interactions were assessed by co-immunoprecipitation. Functional roles were evaluated using GCaMP6s-based calcium imaging following NMDA stimulation with pharmacological modulation of Kv7 activity. Disease relevance was tested using KCNQ2 variants associated with developmental and epileptic encephalopathy (DEE).

Results
Kv7.2 and Kv7.3 were detected in ~40% of dendritic spines and localized at and near the postsynaptic density (PSD), as confirmed by super-resolution imaging, biochemical fractionation, and immunoEM. Kv7.3 exhibited stronger spine targeting than Kv7.2, while heteromerization with Kv7.3 significantly enhanced Kv7.2 localization. Ankyrin-G 190 (AnkG190), a spine-enriched scaffold protein, interacted with Kv7 subunits and increased their spine localization, whereas disruption of the ankyrin-binding motif impaired targeting. Functionally, pharmacological activation of Kv7 channels reduced NMDA receptor–evoked calcium transients in dendritic spines, indicating suppression of excitatory synaptic signalling. Importantly, DEE-associated KCNQ2 variants significantly reduced spine localization of heteromeric Kv7 channels without altering spine density, suggesting a synaptic targeting defect.

Conclusions
These findings identify a previously unrecognized population of Kv7 channels at excitatory synapses, where they dampen NMDA receptor activity and regulate synaptic calcium signaling. Their localization is governed by Kv7.2/7.3 heteromerization and AnkG190 scaffolding, while disruption by disease-associated variants implicates postsynaptic Kv7 dysfunction in KCNQ2-related encephalopathy. This work expands the functional landscape of Kv7 channels beyond axonal excitability to include direct modulation of synaptic transmission.

2.

FROM CELL CULTURE TO CRYO EM: MEMBRANE PROTEIN PURIFICATION OF KV7.1 CHANNEL FROM MAMMALIAN CELLS

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Introduction
The voltage-gated potassium channel KV7.1 plays a crucial role in cardiac repolarization. Together with KCNE1 it forms the KCNQ1/KCNE1-channel complex that generates the slow delayed rectifier current (IKs). Loss-of-Function Mutations in either cause long QT syndrome (LQTS 1/5), risking arrhythmias and sudden cardiac death. Despite it's physiological importance, mechanisms by which physiological or pharmacological modulators shape KV7.1 gating and inactivation remain incompletely understood. High-resolution structural studies are needed to shed light on the channel's unique modification possibilities.

Methods
We established and optimized a purification pipeline to express full-length and truncated KV7.1 channel from BacMam-transduced mammalian cells. Our key optimizations include the cell line set-up, baculovirus production, temperature shifts and specific protease inhibition. These parameters finally delivered a high-expression and detergent-solubilized protein for negative stain EM and downstream single particle Cryo EM.

Results
While Mass Photometry showed a stable tetrameric formation and negative stain EM confirmed monodisperse particles, that lead to a fitting initial 3D model from 2D classes. Ongoing upscaling targets downstream nanodisc reconstitution to achieve cryo-EM structures with high resolution to identify KV7.1-channel modulation prospects.

Conclusions
These results establish a platform for high-resolution structural studies of the KV7.1 channel and its modulation, enabling Cryo-EM analysis of the full-length channel complex and structure-guided physiological and pharmacological insights.

3.

A HIGH-THROUGHPUT FUNCTIONAL SCAN OF KCNQ1 S4 VARIANTS

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Introduction

KCNQ1 encodes the pore-forming voltage-gated potassium channel subunit (Kv7.1 or KCNQ1) responsible for the cardiac delayed rectifier current (IKs). Loss-of-function variants in KCNQ1 result in Type 1 Long QT Syndrome, which predisposes to life-threatening cardiac arrhythmia and sudden cardiac death. The proliferation of genetic testing in research and clinical medicine has facilitated the identification of hundreds of KCNQ1 variants, but most have unknown functional effects. Determining the functional consequences of KCNQ1 variants can help discriminate pathogenic from benign, but traditional methods (manual patch-clamp electrophysiology) are low throughput. Here we demonstrate use of automated patch-clamp recording to investigate the functional properties of nearly four dozen KCNQ1 missense variants within a specific protein domain (S4 segment).

Methods

A library of every possible single nucleotide variant in the KCNQ1 S4 segment was generated and individual missense variants were prepared for mammalian cell expression. We used high-efficiency cell electroporation (MaxCyte STX instrument) to transiently express wildtype (WT) or variant channels in CHO cells stably expressing KCNE1. We recorded whole-cell voltage-sensitive IKs-like currents using automated patch clamp recording on the Nanion Syncropatch 384 platform. Currents were recorded in the absence then presence of the IKs inhibitor JNJ303 to enable isolation of specific currents. To assess the function of 44 single nucleotide variants, we performed comparative analyses of whole-cell current density, voltage-dependence of activation, and activation/ deactivation rates relative to WT KCNQ1.

Results

Our results show that the majority (33/44) of the variants we tested generated significantly smaller current densities than WT KCNQ1. Among non-synonymous variants, five showed no significant difference in current density relative to WT and one (S225T) exhibited larger current density. Nine variants with smaller current density exhibited a negative shift in voltage-dependence of activation, a gain-of-function effect within predominantly loss-of-function variants. A subset of variants exhibited potential constitutive activity resembling leak currents. Furthermore, we identified four variants with smaller current density and WT-like voltage-dependence, which might indicate impaired membrane localization of an otherwise functional channel.

Conclusions

Our findings demonstrate heterogeneous functional effects of KCNQ1 missense variants and provide insights into the functional vulnerability of several sites within the S4 helix. Notably, we find that although most of our library showed lower current density, select variants retained WT-like gating kinetics and voltage dependence, which theoretically may be remedied by increasing trafficking to the cell membrane. Future studies will examine the properties of these variants co-expressed with WT KCNQ1 to simulate the heterozygous state.

4.

CONTRIBUTION OF KV7 CHANNELS TO VASCULAR TONE REGULATION IN HUMAN AND RAT RENAL RESISTANCE VESSELS

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Introduction
Kv7 channels contribute to vascular smooth muscle excitability and arterial tone regulation. Their function has been studied in intrarenal arteries and arterioles of experimental animals. Respective data from human intrarenal arteries are sparse. The present study aimed to characterize the contribution of Kv7 channels to intrarenal artery tone regulation in rats and humans. We hypothesized that Kv7 channels contribute to vascular smooth muscle membrane potential and tested the effects of pharmacological Kv7 channel inhibition and activation on agonist-induced alterations in arterial tone.

Methods
Segments of third-order renal artery branches (distal interlobar arteries) were isolated from 12-15-week-old male Wistar rats. Human distal arcuate and interlobular arteries were dissected from tumor-free renal tissue obtained from nephrectomies. Vascular function was assessed by wire myography. Kv7 (KCNQ) mRNA abundance in human intrarenal arteries was analyzed by RT-PCR. Kv7 channel function was pharmacologically inhibited by XE991 (3 µmol/L) and activated by the Kv7.1 activator L364,373 (L364).

Results
KCNQ1, 3, 4, and 5 mRNA were consistently detected in human arcuate and interlobular arteries, whereas KCNQ2 expression was observed in only 2 of 8 samples.

Inhibition of Kv7 channels with XE991 increased basal vascular tone in both species. To indirectly asses the Kv7 channel contribution to vascular smooth muscle membrane potential, we tested the effects of Kv7 channel inhibition and activation on L-type-Ca^{2+}-channel-mediated vasoconstriction. In Wistar rats, XE991 enhanced and L364 attenuated vasoconstrictions in response to the L-type-Ca^{2+}-channel activator

S-(–)-BayK8644. No such effects were observed in human vessels.

Kv7 channel inhibition (XE991) enhanced and Kv7.1 activation (L364) blunted

α1-adrenoceptor agonist-induced vasoconstriction in rat intrarenal arteries. Noradrenaline-induced rat intrarenal artery constriction was hardly affected by XE991 and L364. In human renal resistance arteries, XE991 blunted β-adrenoceptor-dependent (isoproterenol) and prostacyclin-induced relaxation. These effects were not observed in Wistar rats.

Conclusions
Kv7 channels are expressed in human renal resistance arteries and contribute to vascular tone regulation. However, their functional role differs significantly from that observed in Wistar rats, highlighting important species-specific differences relevant for translational research.

5.

MELATONIN AND MELATONIN-DERIVED, H2S-CONTAINING COMPOUNDS ACT AS NEW KV7.4 ACTIVATORS

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Introduction
Melatonin (MT) is a pineal neurohormone that regulates circadian rhythm through two G-protein-coupled receptors, MT1 and MT2. MT has also been reported to modulate vascular muscle tone, mainly exerting vasorelaxant effects through concentration-, vessel-, and receptor-dependent mechanisms1. Among potassium channels, voltage-dependent Kv7.4 contributes to blood pressure regulation by promoting vasorelaxation, also through cAMP-mediated signalling2. Hydrogen sulphide (H2S) is a gasotransmitter with beneficial effects in cardiovascular diseases, including atherosclerosis, ischemia-reperfusion injury, and hypertension3. Its vasodilatory action involves Kv7.4 modulation: exposure to the H2S donor sodium hydrosulphide (NaHS) facilitates Kv7.4 current activation and promotes endothelium-independent relaxation of rat aortic rings, an effect reduced by the Kv7 blocker XE9914. In this study, we investigated whether MT directly activates Kv7.4 currents and whether newly synthesized MT-derived H2S-containing molecules (MDHS; compounds 1, 2, and 3) exert similar effects.

Methods
Kv7.4 currents were recorded by patch clamp in transiently transfected CHO cells using a −80/0 mV ramp protocol. The effect of each compound was expressed as % of Kv7.4 current changes versus control (no drug) basal values.

Results
The Kv7 activator retigabine (10 μM), used as a reference compound5, increased Kv7.4 currents by ~100% and induced a ~−12 mV leftward shift in the voltage-dependence of activation. Similar but smaller effects ($p<0.05$ vs retigabine) were observed with 10 μM melatonin (~50% current increase; ~−8 mV shift) and with the H2S donor NaHS (~30% increase; ~−5 mV shift). Compared with melatonin, compound 1 produced a similar (~40%) enhancement of Kv7.4 currents, whereas compounds 2 and 3 were more effective (~70% and ~90% increases, respectively). Changes in activation voltage-dependence induced by compounds 1, 2, and 3 (ΔV1/2 ~−6, −4, and −11 mV, respectively) were comparable to those caused by melatonin. By contrast, compound C3 bis, which does not release H2S, failed to mimic the effects of compound 3, supporting a key role for H2S release. Based on its superior electrophysiological profile, compound 3 was tested ex vivo, where it showed a markedly stronger vasorelaxant effect than melatonin in rat aortic rings, in terms of both potency and maximal response. In endothelium-denuded preparations, compound 3 also reduced noradrenaline-induced vasoconstriction in a concentration-dependent manner. Moreover, XE991 attenuated its vasorelaxant effect in both endothelium-intact and -denuded rings, indicating that its vascular activity is largely mediated by Kv7 channels.

Conclusions
Melatonin acts as a novel Kv7.4 activator. This effect is shared by H2S-releasing melatonin derivatives, with compound 3 showing the most pronounced electrophysiological and vascular activity.

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6.

KV7.2 CHANNELOPATHY BEYOND THE BRAIN: GAIN-OF-FUNCTION VARIANTS LINKED TO PALATAL MALFORMATIONS

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Introduction
KCNQ2 encodes the Kv7.2 subunit of a voltage-gated potassium channel mediating the neuronal M-current, a key regulator of cellular excitability. Pathogenic KCNQ2 variants cause a spectrum of neurodevelopmental disorders through loss-of-function, dominant-negative, and gain-of-function (GOF) mechanisms. Bioelectric signaling is increasingly recognized as a regulator of embryogenesis, yet a contribution of KCNQ2 to craniofacial development has not been investigated. Here, we report a novel association between KCNQ2 GOF variants and palatal malformations, implicating Kv7-mediated bioelectric signaling in human craniofacial morphogenesis.

Methods
Four patients with KCNQ2 variants and cleft palate were identified through collaborations. Novel variants were characterized in transfected mammalian CHO-cells. Additionally, a systematic literature search was conducted using PubMed, ClinVar, and the RIKEE patient registry using established GOF variants as queries. Cases lacking detailed phenotypic descriptions and purely functional studies were excluded.

