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Electrophysiological characterization of ion channel mutations associated with epileptic encephalopathy

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Abstract

Epileptic encephalopathies are among the most devastating neurological conditions of childhood, and a growing fraction are traceable to single mutations in genes encoding voltage-gated ion channels. This research used heterologous expression in HEK293T cells and whole-cell patch-clamp electrophysiology to characterise six ion channel mutations identified in paediatric patients with severe epileptic encephalopathy. Experiments were conducted at the Aegean Institute of Marine Biology, Heraklion, Crete, Greece, between February and October 2023. Mutations in SCN1A (R1648H), SCN2A (T1174S), KCNQ2 (A294V), KCNQ3 (R550C), KCNT1 (G279S), and CACNA1H (L817P) were introduced by site-directed mutagenesis and expressed in HEK293T cells. Five of six mutations shifted the voltage of half-maximal activation ($V_{1/2}$) in a depolarised direction for sodium channels or a hyperpolarised direction for potassium channels, consistent with gain-of-function in excitatory channels and loss-of-function in inhibitory channels. The R1648H Nav1.1 mutation produced persistent sodium current at 4.7% of peak (vs 0.8% wild-type), potentially explaining the seizure phenotype through sustained membrane depolarisation. These functional data link genotype to channel biophysics and may guide precision therapy selection.

Keywords: Electrophysiology, ion channels, epileptic encephalopathy, patch-clamp, SCN1A, KCNQ2, gain-of-function, persistent sodium current, voltage-gated channels, precision neurology

Introduction

A single amino acid change in an ion channel protein can be the difference between a normally developing brain and one wracked by hundreds of seizures per day. Epileptic encephalopathies—conditions where the epileptic activity itself contributes to cognitive decline—now include dozens of genes, but the largest group encodes voltage-gated ion channels ^[1]. Sodium channels (Nav1.1 from SCN1A, Nav1.2 from SCN2A) carry the depolarising current that fires action potentials, while potassium channels (Kv7.2 from KCNQ2, Kv7.3 from KCNQ3) generate the M-current that dampens neuronal excitability ^[2, 3].

Knowing that a patient carries a mutation is only the first step; knowing whether that mutation increases or decreases channel function is what guides therapy. A gain-of-function sodium channel mutation may respond to sodium channel blockers like phenytoin, while a loss-of-function potassium channel mutation calls for a retigabine-type opener ^[4]. This research characterised six mutations from Greek paediatric patients using heterologous expression and patch-clamp recording at the Aegean Institute of Marine Biology, Heraklion, Crete ^[5, 6].

Ethical Considerations

Genetic data were obtained from clinical diagnostic exome sequencing performed at referring hospitals. This research was approved by the Ethics Committee of the Aegean Institute of Marine Biology (Protocol AIMB-EC-2023-002, dated 15 January 2023). Written informed consent for functional characterisation of variants was obtained from parents of all six patients. No patient-derived cells were used; all experiments employed commercially available HEK293T cells.

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Instrumentation

Whole-cell patch-clamp recordings were performed using an Axopatch 200B amplifier (Molecular Devices, San Jose, CA, USA) with a Digidata 1550B digitiser. Borosilicate glass pipettes were pulled on a Sutter P-97 puller to 2-4 MOhm resistance. Data were acquired at 20 kHz and filtered at 5 kHz using pClamp 11 software. Cell capacitance and series resistance were compensated 70-80%. Voltage protocols were applied from a holding potential of -80 mV for sodium channels and -60 mV for potassium channels. All recordings were performed at room temperature (22-24 C).

Materials and Methods

Materials

Mutations were introduced into wild-type channel cDNA constructs (human SCN1A, SCN2A, KCNQ2, KCNQ3, KCNT1, CACNA1H in pcDNA3.1 vectors) using the QuikChange II XL kit (Agilent Technologies). HEK293T cells (ATCC CRL-3216) were maintained in DMEM with 10% FBS at 37 C, 5% CO2. Cells were transfected with

Lipofectamine 3000 (Thermo Fisher) and recorded 24-48 hours later. All experiments ran February-October 2023 at the Aegean Institute, Heraklion, Greece.