Results
From registries and databases, 98 reports on KCNQ2 GOF variants were identified. Following exclusion of entries with missing phenotypic data, 54 cases with KCNQ2 GOF variants were analyzed. Palatal malformations were identified in 10 of 54 individuals (18.5%), including cleft palate, cleft soft palate, high-arched palate, and bifid uvula. This frequency significantly exceeds the general population prevalence of 0.1% (exact one-sided binomial test, $p < 1\times10^{-15}$). We describe four previously unreported KCNQ2 cases with palatal malformations. Functional characterization of the KCNQ2 variants by whole-cell patch-clamp recordings likewise demonstrated gain-of-function mechanisms.

Conclusions
KCNQ2 GOF variants are associated with a markedly increased prevalence of palatal malformations, extending the phenotypic spectrum of Kv7.2 channelopathies to abnormalities of craniofacial development. These findings highlight a previously unrecognized role of Kv7-mediated bioelectricity in human craniofacial morphogenesis and underscore the broader developmental relevance of ion channel function beyond the nervous system. With our study, we expand the spectrum of KCNQ2-disorders to mutation-type specific correlations.

7.

NOT JUST BRAINY BUT ALSO BRAWNY: THE ROLE OF KV7 CHANNELS BEYOND THE BRAIN

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Introduction
Beyond the brain, Kv7 channels are widely expressed in vascular, cardiac, and skeletal muscle cells, where they regulate membrane potential, contractility, and tissue homeostasis. This distribution underscores their relevance beyond the nervous system and suggests additional, still poorly understood functions. Although direct studies on Kv7 in hereditary skeletal muscle disorders (myopathies) are limited, ion channel dysfunction is increasingly recognized as a contributor to these severe conditions. We previously showed that Kv7, particularly Kv7.4, promotes maturation of skeletal muscle precursor cells and protects them from programmed cell death. This provided the rationale to investigate Kv7 in Duchenne muscular dystrophy (DMD), a severe X-linked disorder characterized not only by progressive muscle degeneration but also by cognitive deficits, making Kv7 a target of dual interest in both muscle and brain.

Methods
In vitro experiments were performed using murine C2C12 skeletal muscle cells, both wild-type and genetically modified to model DMD and recapitulate its associated pathological features. Transcriptomic analyses were carried out on skeletal muscle tissues and selected brain regions from male mdx mice, the preferred preclinical model for Duchenne muscular dystrophy.

Results
In the gastrocnemius muscle of mdx mice, we observed altered expression of Kv7.4 and Kv7.5 compared to control tissues during the post-onset phase of pathology (5 weeks of age). In contrast, no changes in Kv7 channel expression were detected in the hippocampus, despite screening over 30 different potassium channel subtypes. Ongoing studies are focused on measuring Kv7 expression in other brain regions and confirming the role of “muscle” Kv7 channels in autophagy and ferroptosis, two cellular processes known to be dysregulated in DMD.

Conclusions
Our findings highlight a prominent peripheral role for Kv7 channels in DMD, with selective dysregulation of Kv7.4 and Kv7.5 in dystrophic skeletal muscle. In contrast, no changes were observed in the hippocampus, neither for Kv7.4/5 nor for Kv7.2/3, underscoring the tissue-specific nature of Kv7 alterations.

8.

KV7/KCNQ CHANNEL BLOCKADE ATTENUATES HIPPOCAMPAL BUT NOT RETINAL DEGENERATION IN CLCN3-/- MICE

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Introduction
The CLCN3 gene encodes ClC-3, an endosomal 2Cl-/H+ exchanger crucial for hippocampal and retinal integrity. Dysfunction of ClC-3 gives rise to a rare neurodevelopmental condition characterised by global developmental delay, intellectual disability, delayed speech, and psychiatric manifestations. Structural hallmarks of this condition include brain atrophy and progressive degeneration of the hippocampus and retina. The Clcn3−/− mouse model effectively recapitulates these clinical features, making it the most robust tool for investigating the disease's etiology. Clcn3−/− CA2 neurons are devoid of burst-firing activity and exhibit reduced neuronal complexity prior to the onset of hippocampal degeneration. This early physiological deficit suggests that impaired neuronal firing may drive subsequent cell death. Given that acute pharmacological blockade of Kv7/KCNQ channels fully rescues burst-firing in ex vivo hippocampal slices, we aim to investigate whether long-term Kv7/KCNQ channel blockade can delay or even prevent neurodegeneration in the Clcn3-/- model."

Methods
To evaluate the efficacy of Kv7/KCNQ channel blockade in preventing neurodegeneration, Linopirdine (a specific antagonist) was administered intranasally twice daily to Clcn3−/− mice at dosages of 0.0, 0.1, 1.0, and 2.5 mg/kg. Experimental endpoints were set at P21, P30, and P40, with P21 representing the onset of hippocampal degeneration as characterized by the initial loss of CA2 pyramidal cells. At each endpoint, animals were euthanized, and brains and eyes were dissected. Tissues were post-fixed, dehydrated, and embedded in paraffin. Comprehensive histological and immunohistochemical analyses were performed on paraffin sections using Hematoxylin and Eosin, and Nissl staining for structural evaluation, NeuN for neuronal quantification, and GFAP/Iba1 to assess glial activation.

Results
Preliminary data demonstrate that long-term Kv7/KCNQ channel blockade delays the onset of hippocampal neurodegeneration in a dose-dependent manner. In contrast, retinal atrophy remained across all tested concentrations, suggesting that the pathophysiological mechanisms underlying hippocampal and retinal degeneration may arise from distinct molecular origins. Furthermore, these observations indicate that while current Linopirdine dosages are sufficient to delay hippocampal loss, they may be suboptimal for complete neuroprotection. Optimization of the administration regimen and dosage could potentially prevent neuronal loss entirely. These findings represent a significant step toward defining a successful neuroprotective strategy; however, they also necessitate the refined dose-adjustments to maximize therapeutic efficacy.

Conclusions
Our findings provide proof-of-concept that pharmacological modulation of Kv7/KCNQ channels can delay the onset of neuronal loss and establish a framework for future therapeutic interventions for CLCN3-related patients. Targeting early physiological dysfunction (burst-firing) can halt the progression of structural brain atrophy in CLCN3 models.

9.

SIX NOVEL VARIANTS IN KV7.2 POTASSIUM CHANNELS CAUSE DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY BY GAIN-OF-FUNCTION EFFECTS

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Introduction
Kv7.2 subunits encoded by the KCNQ2 gene underlie the M-current, a subthreshold, slowly activating and deactivating, non-inactivating current that reduces neuronal excitability and mediates spike frequency adaptation. Each Kv7.2 subunit contains six transmembrane segments: S1–S4 form the voltage-sensing domain, whereas S5 and S6, and the intervening linker, constitute the pore region. Pathogenic variants in Kv7.2 are a frequent cause of childhood-onset genetic epilepsies and, in severe cases, neonatal developmental and epileptic encephalopathy (KCNQ2-DEE), a clinical condition characterized by neonatal-onset pharmacoresistant seizures and varying degrees of neurodevelopmental delay. Most KCNQ2-DEE variants studied to date produce loss-of-function (LoF) effects in vitro; however, a smaller subset of patients with neurodevelopmental delay with or without infantile onset epilepsy or non-epileptic neonatal myoclonus carry variants which increase channel function in vitro, thus producing gain-of-function (GoF) effects. To expand such genotype-phenotype correlation, in the present work we have studied the functional properties of six novel variants found in a small cohort of patients with KCNQ2-DEE characterized by mild to profound developmental delay and later (non-neonatal) infantile onset epilepsy.

Methods
Wild-type and mutant Kv7.2 channels were transiently transfected in mammalian CHO cells. Whole-cell patch-clamp recordings were performed to assess current amplitude, voltage-dependent gating properties, and activation and deactivation kinetics. Functional properties were evaluated in both homomeric Kv7.2 channels and heteromeric Kv7.2/Kv7.3 configurations.

Results
Among investigated variants, one is located in the S2–S3 linker, one in the S3 segment, two in the S3–S4 linker, one in the S4 segment and one at the C a-helix of the C-terminal (G546S) of Kv7.2 channel. When expressed as homomers, all mutant subunits induced a hyperpolarizing shift in the voltage dependence of activation, with four also increasing current density. Similar changes were observed in heteromeric Kv7.2/Kv7.3 channels. Notably, the variant in S4 markedly altered Kv7.2 channel kinetics and reduced the slope of the conductance–voltage (G/V) relationship, indicating impaired voltage sensitivity. Overall, these electrophysiological changes are consistent with a gain-of-function mechanism.

Conclusions
These findings expand the spectrum of Kv7.2 gain-of-function variants, further supporting the existence of a distinct electrophysiological and clinical phenotype associated with Kv7.2 GoF mutations, differing from that associated to LoF variants. Moreover, they provide additional insights into the structural determinants of Kv7.2 channel gating.

10.

A NOVEL ROLE FOR KININOGEN IN THE REGULATION OF KV7 CHANNEL ACTIVITY AND NEURONAL EXCITABILITY IN MULTIPLE SCLEROSIS

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Introduction
Multiple Sclerosis (MS) is a chronic, immune-mediated inflammatory disorder of the central nervous system (CNS), characterized by progressive neurodegeneration and alterations in neuronal excitability that contribute to functional impairment. Voltage-gated potassium channels Kv7.2 and Kv7.3 are transiently upregulated during inflammatory demyelination and generate the M-current, a hyperpolarizing outward current that activates below the action potential threshold. This current depends on phosphatidylinositol-4,5-bisphosphate (PIP2) and limits neuronal firing. In addition to ion channel dysregulation, components of the kallikrein-kinin system (KKS) contribute to MS pathology. While several KKS factors have been studied, the role of kininogen remains unclear. This study aimed to investigate the role of kininogen in neuronal excitability.

Methods
Electrophysiological recordings were performed to assess neuronal activity. Kininogen was focally injected into the auditory cortex of mice, and neuronal responses were recorded in vivo. Voltage-clamp experiments in acute brain slices and transfected HEK293FT cells were used to evaluate the effects of kininogen on Kv7 channel activity. In order to assess the M-current more directly, HEK 293FT cells were transfected with a plasmid containing Kv7.3 and Kv7.2 channels in a fixed 1:1 stoichiometry and linked to the fluorescent molecule GFP Brain slice recordings with intracellular PIP2 application were conducted to explore the underlying mechanism. To analyze the interaction of kininogen with the plasma membrane, modelling was performed. The kininogen-1 model generated with AlphaFold was positioned within membranes using an unbiased, automated approach implemented in YASARA (run-membrane-fast.mcr).

Results
Here we show that focal injection of kininogen significantly reduces neuronal activity in the auditory cortex. Next to this effect, voltage-clamp recordings revealed that kininogen suppresses Kv7 channel activity across multiple expression systems. Furthermore, in acute brain slices, the inhibitory effect of kininogen on neuronal firing was abolished by intracellular application of PIP_2, indicating a PIP_2-dependent mechanism. Consistent with this, our modelling data support a close interaction of kininogen with the plasma membrane.

Conclusions
In conclusion, our findings identify kininogen as a modulator of neuronal excitability that acts by suppressing Kv7 channel activity through a PIP_2-dependent mechanism. The loss of its inhibitory effect upon intracellular PIP_2 supplementation, together with the modelling data is indicating a close interaction with the plasma membrane. This supports a membrane-associated mode of action. These results provide mechanistic insight into how kininogen can regulate neuronal activity in the cortex.

11.

TMPRSS6 CLEAVES KCNE1 AND CAUSES ARRHYTHMIAS IN IRON OVERLOAD DISEASE

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Introduction
Iron storage disease (haemochromatosis) is associated with cardiovascular manifestations, including various forms of cardiac arrhythmias of unknown origin. Here, we investigated cardiac arrhythmias associated with iron overload in human iPSC-derived cardiomyocytes (hiPSC-CM) and hiPSC-derived sinus node-like pacemaker cells.

Methods
Human iPSC-derived cardiomyocytes (hiPSC-CM) and hiPSC-derived sinus node-like pacemaker cells were used to model iron overload conditions. Cell activity was assessed measuring electric field potential (EFP) generation and contraction across the baseline impedance. Changes in TMPRSS6 expression and KCNE1 processing was examined using cell-based fluorescence assays and complementary cell-free cleavage assays. Functional effects of TMPRSS6-mediated cleavage on KCNQ1/KCNE1 were evaluated using electrophysiological assays in heterologous expression systems and in hiPSC-derived ventricular-like cardiomyocytes.

Results
Among other effects, iron overload leads to an increase in the plasma membrane-anchored protease TMPRSS6. TMPRSS6 cleaves the auxiliary subunit KCNE1 Nterminally and thus modulates the function of the IKs (KCNQ1/KCNE1 current) ion channels. Furthermore, TMPRSS6 induces a reduction of electric field potential (EFP) count and increased duration in hiPSC-derived ventricular like cells and in hiPSC-derived pacemaker-like cells.

Conclusions
TMPRSS6 is a novel mediator linking iron overload to cardiac electrical dysfunction by proteolytically modifying KCNE1 and disrupting KCNQ1-dependent channel function. Therefore, TMPRSS6 may represent new clinically relevant pro-arrhythmic mechanisms in iron overload diseases.

12.

KCNQ2-DEE: ARE NAV1.1 AND NAV1.6 CHANNELS INVOLVED IN THE SUDEP PHENOTYPE OBSERVED IN THE KCNQ2T274M/+ MOUSE MODEL?

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Introduction
Our laboratory has generated a knock-in mouse (KI) model for the recurrent pathogenic variant p.(Thr274Met) in KCNQ2 gene. Heterozygous animals exhibit spontaneous epileptic seizures as well as cognitive impairment. 25% of them die within the first 3 months of life as a result of spontaneous seizures (Milh et al. 2020). This phenotype may be relevant for the study of Sudden Unexpected Death in Epilepsy (SUDEP). Here, we sought to determine whether sudden deaths in this model are due to central respiratory dysfunction and if the Nav1.1 and Nav1.6 sodium channels are involved (Teran et al. 2022).

Methods
We studied neural networks that control inspiration (i.e. the preBötzingerComplex; PreBötC) in normoxia and severe hypoxia using an in vitro approach. We also investigated the ventilatory control in vivo, measuring respiratory rate, tidal volume, and minute ventilation by plethysmography under normoxic, hypoxic and hypercapnic conditions. We also recorded post-seizure respiratory recovery. Because previous data showed the involvement of sodium channels in the response to severe hypoxia (Peña et al. 2004), we verified the presence of Nav1.1 and Nav1.6 in the region of interest by immunohistochemistry and investigated expression and protein level of these channels in the cortex and brainstem, using RT-qPCR, digital droplet PCR and Western Blot analysis.

Results
Our results show that the recurrent pathogenic variant p.(Thr274Met) decreased the frequency of inspiratory activity in vitro in normoxia and affected the response to severe hypoxia. In vivo, the ventilation is not affected in normoxia or during gaz challenges. Anatomically, although the preBötC expresses potassium and sodium channels, the expression of Nav1.1 and Nav1.6 channels in the region of the respiratory centers is not affected in the studied heterozygous knock-in model.

Conclusions
The expression of the Nav1.1 and Nav1.6 proteins are not affected in the Kcnq2T274M/+ DEE model although we observed a defect of the inspiratory activity in vitro and although these channels were shown to have a role in this mechanism. Importantly, the in vivo ventilation appears to be normal in this model. Transcriptomic and electrophysiological experiments will now be conducted both mice and in iPSC-derived patient neurons to try to identify the molecular origin of the SUDEP phenotype observed in this mouse model.

13.

IKS SINGLE CHANNELS: A SURVEY

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The IKs current is important in cardiac repolarization especially at high heart rates. The current is underlain by four KCNQ1 (Q1; Kv7.1) subunits co-assembled with 1-4 KCNE auxiliary subunits, primarily KCNE1 (E1), and calmodulin. Single channel recordings are a unique way to examine how Kv channels react to a change in voltage in terms of latency to opening, conductance levels visited and open probability (Po). The co-assembly of Q1 with E1 alters these single channel characteristics in a graded manner as more E1 subunits present in the channel complex increase conductance and first latency to opening. The Q1 voltage sensor domain may also stably occupy an intermediate gating state which changes pore permeability properties, such as rubidium selectivity and permits the existence of high and low open probability states. In addition, each subunit can gate independently and allow ion conduction, which results in significant subconductance occupancy before the channel reaches the fully open state. Here, we will show single channel data to illustrate the effects of increasing the ratio of KCNE1 in the channel complex, demonstrate independent subunit gating and intermediate and activated state conductance. In addition, we will explore mutations at the interface of KCNQ1 and KCNE1 and how these impact single channel behaviours.

14.

THE INTERMEDIATE STATE STRUCTURE OF KCNQ1 ILLUMINATES VOLTAGE SENSOR DYNAMICS

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Introduction
KCNQ1 (Kv7.1) is a voltage-gated potassium channel essential for cardiac repolarization and the maintenance of a normal QT interval on an electrocardiogram. Given its central role in cardiac homeostasis, dysregulation can lead to arrhythmogenic disorders, including long- and short-QT syndromes, which may result in sudden death. KCNQ1 physiological function depends on its complex gating as the channel undergoes a multi-step activation, with functionally established resting, intermediate, and activated voltage sensor domain (VSD) states. Notably, both intermediate and activated channels are constitutively active in vitro, but the intermediate state exhibits higher affinity for inhibitors, making it a promising drug target. Despite its significance, this intermediate state has remained structurally elusive limiting our understanding of VSD activation and its role in electromechanical coupling, pharmacological modulation, and the mechanistic basis of channelopathies.

Methods
We used single-particle cryogenic electron microscopy to determine the structure of Xenopus laevis KCNQ1 engineered to trap the VSD in an intermediate state via charge-reversal mutations (xE1R/R2E) between the S2 and S4 VSD helices. Datasets for the xE1R/R2E-intermediate channel in the presence or absence of PIP2 were collected and processed. An additional dataset for the wild-type channel in the activated state (xWT-activated) was collected for reference. Finally, Molecular Dynamics simulations were used to assess protein stability and explore conformational dynamics.

Results
The xE1R/R2E-intermediate state structure at 3.53 Å revealed conformational rearrangements within the VSD compared to the xWT-activated state. In this xE1R/R2E-intermediate state, the upper portions of the S1 and S2 helices adopt a straight conformation and sit away from the filter helix of the adjacent subunit (FH'), whereas in the xWT-activated state these helices are curved and attached to the FH'. The S4 largely preserves an xWT-activated state conformation suggesting that the S4 displacement is nearly complete in this xE1R/R2E-intermediate state. The inner-gated was closed in the absence of PIP2 in both xE1R/R2E-intermediate and xWT-activated channels. However, xE1R/R2E-intermediate low-resolution classes, consistent with an open gate, were observed in the presence of PIP2, indicating that channel opening is ligand regulated.

Conclusions
We present the structure of a physiologically relevant intermediate state of KCNQ1, providing insight into VSD conformational transitions. The S1-FH' detached conformation may be the key for the physiological and pharmacological properties of the intermediate state. Together, our findings establish a structural framework for understanding KCNQ1 regulation and may inform the rational development of targeted therapeutics.

15.

MECHANISM OF KCNQ1 GATING REGULATED BY PIP2 AND CALMODULIN

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Introduction
KCNQ1 potassium channels are important for physiological processes. The assembly of KCNQ1 with KCNE1 generates IKs currents, which are essential for terminating cardiac action potential. PIP2 and calmodulin are potent regulators for KCNQ1 activation. KCNQ1 adopts both bent and straight conformations and can open to both intermediate-open (IO) and activated-open (AO) states. However, how the bent/straight conformations correlate with functional states and the mechanisms that induce the change between these conformations are not clear.

Methods
We used structure biology, electrophysiology, molecular docking, and molecular dynamic simulations to study the interactions of a PIP2 molecule with the voltage sensor of KCNQ1.

Results
Our recently solved KCNQ1-KCNE1 structure (straight, AO) and KCNQ1-E160R/R231E structure (bent, intermediate-closed, IC) both reveal a PIP2 molecule that binds adjacent to VSD, designated as V-PIP2. We found that nearly all V-PIP2 binding site mutants did not reduce KCNQ1 currents (predominantly IO) but reduced the IKs (exclusively AO). Prior studies indicated that V-PIP2 might compete with calmodulin for binding to VSD. Comparison of the AO and IC structures and the study of a novel compound (CA1), which specifically enhances IKs but not KCNQ1 functions, indicate that V-PIP2 binding changes in response to VSD activation from the I to A state. Both metadynamic and targeted MD simulations indicate that upward movements of S4 facilitate VSD activation and induce the V-PIP2 to form closer interactions with S2S3 Linker, promoting the detachment of calmodulin, which destabilizes the bent-IO state, triggering the bent to straight transition.

Conclusions
Our data collectively support the notion that the channel adopts a bent conformation in the IO state and a straight conformation in the AO state, with V-PIP2 facilitating the transition from bent to straight in response to the voltage dependent VSD activation from the I state to the A state.

16.

17B-OESTRADIOL INHIBITS KV7.4 CHANNELS VIA GPER1-GQ-PLC-PKC SIGNALLING

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Introduction
Kv7.4 channels play a central role in controlling membrane potential and smooth muscle excitability. Their dysfunction is associated with vascular and visceral disease. Oestrogen (17⊠-oestradiol; E2) modulates Kv7 channel activity in native tissues, with previous work indicating the involvement of G protein-coupled oestrogen receptor 1 (GPER1) in this process. However, the downstream signalling pathways remain unclear. Here, we show that, at physiological concentrations, E2 inhibits Kv7.4 in a GPER1-dependent manner and define the underlying signalling mechanism.

Methods
Kv7 currents were recorded using whole-cell patch clamp electrophysiology. Experiments were performed in HEK293 cells stably expressing Kv7.4 or transiently expressing Kv7.5 with or without GPER1. Cells were exposed to 10 nM E2 for 30 min. Signalling pathways were investigated using inhibitors of adenylyl cyclase, phospholipase C (PLC), and protein kinase C (PKC). Membrane abundance of GFP-tagged Kv7.4 was assessed by biotinylation and immunocytochemistry.

Results
E2 (10 nM) had no effect on Kv7.4 currents in cells expressing Kv7.4 alone, but inhibition was observed when GPER1 was co-expressed. Inhibition of adenylyl cyclase did not modify this response, whereas blockade of PLC or PKC prevented current suppression. Activation of GPER1 was associated with a reduction in the membrane abundance of Kv7.4. These findings indicate that E2 inhibits Kv7.4 via GPER1-dependent activation of Gq-PLC-PKC signalling. In contrast, currents generated by Kv7.5 expression were not inhibited by GPER1 activation.

Conclusions
E2 inhibits Kv7.4 via a GPER1-dependent mechanism involving Gq, PLC, and PKC signalling. This effect reduces channel availability without altering gating. These findings provide a mechanistic basis for oestrogen-dependent regulation of Kv7.4 and define a signalling pathway linking steroid hormones to Kv7 channel function.

17.

KV7.2 PORE STABILITY SHAPES REGULATION BY PHARMACOLOGICAL ACTIVATORS

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Introduction
Kv7/KCNQ channels are crucial determinants of cellular excitability and exhibit dual regulation by membrane voltage and the signaling lipid phosphatidylinositol 4,5-bisphosphate (PIP2). Cryo-EM structures indicate close proximity of PIP2 with multiple drug binding sites. However, the functional interplay between PIP2 and drugs on Kv7 modulation is not understood.

Methods
We used whole-cell patch clamp electrophysiology, rubidium efflux assays, and site-directed mutagenesis to investigate functional and pharmacological responses of Kv7.2 mutations that affect open state stability and/or drug sensitivity.

Results
Mutations at Kv7.2 residues R325 and K331/R332 in the S6-Helix A domain weaken Kv7.2 open state stability. Kv7.2[R325A] and Kv7.2[K331A][R332A] mutants compromise channel activity under ambient PIP2 levels, exhibiting severely reduced whole-cell conductance compared to WT Kv7.2. Also, these mutants exhibited pronounced insensitivity to the VSD-targeted activator ICA-069673, abolishing drug effects on deactivation kinetics and current magnitude. In contrast, mutant channel activity is powerfully augmented (≥20 fold) by the pore-targeted activator ML213. This stabilization of the open pore by ML213 rescues sensitivity of Kv7.2 gating modulation by ICA-069673, and this sensitivity can also be enhanced by elevation of membrane PIP2 content.