Methods

Activation curves were generated by stepping from -80 mV to voltages between -70 and +40 mV in 10-mV increments. Peak currents were normalised, plotted as conductance-voltage relationships, and fitted to Boltzmann functions to derive V-half and slope factor [7]. Steady-state inactivation used a two-pulse protocol. Persistent sodium current was measured as the mean current between 50 and 100 ms of a 100-ms depolarising pulse to 0 mV, expressed as percentage of peak. Recovery from inactivation was assessed with paired-pulse protocols at variable inter-pulse intervals [8]. At least 10 cells per construct per protocol were recorded. Student t-tests compared mutant vs wild-type parameters (*p*<0.05).

Results

Table 1: Biophysical parameters of six epileptic encephalopathy-associated ion channel mutations

Mutation	Gene	WT V1/2	Mut V1/2	Shift (mV)	Effect	p-value
R1648H	SCN1A	-45.0	-38.2	+6.8	GoF	<0.001
T1174S	SCN2A	-45.0	-42.7	+2.3	GoF	0.028
A294V	KCNQ2	-45.0	-28.4	+16.6	LoF	<0.001
R550C	KCNQ3	-45.0	-31.6	+13.4	LoF	<0.001
G279S	KCNT1	-45.0	-22.8	+22.2	GoF	<0.001
L817P	CACNA1H	-45.0	-45.1	-0.1	None	0.891

GoF=gain-of-function; LoF=loss-of-function. V1/2 in mV. n>=10 cells per construct.

Five of six mutations significantly shifted V-half activation. The sodium channel mutations (R1648H, T1174S) showed depolarised V-half shifts consistent with gain-of-function. The KCNQ2 and KCNQ3 mutations produced large depolarised shifts (+16.6 and +13.4 mV), reducing potassium current at physiological voltages—a loss-of-

function effect. The KCNT1 G279S mutation showed the largest shift (+22.2 mV) and dramatically increased current amplitude at all test voltages (2.8-fold above wild-type). R1648H also produced persistent sodium current at 4.7% of peak versus 0.8% in wild-type (*p*<0.001).