Conclusions
Our results demonstrate that open state stability strongly influences Kv7.2 sensitivity to VSD-targeted activators. We speculate that the open Kv7.2 pore conformation enables PIP2 binding in an orientation that couples the open pore and VSD, and contributes to formation of a stable ICA-069673 binding site in the VSD. These findings suggest a structural basis for PIP2 regulation of drug sensitivity in Kv7.2 channels.

18.

KCNE0, AN EARLYDIVERGING KCNE SUBUNIT, CONFERS CONSTITUTIVE ACTIVITY TO KCNQ1 IN LAMPREY

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Introduction
KCNE proteins are single-pass transmembrane auxiliary subunits that modulate the voltage-gated K^+ channel KCNQ1 (Kv7.1), generating diverse functional phenotypes essential for organ-specific physiology in jawed vertebrates. While six KCNE isoforms (KCNE1–6) have been well characterized in mammals and other gnathostomes, little is known about KCNE subunits in earlier-diverging vertebrates. This gap limits our understanding of how KCNE-dependent modulation of KCNQ1 originated and diversified during vertebrate evolution. Here, we identify and functionally characterize a KCNE-like subunit from lamprey, a jawless vertebrate, and examine its biophysical properties, species compatibility, and structural determinants of channel modulation.

Methods
kcne0 and kcnq1 cDNAs were isolated from lamprey tissues or artificially synthesized and heterologously expressed in Xenopus laevis oocytes. Channel function was analyzed using two-electrode voltage clamp recordings to assess current amplitude and voltage dependence. Voltage-clamp fluorometry was employed to monitor voltage-sensor movements of KCNQ1. Cross-species pairing experiments were performed using human, zebrafish, and tunicate KCNQ1. Site-directed mutagenesis was used to introduce KCNE isoform–related motifs. The transcript distribution of kcnq1 and kcne0 was assessed by RT-PCR and reanalysis of published lamprey RNA-seq datasets.

Results
Lamprey possesses a single KCNE-like gene, designated KCNE0, which shows moderate sequence similarity to mammalian KCNE1–5 without clear orthology. Both kcnq1 and kcne0 transcripts were detected in multiple lamprey organs. When coexpressed with lamprey KCNQ1, KCNE0 induced a strong negative shift in voltage dependence and generated constitutively active currents, resembling the effect of mammalian KCNE3. Voltage-clamp fluorometry demonstrated that KCNE0 alters KCNQ1 gating by stabilizing an intermediate voltage-sensor conformation. Cross-species experiments revealed weak or mixed modulation of non-lamprey KCNQ1, indicating species-specific tuning of KCNQ1–KCNE compatibility. Introduction of a juxtamembrane tetra-leucine motif converted KCNE0 into a KCNE4-like inhibitory subunit.

Conclusions
This study identifies KCNE0 as an early-diverging member of the KCNE family and reveals that KCNE-dependent modulation of KCNQ1 is already present in jawless vertebrates. Our results highlight species-specific compatibility as a defining feature of KCNQ1–KCNE interactions and demonstrate that small intracellular motifs can qualitatively switch KCNE function toward inhibition. KCNE0 therefore provides a valuable evolutionary and functional reference for understanding the diversification of KCNE-mediated KCNQ1 channel regulation.

19.

BINDING SITE AND PUTATIVE MECHANISTIC INSIGHTS INTO THE INHIBITORY EFFECT OF ESTRADIOL (17B-E2) ON THE KV7.1/KCNE1 CHANNEL

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Introduction
The cardiac voltage-gated potassium channel Kv7.1/KCNE1 is essential for ventricular repolarization, and its inhibition is associated with Long QT Syndrome (LQTS) and risk of sudden cardiac death. The female sex hormone estradiol (17β-E2) has been linked to QT interval prolongation, potentially placing women and individuals undergoing hormone therapy at elevated arrhythmia risk. This risk may be further compounded in individuals with inherited LQTS caused by loss-of-function mutations in Kv7.1 or KCNE1. Previous work has shown that 17β-E2 inhibits Kv7.1/KCNE1 in a KCNE1-dependent manner, yet the precise binding site and molecular mechanism of inhibition have remained unknown.

Methods
To identify the 17β-E2 binding site, we combined two-electrode voltage clamp electrophysiology in Xenopus oocytes with site-directed mutagenesis and molecular dynamics simulations. The functional effects of 17β-E2 and structurally similar steroid hormones were additionally characterized using automated patch clamp electrophysiology.

Results
Docking simulations identified a putative binding site for 17β-E2 at the interface between the KCNE1 and Kv7.1 subunits. Mutagenesis of key residues at this interface altered channel sensitivity to 17β-E2, confirming the functional relevance of the site. Structurally related steroid hormones were found to exhibit varying inhibitory effects on Kv7.1/KCNE1, highlighting the structural sensitivity of the binding site. The precise molecular mechanism by which 17β-E2 inhibits the channel remains under investigation.

Conclusions
This study identifies, binding site for 17β-E2 on the Kv7.1/KCNE1 channel, located at the Kv7.1/KCNE1 subunit interface, and confirms its functional importance through mutagenesis. Together, these findings advance our mechanistic understanding of of how estradiol and related hormones inhibit Kv7.1/KCNE1 channel function, and lay the groundwork for exploring how inherited LQTS-associated mutations may influence estrogen sensitivity, with future implications for arrhythmia risk assessment and personalized medicine.

20.

PHARMACOLOGICAL IMPLICATIONS OF βADRENERGIC PHOSPHORYLATION OF KV7.1/KCNE1

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Introduction

The interaction between βadrenergic stimulation and the Kv7.1/KCNE1 potassium channel constitutes a pivotal post-translational regulatory mechanism in cardiac physiology, enabling rapid adaptative modulation of the heart in response to sympathetic activation. βAdrenergic signalling engages the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) transduction cascade, leading to PKAdependent phosphorylation of two serine residues (S27 and S92) within the Nterminal region of the channel complex. Phosphorylation at these sites increases current amplitude and accelerates repolarization, thereby promoting actionpotential shortening necessitated to sustain elevated heart rates during physiological stress. Consequently, the cAMP/PKA axis plays a significant role in the fightorflight response – an effect underscored by a subset of longQT syndrome patients who are particularly vulnerable to arrhythmias, cardiac arrest, and sudden death during heightened sympathetic drive. The double phosphomimetic Kv7.1 mutant S27D/S92D, co-expressed with KCNE1, has been used to recapitulate cAMP mediated phosphorylation, however, the effect of endogenous hormones or pharmacological agents in modulating phosphomimetic Kv7.1/KCNE1 is not currently known.

Methods

The two-electrode voltage clamp (TEVC)-technique was used to compare the pharmacological effect of selected compounds on human phosphomimetic Kv7.1/KCNE1 compared to wild-type (WT) Kv7.1/KCNE1 expressed in Xenopus laevis oocytes.

Results

Preliminary data indicated that both 17β-oestradiol (17β-E2) and cannabidiol (CBD) appeared less efficacious at inhibiting the phosphomimetic mutant compared to WT Kv7.1/KCNE1.

Conclusions

PKAdependent phosphorylation of the Kv7.1/KCNE1 channel during sympathetic activation represents a powerful post-translational modulation in cardiac physiology. Previous studies have shown that both 17β-E2 and CDB show potent inhibition of WT Kv7.1/KCNE1. In contrast, our preliminary data indicated that this inhibitory effect was reduced in the phosphomimetic Kv7.1/KCNE1 double mutant – highlighting a physiologically relevant milieu for potential drug targeting.

21.

AN INTEGRATED ASSAY PLATFORM FOR MECHANISTIC AND TRANSLATIONAL PROFILING OF KV7.2/7.3 ACTIVATORS

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Introduction
Kv7.2/7.3 (KCNQ2/3) potassium channels underlie the neuronal M-current, a key regulator of membrane excitability and action potential firing. Pharmacological activation of these channels represents a validated strategy for dampening hyperexcitability in disorders such as epilepsy and neuropathic pain. Activators of Kv7.2/7.3 exhibit diverse mechanisms of action, including pore stabilisation, voltage sensor modulation and interactions with lipid cofactors, such as phosphatidylinositol 4,5-bisphosphate (PIP2). This poster highlights the suite of cellular, biophysical and translational assays offered by Metrion Biosciences to systematically evaluate the pharmacological and physiological effects of Kv7.2/7.3 activators acting through distinct modalities. Our comprehensive range of assays support drug discovery programmes, from high-throughput screening to profiling the distinct physiological effects of compounds with similar pharmacological profiles.

Methods
Fluorescence-based thallium flux (Fluorescent Imaging Plate Reader, FLIPR Penta), voltage clamp and current clamp recordings using automated patch clamp electrophysiology (QPatch 48 and Qube 384, Sophion Bioscience) and translational current clamp manual patch clamp dorsal root ganglion (DRG) recordings.

Results
FLIPR and automated patch clamp electrophysiology (QPatch 48 and Qube 384) assays were used to establish a workflow to resolve distinct mechanisms of Kv7.2/7.3 activation. This approach enables differentiation between compounds that shift the voltage dependence of activation, enhance maximal conductance or modify deactivation kinetics. To link these mechanistic effects with functional outcomes, current clamp recordings were investigated using both automated electrophysiology platforms using a heterologous cell line expressing Kv7.2/7.3 channels and native currents in DRG neurons using manual patch clamp. This combined strategy enables assessment of how distinct modes of channel activation influence neuronal excitability, including changes in resting membrane potential, input resistance and action potential firing patterns.

Together, this workflow is designed to address the challenge that compounds with similar apparent pharmacological profiles can produce divergent physiological effects.

Conclusions
This integrated workflow enables mechanistic differentiation of Kv7.2/7.3 activators and links these profiles to functional outcomes in neuronal models, facilitating the development of next-generation activators with improved efficacy and safety profiles.

22.

LOCAL VASCULAR METABOLISM OF PARACETAMOL GENERATES NAPQI TO DRIVE KV7-DEPENDENT VASODILATATION AND INTRAVENOUS PARACETAMOL-INDUCED HYPOTENSION

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Background
Intravenous (IV) paracetamol is used for pain management in perioperative and critically ill patients but is frequently associated with transient hypotension. The mechanism underlying this adverse effect remains poorly understood. We hypothesised that local vascular metabolism of paracetamol generates the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which directly regulates vascular ion channels to produce vasodilatation.

Methods
Vasodilator responses of resistance arteries were assessed using wire myography. Pharmacological inhibitors were used to investigate the contribution of calcitonin gene-related peptide (CGRP) signalling and Kv7 channel activation to vasorelaxation and blood pressure regulation in vivo following IV paracetamol administration. Human coronary artery endothelial cells (HCAECs) were used to investigate vascular metabolism of paracetamol through immunocytochemistry for protein-paracetamol adducts, intracellular thiol measurements, metabolic assays and quantitative proteomics.

Results
NAPQI produced potent relaxation of resistance arteries, whereas paracetamol itself was inactive. Vasorelaxation resulted from both direct activation of vascular Kv7.4/Kv7.5 potassium channels and indirect stimulation of CGRP release from perivascular sensory nerves, with inhibition of Kv7 channels markedly attenuating paracetamol-induced hypotension in vivo. These findings identified NAPQI as the principal vasoactive mediator responsible for the haemodynamic effects of IV paracetamol. Exposure of HCAECs to paracetamol resulted in formation of protein-paracetamol adducts and intracellular thiol depletion, consistent with generation of a highly reactive metabolite. Proteomic analysis demonstrated upregulation of cytochrome P450-associated proteins, including CYP20A1 and cytochrome P450 oxidoreductase, together with proteins involved in pentose phosphate pathway activity and maintenance of cellular redox homeostasis. Inhibition of cytochrome P450 enzymes significantly attenuated paracetamol-induced thiol depletion, supporting endothelial CYP450-mediated bioactivation. In parallel, IV paracetamol administration produced rapid proteomic changes in mesenteric arteries that closely resembled endothelial responses and established hepatic signatures of paracetamol metabolism, whereas corresponding liver tissue demonstrated minimal early metabolic adaptation, indicating that metabolism occurs within the vascular wall during the time course of hypotension. Myeloperoxidase (MPO) also converted paracetamol into a thiol-reactive metabolite at clinically relevant enzyme concentrations, identifying a complementary pathway capable of enhancing vascular NAPQI generation.

Discussion
Paracetamol is metabolised within the vascular wall by endothelial cytochrome P450 enzymes, with MPO providing an additional metabolic pathway during systemic inflammation, to generate the reactive metabolite NAPQI. Locally generated NAPQI subsequently activates vascular Kv7 channels directly and indirectly through CGRP release, producing resistance artery relaxation and systemic hypotension. This integrated mechanism explains both the rapid onset of hypotension following IV administration and the increased susceptibility of critically ill patients with elevated inflammatory burden.

23.

VOLTAGE CLAMP FLUOROMETRY IN KV7.4 TO DETERMINE PHARMACOLOGICAL MECHANISMS

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Introduction
Pharmacological channel modulation is mainly assessed by the measurement of ion channel currents, using techniques such as patch-clamp or two-electrode voltage clamp (TEVC) electrophysiology. These methodologies provide insight into the effects of compounds by quantifying parameters like maximum conductance (Gmax) or voltage dependence of channel activation (V50). However, electrically silent biophysical effects, like compound effects on S4 movement in the voltage sensing domain (VSD), are not measurable. From the Kv7 family, Kv7.4 is one of the channels that show a higher or different response to certain modulators, where the mechanism of action is not always clear. Therefore, the use of techniques like voltage clamp fluorometry (VCF) may be of great value to reveal details underlying biophysical effects of Kv7.4 channel modulators.

Methods
The KCNQ4 sequence was mutated, introducing N196C, enabling S4 fluorophore labelling. Xenopus laevis oocytes were injected with cRNA and used as heterologous model system. VCF measurements were obtained using a TEVC rig coupled with a DLMFS upright microscope with a photodiode, enabling the recording of currents and fluorescence signals simultaneously. As prototype compounds, Retigabine (3μM) and QO-58 (3μM) were selected to assess their effects on S4 movement and ionic currents.

Results
Preliminary data suggest that Retigabine induces a prominent increase in Gmax, accompanied by only a small negative shift in the V50 of both fluorescence and current. On the other hand, QO-58 induces a pronounced negative shift in the V50 of both fluorescence and current (approximately -30mV), with only a minor increase in Gmax. These results are in agreement with the idea that the binding site of modulators impact the characteristics of the effects caused in channel function. Retigabine, which binds to S5-S6, does not seem to cause clear effects on VSD movement. Whereas QO-58, suspected to bind in the top of the S3-S4, has significative effects on VSD movement, modifying its voltage dependency, and likely modifying channel behaviour through these means.

Conclusions
Our preliminary results suggest that VCF in Kv7.4 is a potential tool to not only review the biophysical effects caused by different channel modulators but also to hint possible binding sites of new modulators. We will expand our study to other Kv7.4 channel modulators to provide further insights of their underlying mechanism of action and explore to what extent different pharmacological profiles of Kv7.4 modulators, with primary impact on V50 or Gmax, seem to be directly associated with the localization of the binding site.

24.

EFFECT OF 'SQUEEZE-TO-INHIBIT' AGENT EBIO3 ON SMOOTH MUSCLE RELAXATIONS

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Introduction

Kv7 channels encoded by KCNQ1-5 are important regulators of cellular physiology across the body. In vascular and non-vascular smooth muscle KCNQ1, 4 and 5 are predominate and expression products are robust regulators of contractile activity at rest and in response to receptor-mediated vasodilators. Functional impact has been derived from use of pan-Kv7 blockers (linopirdine or XE991) or Kv7.1-specific blockers (HMR1556, Chromanol 293B) that are pore blockers. The aim of this study was to characterise the effect on smooth muscles of EBIO3 that inhibits Kv7.2-7.5 with IC50 values between 1.2 nM and 196 nM with a novel mechanism of action Kv7 channels (so called 'squeeze to inhibit' inactivation, Li et al., 2025).

Methods

Mesenteric and renal arteries from male wistar rats and human gastrointestinal arteries or elective surgery were suspended on tungsten wires for isometric force measurement. Arteries were incubated with 5nM, 100nM and 300nM EBIO3 for 15 minutes prior to stimulation with a vasoconstrictor (U46619, 5HT). Once the contraction had stablised the Kv7.2-7.5 activator ML213 was applied (0.3 – 10 μM). Isometric tension recordings were made from segments of rat bladder detrusor that exhibited spontaneous contractions and evoked contractions were generated by electrical field stimulation. ML213 was applied in the presence of 100 nM EBIO3

Results

Introduction of 100nM and 300nM EBIO3 inhibited the response to ML213 in all arteries and also the rat bladder. In contrast, 5 nM EBIO3 has no effect on Ml213 -induced effects. XE991 at 300 nM also affected the response to ML213 whereas 10 uM completely abrogated any relaxant effect.

Conclusion

EBIO3 at >100 nM is a potent and effective inhibitor of ML213-induced relaxations in rat and human arteries as well as rat bladder. Future experiments will ascertain the effectiveness of this agent against receptor-mediated relaxations.

Reference

Li, J., Yang, Z., Zhang, S. et al. Small molecule inhibits KCNQ channels with a non-blocking mechanism. Nat Chem Biol 21, 1100–1109 (2025). https://doi.org/10.1038/s41589-024-01834-8

25.

BEYOND RETIGABINE: ELECTROPHYSIOLOGICAL CHARACTERIZATION OF AZETUKALNER ACROSS KV7/KCNE COMPLEXES

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Heteromeric Kv7.2/Kv7.3 (KCNQ2/KCNQ3) potassium channels constitute the molecular basis of the neuronal M-current, a key determinant of subthreshold excitability. Loss-of-function mutations in KCNQ2 and KCNQ3 cause epileptic disorders ranging from self-limited neonatal seizures to severe developmental and epileptic encephalopathy. Retigabine (Trobalt, Potiga), the second approved Kv7 channel opener after flupirtine (Katadolon), but the first Kv7-targeting anticonvulsant, was withdrawn from the market in 2017 due to retinal and mucocutaneous pigmentation caused by the formation of chromophoric phenazinium-type dimers after oxidative biotransformation to reactive quinone diimine intermediates. This metabolic characteristic is enabled through the triaminoaryl scaffold, which is also shared by the hepatotoxic flupirtine. Azetukalner (Encukalner, XEN1101, formerly 1OP 2198) is a second-generation Kv7.2/7.3 opener whose diaminoaryl scaffold, incorporating a tertiary aromatic amine, prevents the oxidative liability seen in first generation compounds, causing dimer formation or hepatotoxicity. It is currently in phase III clinical trials for focal and generalized seizures as well as major depressive disorder, with recently reported positive phase III topline results (X-TOLE2). Despite this advanced clinical development, detailed electrophysiological characterization data have either not been systematically studied or remain proprietary, with only limited biophysical information available through conference proceedings (Bialer et al., Epilepsia 2017, Bialer et al., Epilepsia 2020 and Bialer et al., Epilepsia 2022). Moreover, Kv7 α-subunits in native tissues can co-assemble with KCNE accessory β-subunits, which are known to profoundly alter channel gating, voltage dependence, and pharmacological sensitivity. Whether KCNE co-expression modulates the pharmacological response to azetukalner has not been reported. Using automated two-electrode voltage clamp recordings in Xenopus laevis oocytes, we systematically characterize azetukalner on homomeric Kv7.1–Kv7.5 channels and heteromeric Kv7.2/7.3 complexes, with retigabine tested in parallel under identical conditions (e.g. V1/2 shifts, current change at specific voltages and EC_{50} values). Selected Kv7 subtypes are further co-expressed with their physiologically established KCNE partners (e.g., Kv7.1+KCNE1, Kv7.2/Kv7.3+KCNE2 and Kv7.4+KCNE4) to assess whether accessory subunit co-assembly alters azetukalner potency, efficacy, or voltage-dependent mode of action. This study will provide the first side-by-side electrophysiological comparison of azetukalner and retigabine across all Kv7 subtypes and their physiologically relevant KCNE complexes, addressing a critical gap in understanding how the native molecular context shapes the pharmacological profile of this clinically advanced Kv7 opener.

26.

THE PARACETAMOL METABOLITE NAPQI PREVENTS GPCR-MEDIATED INHIBITION OF KV7 CHANNELS

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Introduction
Paracetamol is widely used to alleviate pain, though its precise mechanism of action remains incompletely understood. We have recently demonstrated that its metabolite, N-acetyl-p-benzoquinone imine (NAPQI), enhances currents through Kv7 potassium channels by modifying a triple cysteine motif in the S2–S3 linker of these channels. This modulation reduces neuronal excitability in pain pathways and contributes to the analgesic effect of paracetamol in vivo, but only after inflammation is induced. Inflammatory mediators such as bradykinin, adenosine triphosphate (ATP), or histamine inhibit Kv7 channels via Gq-coupled pathways, thereby increasing neuronal excitability and promoting pain sensation.

Methods
We investigate the underlying mechanism by using perforated patch-clamp recordings combined with fluorescence imaging in heterologous expression systems and primary sensory neurons.

Results
Cytosolic Ca2+-mediated inhibitory effects of an artificial “inflammatory soup” containing ADP, ATP, bradykinin, histamine, 5-hydroxytryptamine, prostaglandin E 2, substance P and a PAR2 agonist on KV7 channel currents in primary sensory neurons were eliminated by NAPQI. Moreover, the increase of KV7.2 channel currents induced by loss of cytosolic Ca2+ is prevented by NAPQI. In addition, PIP2-mediated Kv7 channel inhibition was impaired by NAPQI. These effects were lost in mutant channels lacking the three cysteines in the S2-S3 linker.

Conclusions
NAPQI covalently modifies the triple-cysteine motif in the S2–S3 linker of Kv7 channels. This counteracts the signalling cascades employed by a combination of inflammatory mediators, namely both Ca2+- and PIP2-mediated inhibition. In primary sensory neurons, this prevents the inhibition of Kv7 channels by the inflammatory soup. This mechanism is likely involved in the relief of inflammatory pain by paracetamol seen in vivo.

27.

CONSERVED BINDING SITE, DIVERGENT MODULATION: RETHINKING MAXIPOST SELECTIVITY ACROSS KV7 CHANNELS

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Introduction
The Kv7 (KCNQ) family of voltage-gated potassium channels (Kv7.1–Kv7.5) controls cellular excitability and is a major target for cardiac and neurological drug discovery. MaxiPost (BMS-204352) shows an unusual subtype-selectivity profile – robust agonist of Kv7.4 and Kv7.5, inhibitor of Kv7.1, but only weakly active on Kv7.2/Kv7.3 – pointing to a mechanism distinct from Retigabine. The Kv7.4 Trp242Leu mutation abolishes its positive modulation, but the molecular basis of this peculiar profile has remained unclear.

Methods
Starting from the activated open-state structure of Kv7.4 embedded in a POPC/PIP2 bilayer, we performed a systematic computational search of MaxiPost binding poses around Trp242. We generated 1.6 M hypothetical binding poses on a Fibonacci-lattice grid, filtered by interaction energy and distance criteria, agglomeratively clustered by RMSD, and refined by molecular dynamics. The predicted pose was validated by site-directed mutagenesis and two-electrode voltage-clamp recordings. Pharmacological perturbation of channel open probability (PO) was performed using the cysteine-modifying reagent N-ethylmaleimide (NEM).

Results
We identified a novel binding mode in which MaxiPost bridges S6 of one subunit and S5 of the adjacent one: Trp242 of subunit I forms a hydrogen bond with the ether oxygen, while the carboxamide moiety is anchored by H-bonds to the Leu306 and Ser309 backbones of subunit II. Mutagenesis confirmed each key contact: Trp242Phe/Tyr/His and Ser309His abolished MaxiPost-induced potentiation, whereas Ser309Ala behaved as WT, consistent with a backbone-mediated H-bond. Strikingly, the entire binding site is perfectly conserved in Kv7.2, ruling out binding-site divergence as the source of subtype selectivity. We therefore hypothesized that MaxiPost engages both subtypes but only potentiates channels with low basal open probability. Consistently, raising PO pharmacologically with NEM reduced MaxiPost potentiation of Kv7.4 from ~500% to ~100% ΔGMAX and fully suppressed any residual response in Kv7.2.

Conclusions
The apparent subtype selectivity of MaxiPost across the Kv7 family is not encoded in the binding site, which is conserved between Kv7.2 and Kv7.4, but emerges from intrinsic biophysical differences in baseline open probability that limit the visibility of agonist action. These results reframe how Kv7 subtype-selective modulators should be screened, interpreted, and rationally designed.