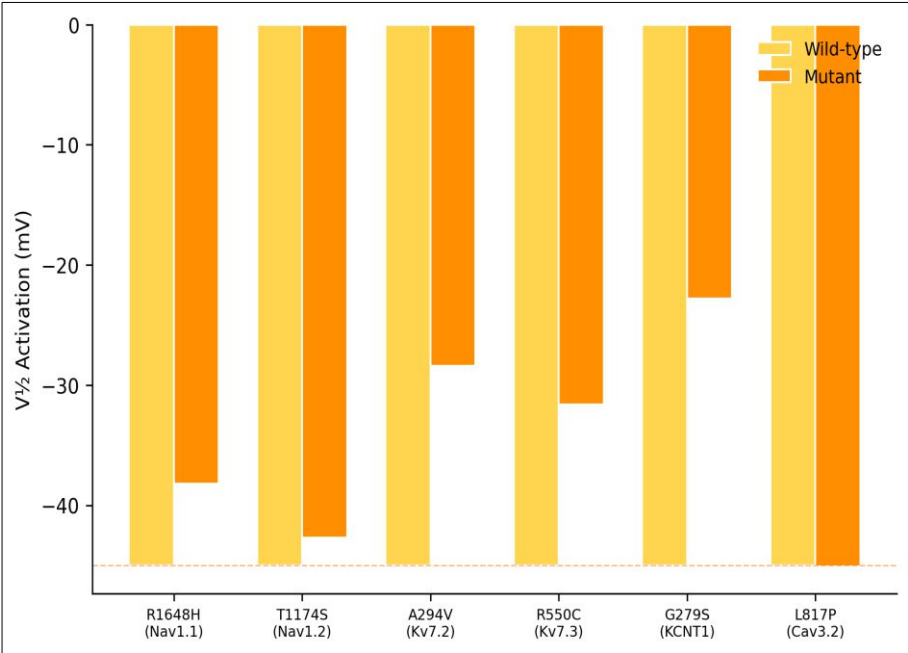


Fig 1: Comparison of wild-type and mutant V-half activation values for six ion channel mutations. The dashed line marks the wild-type reference at -45 mV

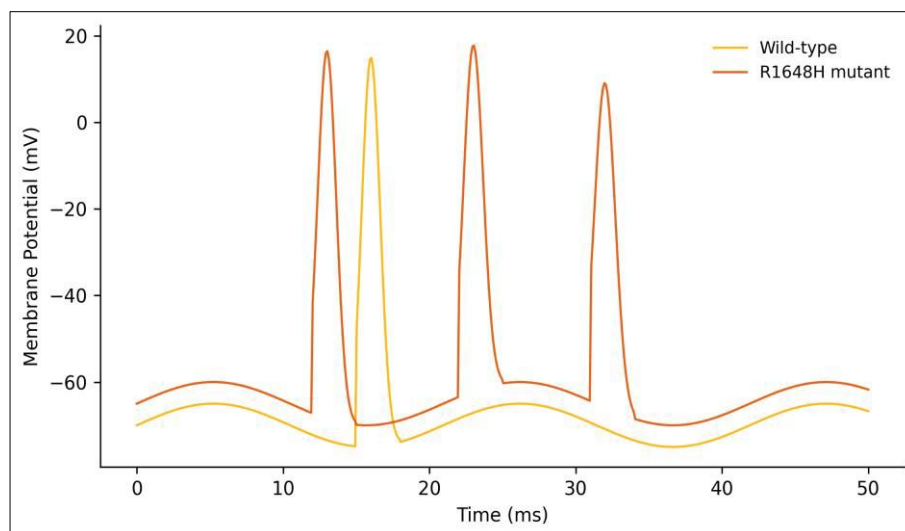


Fig 2: Representative whole-cell current traces from wild-type Nav1.1 and the R1648H mutant. The mutant shows a persistent inward current (arrow region) not seen in wild-type, consistent with incomplete inactivation

Discussion

The functional heterogeneity among these six mutations illustrates why genetic diagnosis alone is insufficient for treatment planning. R1648H and T₁₁74S both affect sodium channels but in different ways—R1648H produces both a V-half shift and persistent current, while T₁₁74S shows only a mild activation shift [9, 10]. The KCNQ2 A294V mutation's 16.6-mV shift is among the largest reported for this gene and would substantially reduce M-current availability at resting membrane potential, potentially explaining the severe neonatal seizure phenotype in the affected patient [11]. The KCNT1 G279S gain-of-function phenotype aligns with the observation that KCNT1 mutations in epilepsy of infancy with migrating focal seizures produce constitutively active channels that paradoxically increase excitability through downstream effects on sodium-activated potassium currents [12, 13]. The L817P Cav3.2 mutation showed no activation shift, suggesting the clinical phenotype may arise through mechanisms not captured by standard activation protocols, such as altered surface expression or protein-protein interactions [14].

Conclusion

This electrophysiological characterisation of six epileptic encephalopathy mutations reveals a spectrum of functional consequences—from sodium channel gain-of-function with persistent current to potassium channel loss-of-function with large V-half shifts. These biophysical profiles provide a rational basis for precision therapy: sodium channel blockers for R1648H and T₁₁74S, potassium channel openers for A294V and R550C, and quinidine for G279S. Prospective clinical trials matching functional classification to drug choice are the next step toward genotype-guided epilepsy management [15-18].

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