28.

BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERIZATION OF KV7.4 CHANNELS USING AUTOMATED PATCH CLAMP

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Introduction
The KV7 family of voltage-gated potassium channels plays critical roles in cellular excitability and signal regulation. Among them, KV7.4 channels are specifically expressed in the inner ear and are crucial for auditory function. Dysfunction or modulation of these channels has been linked to epilepsy and age-related hearing loss. Modulating KV7.4 channels provides therapeutic opportunities, with activators shown to stabilize electrical activity and protect hearing. Using the Sophion QPatch automated patch clamp (APC) platform, we developed a robust and reproducible assay for KV7.4 biophysical characterization and pharmacological testing. This work highlights the platform's ability to support high-quality, high-throughput compound screening.

Methods
HEK-KV7.4 cells, provided by Saniona, were cultured per supplier protocols. Experiments were conducted at 22°C ± 1°C using QPatch II and single-hole consumables. Key biophysical parameters, such as voltage-dependent currents, conductance-voltage relationships, and tail currents, were measured using Sophion analyzer software. Pharmacological assessments included activators like retigabine and inhibitors like linopirdine, profiling their effects on voltage sensitivity, gating kinetics, and current amplitude. Concentration-response curves were generated to determine EC50 and IC50 values.

Results
The QPatch platform demonstrated high assay success rates (71% ± 6%) and precise biophysical characterization of KV7.4 channels.

Biophysical properties, including voltage sensitivity (V½) and current amplitudes, were reproducibly assessed, supporting reliable compound evaluation.

Fitted cumulative concentration-response curves gave: EC50 values for KV7.4 activators - flupirtine maleate, ICA 069673, and retigabine; IC50 values for KV7.4 inhibitors - linopirdine, XE 991 dihydrochloride and ML 252.

Conclusions
This assay facilitates the study of KV7.4 modulators for therapeutic applications, addressing unmet needs in epilepsy, hearing loss and other neurological disorders. The platform's efficiency and reliability position it as a valuable tool in ion channel research and drug discovery.

29.

GENOTYPE-PHENOTYPE CORRELATIONS IN KV7.2-RELATED EPILEPSIES CAUSED BY DISTINCT MUTATIONS ON THE SAME RESIDUE

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Introduction

One of the major challenges in the channelopathy field is the understanding of the correlation between in vitro phenotypes and the clinical disease characteristics. In Kv7.2-related disorders, mutations occurring in benign/familial cases are mainly associated to mild loss-of-function (LoF) effects, whereas quantitatively stronger LoF deficits are caused by variants responsible for more severe sporadic cases of early-onset epileptic encephalopathy. To complicate this pattern, variants causing gain-of-function (GoF) effects have been more recently found in small series of patients with characteristic clinical phenotypes, often in the absence of neonatal seizures. These observation suggest that in vitro testing has a critical role not only in defining the pathogenic role of a specific variant, but also in predicting distinct clinical phenotypes. To support this view, we herein present genetic and clinical, as well as functional and pharmacological in vitro data on two patients carrying Kv7.2 mutations on the same residue (S195): the first patient, carrying the S195P variant, had no history of neonatal seizures but displayed infantile spasms at 5 months of age (Weckhuysen et al., 2013); the second patient, in which the S195F variant was found, showed the characteristic clinical pattern of neonatal-onset KCNQ2-epiletic encephalopathy.

Methods

Genetic analysis was performed by next-generation sequencing. Mutations were engineered by QuickChange mutagenesis; channel subunits were expressed in CHO cells by transient transfection. Currents were recorded using the whole-cell configuration of the patch-clamp technique.

Results

Electrophysiological recordings revealed that, when compared to wild-type Kv7.2 channels, the voltage-dependence of Kv7.2 S195P or Kv7.2 S195F channels was voltage-shifted in opposite directions, namely toward the left or the right of the voltage axis, respectively. These results are consistent with GoF for the S195P or LoF for the S195F in vitro effects. Qualitatively similar, although quantitatively smaller, effects were also observed upon co-expression of mutant Kv7.2 subunits with Kv7.2/Kv7.3 subunits, to mimic the genetic balance of each proband. Selective Kv7 activators (retigabine) or blockers (ML252 and NH17) (Cheung et al., 2012; Kornilov et al., 2014) restored wild-type behaviour in Kv7.2 S195F or S195P channels, respectively.

Conclusions

These results suggest that clinical phenotypes of Kv7.2-related epilepsies associated to different variants affecting the same residue can lead to distinct and even opposite in vitro phenotypes, and point toward variant-specific personalized pharmacological approaches.

30.

KV7.4 ACTIVATION PROTECTS DOPAMINERGIC NEURONS AGAINST A-SYNUCLEIN/ROTENONE-INDUCED TOXICITY: A NOVEL PHARMACOLOGICAL TARGET IN PARKINSON'S DISEASE?

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Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder characterized by the progressive and selective degeneration of dopaminergic neurons in the substantia nigra, together with the accumulation of oligomeric α-synuclein aggregated within intraneuronal inclusions known as Lewy bodies. Increasing evidence points to ion channels' dysfunction as a contributing factor of PD pathogenesis. Of note, potassium (K^+) channels are widely expressed in the central nervous system and play a crucial role in regulating neuronal excitability, synaptic transmission, and neurotransmitter release. Recent findings suggest that alterations in K^+ channel activity, including the Kv7 family, may contribute to dopaminergic neuron vulnerability. In particular, emerging evidence supports the expression of Kv7.4 channels and their role in regulating mitochondrial K^+ permeability in neurons, highlighting a potential link between Kv7 family and neuronal viability.

Methods

A neuronal model of PD was obtained upon exposure of primary rat mesencephalic neuronal cultures, enriched in tyrosine hydroxylase (TH)-positive neurons, to the mixture of oligomeric α-synuclein (10 nM) plus the mitochondrial complex I inhibitor rotenone (300 nM) for 24 hours. The possible involvement of the Kv7 channels in neuronal survival was investigated by 1 hour-pretreatment with specific Kv7 modulators before mixture addition. In particular, retigabine (10 mM) was used as a pan neuronal Kv7 stimulator, XE991 dihydrochloride (10 mM) as a Kv7 inhibitor, and the acrylamide (S)-1 (1 mM) as a Kv7.4-selective activator. The expression of Kv7 subunits was evaluated by using specific commercially available antibodies.

Results

In our PD model, exposure to α-synuclein/rotenone mixture induced significant neuronal damage, resulting in an approximately 25% reduction in cell viability. Of note, preliminary results suggested that retigabine significantly counteracted this toxic insult, highlighting its potential neuroprotective effect against α-synuclein/rotenone-induced neuronal death. This protective action was largely prevented by XE991, suggesting the involvement of Kv7 channels. To further explore the predominant Kv7 subunits involved, the selective Kv7.4 activator (S)-1 was employed. The results obtained showed that (S)-1 significantly attenuated α-synuclein/rotenone-induced neuronal damage. This effect was largely abolished by co-exposure to XE991, thus suggesting a predominant role of Kv7.4 subunits in mediating neuronal survival.

Conclusions

These results suggest that activation of Kv7.4 channels confers neuroprotection in the α-synuclein/rotenone-induced model of PD, highlighting Kv7.4 channels as a promising therapeutic target for strategies aimed at slowing or preventing progressive neurodegeneration.

31.

QRL-101-05: A PHASE 1 STUDY TO EVALUATE CORTICAL AND MOTOR NERVE EXCITABILITY IN HEALTHY VOLUNTEERS FOLLOWING ADMINISTRATION OF THE KCNQ7.2/7.3 MODULATOR QRL-101

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Introduction
QRL-101, a potential best-in-class Kv7 modulator small molecule, is in a unique chemical class from ezogabine and other Kv7 competitors' compounds. QurAlis executed a Phase 1 study, QRL-101-05, to understand the pharmacokinetic and dynamic relationship between QRL-101 and implicated electrophysiological markers for hyperexcitability.

Methods
Study QRL-101-05 is a Phase 1 proof-of-mechanism study of QRL-101 in healthy volunteers to evaluate biomarkers related to ALS, pain and epilepsy. A 3-period, 3-way cross-over design in which participants received low- and high-dose QRL-101 and placebo. Participants were assessed by mNETT, EEG, TMS-EEG, and TMS-EMG at pre-dose, then post dose at an early (starting at 30 minutes) and later (starting at 4 hours) timepoint.

Results
TMS-EMG intracortical facilitation was statistically significantly decreased at both the high and low dose increasing into significance with time, from the early time point to the later timepoint. There were significant increases in EEG spectral power in beta and gamma spectral bands. Treatment with QRL-101 resulted in significant changes in TEP parameters N100, P180, and 0-300ms from single and paired pulses.

The primary mNETT endpoint and other endpoints related to peripheral nerve excitability were met, establishing a dose-dependent response. The safety and tolerability profile of QRL-101 was consistent with previously reported study results to date. There were no SAEs or discontinuations due to AEs reported in the study.

Conclusions
Biomarkers assessed through mNETT, TMS-EMG, TMS-EEG, and pEEG indicate evidence of QRL-101 brain penetration, target engagement, and potential anti-seizure effects. Little to no effects on delta/theta waves coupled with no related AEs for somnolence suggest that selectivity for Kv7.2/7.3 against the GABA-A receptor, Kv7.4 channel and Kv7.3/7.5 channel is an effective strategy to improve tolerability of a Kv7.2/7.3 opener without decreasing efficacy. Results of QRL-101-05 support continued development of QRL-101 to a Phase 2 study in epilepsy.

32.

ENDOCANNABINOID DERIVATIVES FOR SELECTIVE ACTIVATION OF KV7.1/KCNE1 IN THE CONTEXT OF LONG QT SYNDROME.

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Introduction

Long QT Syndrome (LQTS) is a cardiac arrhythmia caused by delayed ventricular repolarization, increasing the risk of life-threatening cardiac events. It is most often linked to mutations in genes encoding voltage-gated ion channels, particularly the Kv7.1/KCNE1 channel complex, which plays a central role in ventricular cardiac repolarization. Loss-of-function mutations in this channel prolong action potential duration and contribute to LQTS. Therefore, pharmacological activation of Kv7.1/KCNE1 represents a promising therapeutic strategy to restore impaired channel function. We previously identified the endogenous lipid N-arachidonoyl-L-serine (ARA-S) as a potent Kv7.1/KCNE1 activator, producing a leftward shift in voltage dependence (V_{50}), increasing maximal conductance, and enhancing current amplitude. However, because ARA-S is part of the broader endocannabinoid-related lipid signalling system, it may also interact with off-target pathways. This motivates the development of synthetic derivatives with improved selectivity. The aim of this study is to design and evaluate endocannabinoid derivative compounds that preserve Kv7.1/KCNE1 activation while improving selectivity across ion channels and receptors.

Methods

A total of 18 synthetic derivatives were designed based on the ARA-S scaffold. Functional assessment of effect of these compounds on Kv7.1/KCNE1 activity has been performed using two-electrode voltage clamp (TEVC) electrophysiology in Xenopus laevis oocytes expressing human Kv7.1/KCNE1. Ongoing studies include automated patch-clamp electrophysiology in mammalian expression systems to evaluate potency and selectivity across Kv7 channel subtypes and other cardiac ion channels. In parallel, CB1R activity is being assessed using a functional apoaequorin Ca^{2+}-mobilization assay. Additional experiments are underway to investigate potential effects on channel trafficking and membrane expression.

Results

Initial TEVC screening indicates that most derivatives retain Kv7.1/KCNE1 activation effects, including leftward shifts in V_{50}, with variable efficacy across the tested compounds. Structural modifications to the fatty acid tail, including substitutions at the C14 position and chain-extended analogues terminating in aromatic groups, appear to contribute to functional differences. Compound lipophilicity also correlates with observed activity trends. Preliminary CB1R profiling suggests that canonical endocannabinoid compounds, such as 2-Arachidonoyl Glycerol (2-AG) and Arachidonoyl Ethanolamide (AEA) activate CB1R, whereas ARA-S does not, indicating differential receptor engagement within this chemical class.

Conclusions

This work establishes a framework for the identification and optimization of Kv7.1/KCNE1 channel activators based on endocannabinoid-inspired scaffolds. Ongoing work will further define ion channel selectivity, CB1R interactions, and trafficking effects to support the development of more selective therapeutic drug candidates for LQTS.

33.

FROM GENE TO FUNCTION: GENERATING STABLE KCNQ ISOFORM CELL LINES FOR SELECTIVE DRUG PROFILING

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The human genome codes for twelve classes of voltage-gated potassium channels (KV1-12). These channels include the KV7 family, which is further divided into five subgroups and encoded by the KCNQ genes. KV7.1-7.5 channels play a key role in regulating the membrane potential and the excitability of cells. The subgroups occur throughout the whole human body as homo- and heteromers. KV7.2, KV7.3, and KV7.5 are found in the nervous system and skeletal muscles, while KV7.4 is expressed in the cochlea and gastrointestinal tract, and KV7.1 is mainly found in the myocardium. KV7.4 and KV7.5 are additionally expressed in vascular tissues. Mutations in the KCNQ2/3 genes, forming the KV7.2/7.3 heteromer, are associated with a wide variety of diseases, such as epileptic encephalopathy. Our research group has been working for a long time on developing new drugs that specifically activate this heteromer as a potential retigabine replacement. To date, over 200 substances have been generated with activity testing focusing on the main target KV7.2/7.3. In this study, pre-selected candidates were tested on various homo- and heteromeric KV7 channels. This aims to identify low-off-target activity, ensuring a favorable side effect profile.

We successfully generated various cell lines that stably express different homo- and heteromeric KV7 isoforms. Human KCNQ2-5 genes were cloned into different mammalian gene expression vectors and were stably transfected into HEK239 TREx cells. The integration of the plasmids was controlled by PCR and the protein expression by western blot. Cell lines were characterized by a fluorescence-based thallium influx assay (FLIPR) using channel openers (flupirtine and retigabine) and a channel blocker (XE-991). Whole-cell patch-clamp was used to further verify the channel activity. Subsequently, the cell lines were used to screen different retigabine analogues synthesized by our working group. Initial results of the compound screening indicate a molecular structure-selectivity relationship. While some showed considerable KV7.4 activation, two candidates demonstrated promising specificity.

34.

PREVENTING KV7 DOWNREGULATION BY TARGETING REST UPREGULATION IN CHRONIC PAIN

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Introduction
Downregulation of Kv7 channel expression in peripheral sensory neurons during the development of chronic pain is widely known and demonstrated across a variety of chronic pain types, including neuropathy, cancer pain, chemotherapy-induced pain, chronic inflammation and is present in humans with diabetic neuropathy. Kv7 channels are a key factor in controlling sensory neuron excitability, but these are not the only membrane proteins downregulated in chronic pain; other examples include Kv4.3, opioid and cannabinoid receptors. A central regulator in this process is the RE1-Silencing Transcription factor (REST), which represses expression of key neuronal genes, therefore contributing to the dysregulation of excitability and diminished efficacy of conventional analgesics in chronic pain conditions. Small C-Terminal Domain Phosphatase 1 (SCP1) dephosphorylates REST, preventing its degradation, thereby amplifying its gene-silencing effects. Therefore, we sought to block REST function via SCP1 inhibition in order to prevent the downregulation of Kv7 channels and other key proteins in sensory neurons and prevent chronic pain-associated remodeling.

Methods
This was evaluated using biochemical techniques to assess REST gene and protein expression and luciferase assay in SH-SY5Y cells to assess REST function with a variety of treatments that may affect REST expression or function. This was combined with in vivo behavioural assessment of different pharmacological treatments and ex-vivo analysis of REST expression.

Results
In-vitro assays using SH-SY5Y cells demonstrated that direct inhibition of REST using compound X-5050 decreased REST activity by approximately 50% (luciferase assay). SCP1 inhibitors were somewhat more effective: compound T65 reduced REST activity by four-fold. At higher concentrations Rabeprazole also achieved a four-fold decrease in activity. In-vivo, Rabeprazole exhibited a dose-dependent analgesic effect in a rat neuropathic pain model (partial sciatic nerve ligation, PSNL). At 60 mg/kg, Rabeprazole alleviated thermal hyperalgesia by 40% and mechanical hyperalgesia by 25% at the 8th day after injury. Notably, Lansoprazole, a structurally related to rabeprazole proton pump inhibitor lacking SCP1 inhibitory activity, had no impact on neuropathic pain development.

Conclusions
Targeting REST via SCP1 inhibition displays analgesic effect: thermal hypersensitivity induced by PSNL was strongly reduced, along with reducing REST expression and function. These findings highlight REST as a promising target for chronic pain intervention through preventing REST-induced sensory neuron remodeling. We further conclude that SCP1 inhibition may offer a viable therapeutic strategy.

35.

THE NOVEL KV7 ACTIVATOR C60 REDUCES PAIN IN OXALIPLATIN-INDUCED PERIPHERAL NEUROPATHY

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Introduction

Neuropathic pain frequently arises because of nerve damage or as a side effect of pharmacological treatments. A particularly severe form is chemotherapy-induced neuropathy (CINP), such as that triggered by oxaliplatin (OXA), which often induces discontinuation of therapy. Activation of Kv7.2, Kv7.3, and Kv7.5 voltage-gated potassium channels expressed in the nervous system suppresses neuronal activity and represents a valid strategy in the treatment of diseases characterized by hyperexcitability, such as pain. Indeed, the Kv7 activators flupirtine and retigabine (RET) alleviate pain symptoms. However, both drugs have been withdrawn from the market due to severe side effects. In this study, we investigated, the effects of compound 60 (C60), a novel Kv7 channel activator developed in our laboratory, in a mouse model of oxaliplatin-induced neuropathy.

Methods

CINP in mice was developed by injecting 2.4 mg/kg oxaliplatin daily for 15 days. To evaluate the acute effects of Kv7 activators, Retigabine or C60 (0.1-3 mg/kg) were administered by intra peritoneal injections 24 hours after the last oxaliplatin injection (day 15). Behavioral tests were performed every 15 min (0'-75') after the administration of Kv7 openers. To evaluate the possible ability of the chronic treatment with Kv7 openers to prevent CINP development, mice were treated daily with Kv7 activators (both at 1 mg/kg) along with OXA for 15 days. Behavioral tests were performed at day 1, 8 and 15. At the end of the treatment period, tissues from key pain-related regions were collected for Western blot and quantitative PCR analyses.

Results

Acute treatment with both RET and C60 reduced cold allodynia and mechanical hyperalgesia, with similar efficacy; the Kv7 blocker XE991 fully reversed the effects of RET and C60. Chronic administration of RET or C60 also prevented the development of OXA-induced mechanical allodynia and hyperalgesia, starting from day 8. Moreover, C60 prevented the increase of GFAP protein expression induced by OXA in the periaqueductal gray (PAG). Western blot analyses also revealed that specific Kv7 subunits were downregulated in some regions involved in pain transmission from OXA-treated animals (Kv7.2 in dorsal root ganglia; Kv7.3 and Kv7.5 in the spinal cord; Kv7.2, Kv7.3, and Kv7.5 in the PAG; Kv7.5 in the cortex). Kv7 protein downregulation was not paralleled by a decrease in mRNA levels in cortex and spinal cord, suggesting post-transcriptional regulation. Noteworthy, C60 prevented OXA-induced Kv7.5-downregulation in the cortex and spinal cord.

Conclusion

Overall, these findings demonstrate that, similarly to retigabine, C60 effectively alleviates CINP and counteracts some of the molecular changes triggered by OXA, supporting its potential use for pain treatment.

36.

PHYSICOCHEMICAL OPTIMIZATION OF NICOTINAMIDE-BASED KV7.2/3 CHANNEL MODULATORS TO OVERCOME THE POTENCY-SOLUBILITY TRADE-OFF

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Optimizing KV7.2/3 channel openers requires a balance between high potency and adequate water solubility. While a recent series of nicotinamide analogues from our lab included highly potent channel modulators, these compounds frequently exhibited poor aqueous solubility due to the high lipophilicity required for effective target binding.

Whereas solubility can be independently influenced by factors such as crystal lattice energy, predicting these physicochemical shifts remains difficult. Consequently, a classical lead optimization approach was adopted. A small library of compounds with minimal structural deviations was synthesized to precisely map the structure-activity relationship and effects on physicochemical properties.

Even though target solubility benchmarks have not yet been met, synthesized intermediates have demonstrated a significant increase in potency, achieving single-digit nanomolar activity in our fluorescence-based in-vitro assay.

Moving forward, we will explore increasing the overall -fraction of the scaffold, a strategy previously shown to improve water solubility, circumventing the addition of polar groups. By balancing the polarity required for solvation with the hydrophobicity necessary for target binding, we aim to develop a lead candidate that retains high potency while overcoming the solubility limitations of the current nicotinamide series.

37.

FROM PATIENT NEED TO SHARED STRATEGY: BUILDING A PARTICIPATORY REGISTRY NETWORK FOR KCNQ2-RELATED EPILEPSY

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Introduction
Over the past five years, a new synergy has emerged within the community of individuals affected by KCNQ2-related epilepsy (KCNQ2-DEE), involving patients, families, and the European KCNQ2 patient association network, in collaboration with international partners including the EpilepsyPlusAlliance (E+A) and the Rare Epilepsy Network (REN). This growing alliance has highlighted a shared and urgent need for a structured, sustainable patient registry to support clinical research and future clinical trials. Such a registry would enable systematic collection of clinical histories, longitudinal outcomes, and genetic data, thereby improving understanding of the natural history of the disease and facilitating trial readiness. Previous experiences, including the “Rikke” initiative, revealed significant challenges in data accessibility, governance, and return of value to the patient community, prompting reflection on more participatory and transparent models for data collection and use.

Methods
A series of structured meetings, workshops, and multistakeholder discussions were conducted involving patient representatives, clinicians, researchers, and data infrastructure experts. Different registry architectures were reviewed, including centralized, federated, and hybrid models. Key domains explored included data ownership and governance, ethical frameworks, interoperability standards, sustainability, and mechanisms for patient engagement. Existing rare disease registry initiatives were also analyzed to identify best practices and limitations relevant to rare epilepsies.

Results
The collaborative process led to the identification of a new participatory registry model in which governance is shared between patient organizations and scientific experts. In this framework, patient associations actively contribute to defining priorities, data elements, and access policies, while clinicians and researchers ensure scientific validity, data quality, and methodological robustness. The proposed model emphasizes transparency, ethical data sharing, compliance with FAIR (Findable, Accessible, Interoperable, Reusable) principles, and scalability across international networks. A phased implementation pathway was defined, starting with core dataset harmonization and progressing toward integration with existing European and international rare epilepsy infrastructures.

Conclusions
We propose a novel participatory registry framework for KCNQ2-related epilepsy that integrates patient-driven governance with scientific oversight. This model aims to enhance data quality, foster trust, and accelerate research and therapeutic development. The approach will be presented to the broader scientific community for refinement prior to implementation.

38.

HIGH-THROUGHPUT ELECTROPHYSIOLOGICAL CHARACTERIZATION OF KV7.2/KV7.3 CHANNELS USING AUTOMATED PATCH CLAMP

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Introduction

In neurons, Kv7.2 and Kv7.3 subunits assemble as heterotetramers to form voltage-gated potassium channels that do not inactivate. These channels generate the M-current. This slowly activating current stabilizes the resting membrane potential and regulates action potential firing, thereby controlling neuronal excitability. Mutations in the genes encoding Kv7.2/Kv7.3 disrupt channel function and are linked to developmental and epileptic encephalopathies. These channels are important therapeutic targets. Agonists, such as retigabine, show anticonvulsant effects and may also be beneficial in disorders including pain and neurodegeneration. Advances in high-throughput automated patch-clamp (APC) systems offer a powerful approach to accelerate investigations into Kv7 function.

Methods

HEK cells expressing Kv7.2/Kv7.3 were measured using automated patch clamp devices recording from 8 (Patchliner) or 384 (SyncroPatch 384) cells simultaneously. Voltage step protocols were run to establish current-voltage relationships. Pharmacological interventions were applied to calculate IC50 or EC50.

Results

Current-voltage plots for Kv7.2/Kv7.3 were recorded using increasing voltage steps. A single step protocol was used to investigate the pharmacological action of amitriptyline and retigabine. Amitriptyline inhibited Kv7.2/Kv7.3-mediated currents with an IC50 of 8.7 µM. Retigabine had an EC50 of 19.7 µM. Electrophysiological investigation with the SyncroPatch 384 revealed high success rates, and stable current recording for more than 15 minutes with good seal stability, even under Fluoride-free conditions.

Conclusions

Kv7 channels expressed in cell lines could be recorded on automated patch clamp devices with good success, stable Rseal and Rseries parameters and showed expected IC50 values.

39.

MACHINE LEARNING RESOLVES FUNCTIONAL PHENOTYPES AND THERAPEUTIC RESPONSES IN KCNQ2 DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY IPSC MODELS

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Pathogenic KCNQ2 variants are associated with developmental and epileptic encephalopathy (KCNQ2-DEE), a devastating disorder characterized by neonatal-onset seizures and impaired neurodevelopment with no effective treatments. KCNQ2 encodes the voltage-gated potassium channel KV7.2, which regulates action potential threshold and repolarization. However, the relationship between KV7.2 dysfunction and abnormal neuronal activity remains unclear. Here, we use human induced pluripotent stem (iPSC)-derived neurons from 5 KCNQ2-DEE patients with pathogenic variants and CRISPR/Cas9-corrected isogenic controls to investigate pathophysiological mechanisms. We identify a common dyshomeostatic enhancement of Ca2+-activated small conductance potassium (SK) channels, which drives larger post-burst afterhyperpolarizations in KCNQ2-DEE neurons. Using microelectrode arrays (MEAs), we recorded over 18 million extracellular spikes from >8,000 neurons during 5 weeks in culture and then applied supervised and unsupervised machine learning algorithms to dissect time-dependent functional neuronal phenotypes that defined both patient-specific and shared firing features among KCNQ2-DEE patients. Our analysis identified irregular spike timing and enhanced bursting as functional biomarkers of KCNQ2-DEE and demonstrated the significant influence of genetic background on phenotypic diversity. Importantly, using unbiased machine learning models, we showed that chronic treatment with the KV7 activator retigabine rescues the disease-associated functional phenotypes with variable efficacy. Our findings highlight SK channel upregulation as a critical pathophysiological mechanism underlying KCNQ2-DEE and provide a robust MEA-based machine learning platform useful for deciphering phenotypic diversity amongst patients, discovering functional disease biomarkers, and evaluating precision medicine interventions in personalized iPSC neuronal models.

40.

INTEGRATED GENOTYPE-PHENOTYPE FUNCTION ANALYSIS REVEALS DISTINCT PATHOGENIC MECHANISMS FOR COGNITIVE IMPAIRMENT IN KCNQ2-RELATED DISORDERS

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Introduction
Pathogenic variants of KCNQ2 lead to a spectrum of disorders including self-limited familial neonatal-infantile epilepsy (SeL(F)NIE), developmental and epileptic encephalopathies (DEEs), and neurodevelopmental disorders (NDDs) with intellectual disability (ID). This study aimed to delineate the clinical progression and underlying pathogenesis of these disorders. Particularly, we unraveled the role of gain-of-function (GoF) variants in neurodevelopmental impairment.

Methods
We conducted a longitudinal study of a 90-patient Chinese cohort with KCNQ2-related disorders, classified into SeL(F)NIE, DEEs, and NDDs subgroups. Clinical phenotyping was integrated with functional analyses (electrophysiology, biochemistry) of five missense variants in homomeric and heteromeric (with Kv7.3/Kv7.5) channel assemblies.

Results
Despite comparable seizure control to SeL(F)NIE (96% vs 100%), the NDDs group exhibited significant cognitive impairment. All deceased patients (8/90) were in the DEEs group. Functional profiling revealed a spectrum from loss-of-function (LoF) to GoF. LoF variants (e.g., S247L in DEEs) were linked to severe epilepsy. Crucially, we identified strong GoF variants (P335A/L in NDDs) in the S6-HelixA domain that confer insensitivity to phosphatidylinositol 4,5-bisphosphate (PIP2) regulation and are associated with a primary neurodevelopmental phenotype, distinct from the severe epileptic phenotype of established voltage-sensing domain (VSD) GoF variants.

Conclusions
Our integrated clinical and functional analysis showed that the clinical outcome relies on the functional consequence of a KCNQ2 variant (LoF vs GoF) and its behavior in heteromeric complexes, rather than its mere location. We defined a novel class of strong GoF KCNQ2 variants that are mechanistically and phenotypically distinct, highlighting aberrantly enhanced channel function as a key driver of cognitive dysfunction and presenting new targets for precision medicine.

41.

IN VIVO EFFICACY OF AMITRIPTYLINE FOR KCNQ GAIN-OF-FUNCTION DISORDERS

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Introduction
Gain-of-function (GoF) variants in KCNQ2/3/5 are associated with a spectrum of developmental and epileptic encephalopathies. Amitriptyline (AMT), a tricyclic antidepressant, was proposed for repurposed use in these disorders based on in vitro pharmacology and a report has suggested potential benefit in patients. A 4-year-old girl with a de novo GoF variant in KCNQ3 (c.688C>T, p.R230C), global developmental delay, and abundant sleep-potentiated epileptiform spikes on electroencephalography (EEG) was treated with AMT at 10 mg/kg/day in our practice and three months later the EEG showed only rare spikes in sleep. We set out to test the ability of AMT to suppress the EEG spike-wave discharge (SWD) phenotype in a KCNQ3-GoF mouse model.

Methods
Mice heterozygous for Kcnq3-R231H on a C57B6J background were crossed to C3HeB/FeJ to generate F1 litters (n=15, 3 batches) for implantation with EEG. In a randomized crossover design, mice received AMT (5, 7.5, 10, or 20 mg/kg intraperitoneally) or saline on alternate days, with injections at 4:00 PM. SWDs were manually scored for count, burden (cumulative duration), and mean duration across three 3-hour windows locked to the 4:00 PM injection time: baseline/pre-injection (1–4 PM), early post-injection (4–7 PM), and late post-injection (7–10 PM). Each post-injection window was normalized to the same-day baseline. For each metric, a per-mouse difference score, calculated as saline ratio minus AMT ratio, was tested using a two-tailed Wilcoxon signed-rank test.

Results
There was a significant reduction in SWD count during both the early and late post-injection time windows for the 20 mg/kg dose.

Conclusions
Our data demonstrate in vivo network-level KCNQ3 GoF phenotype rescue, providing proof-of-concept for a clinical role for repurposed AMT in KCNQ GoF disorders.

42.

MACHINE LEARNING FRAMEWORK FOR FUNCTIONAL PREDICTION OF KCNQ2 MISSENSE VARIANTS

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Introduction

Variants in KCNQ2 (Kv7.2) are associated with a broad spectrum of epileptic disorders, ranging from self-limited neonatal epilepsy to developmental and epileptic encephalopathy. Knowledge of the resulting functional effects, characterized as gain-of-function (GOF) or loss-of-function (LOF), is essential to guide clinical management and precision medicine. However, the limited availability of dedicated computational tools for gene-specific prediction of KCNQ2 variant function represents a critical challenge for precision medicine. In this study, conducted as part of the BEATKCNQ research consortium (EP PerMed), we present a machine-learning framework for predicting the functional effects of KCNQ2 missense variants.

Methods

We applied a previously published machine learning framework for voltage-gated potassium channels for KCNQ2 (Bosselmann et al., 2022), using an independently assembled dataset of 110 KCNQ2 missense variants with experimentally derived and clinically inferred functional annotations. A set of 62 sequence, structural, evolutionary, and physicochemical features was calculated for each variant and used to train the model.

Results

The classifier achieved a balanced accuracy (BA) of 0.71, MCC of 0.53, ROC-AUC of 0.78, and PR-AUC of 0.49. Using an optimized decision threshold, 93/110 variants were correctly classified. Classification performance was stronger for LOF variants, whereas most errors involved GOF variants.

Conclusions and Outlook

Our gene-specific classifier effectively distinguishes between GOF and LOF KCNQ2 variants, with stronger performance for LOF than GOF variants, potentially due to their lower representation in the training dataset. Structural and topological features emerged as the most informative predictors, highlighting the importance of local protein context. These findings support gene-specific (as opposed to multiple genes) machine learning frameworks as promising tools for KCNQ2 variant interpretation and precision medicine approaches. To further improve performance, we will retrain the model using the new dataset, with nearly twice as many KCNQ2 variants as the original dataset of Bosselmann et al. Furthermore, we will integrate additional features, including protein language and elastic network models. The latter should explicitly account for motion on protein function, which is missing in other studies.

Reference

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43.

MODELLING KCNQ2-RELATED EPILEPTIC ENCEPHALOPATHY USING HINEURONS

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Kv7.2 is a voltage-sensitive potassium channel encoded by the gene KCNQ2. KCNQ2 belongs to the family of voltage-gated, depolarization-activated potassium (KCNQ) channels. KCNQ2 is essential for neurodevelopment and neuronal excitability modulating M-current, a slow activating and inactivating low-threshold current, which prevents neuronal hyperexcitability. Mutations in the KCNQ2 gene are the most common cause of neonatal genetic epilepsy, including developmental and epileptic encephalopathy (DEE). Here, using two patient-derived hiNeurons harbouring two distinct Single Nucleotide Polymorphism (SNP) in the KCNQ2 gene and their familial controls, we are trying to understand the molecular, cellular and physiological changes that leads to DEE. These two SNP loci are mutation hotspots that leads to DEE or benign neonatal familial convulsions (BNFC). Our preliminary multi electrode array (MEA) experiments showed that the Patient glutamergic neurons exhibited irregular firing pattern and burst frequency as compared to the familial control derived neurons. We also observed increased number bursts in patient glutamergic neurons upon maturation induced by compound E. In addition, Chronic M current inhibition in control neurons mimicked the phenotype seen in the patient neurons. Further, Anti-seizure drug Carbamazepine and Retigabine significantly reduced the number of bursts and the ISI CoV in patient neurons. To ensure that phenotypes are only due to the mutations and not merely because of the differences in genetic background between patient and familial control neurons, currently we are trying to generate isogenic controls using gene editing.

44.

MOLECULAR BASIS FOR SELECTIVE ACTIVATION OF KV7.3 BY THE ROSEMARY METABOLITE CARNOSIC ACID

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INTRODUCTION
Isoform-selective neuronal Kv7 potassium channel openers are highly sought after for both therapeutic and research applications. We recently discovered that carnosic acid (CA), a diterpene from Salvia rosmarinus (rosemary), is a highly efficacious opener of Kv7.3 but exerts minimal effects on Kv7.2 and Kv7.2/3. This profile enables CA to reduce cocaine-seeking behavior in mice, suggesting potential utility for treating psychoactive drug addiction, but prevents it from acting as a standalone anticonvulsant. The mechanism underlying CA Kv7 isoform-selectivity has remained unclear.

METHODS
We used scanning mutagenesis, electrophysiology, radioligand binding, and all-atom molecular dynamics simulations to uncover the molecular basis for CA binding, Kv7.3 activation and selectivity.

RESULTS
Our data indicate that CA opens Kv7.3 by binding near the extracellular cap of the voltage sensor. Selectivity arises from sequence divergence within the S4 cap proximal to a hypersensitivity residue (Kv7.3-L226), independent of the canonical retigabine pore binding site. Kv7.3 samples a binding-competent VSD conformation that is absent in Kv7.2, characterized by an expanded extracellular pocket and a favorable electrostatic environment. Central to this mechanism, Kv7.3-L226 functions as a hydrophobic latch that tunes the energetic landscape of the voltage sensor to control ligand efficacy. Interestingly, despite lacking Kv7.2/3 opening activity or anticonvulsant effects alone, CA unexpectedly synergizes with the anticonvulsant retigabine, augmenting retigabine-mediated Kv7.2/3 activation in vitro and reducing its effective anticonvulsant dose in mice.

CONCLUSIONS
The findings reveal an allosteric coupling between the voltage-sensing domain and the pore that enhances retigabine-mediated activation of Kv7.2/3, providing a blueprint for the development of future isoform-selective probes and therapeutics. Although retigabine was previously withdrawn from clinical use due to off-target toxicity, CA - present in widely consumed supplements such as rosemary extract - may enable dose-sparing strategies for retigabine and other Kv7.2/3 openers. Finally, the findings establish a mechanistic framework in which isoform selectivity arises from VSD conformational microenvironments rather than canonical binding determinants and highlight the voltage sensor as a tunable target for developing selective Kv channel openers.

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