Functional Annotation of Human Ion Channel Variants

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Genetic Arrhythmia Syndromes

Congenital Long QT Syndrome
- KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, ANK2, SCN4B, CAV3, AKAP9, SNTA1

Brugada Syndrome
- SCN5A, GPD1L, CACNA1C, CACNB2, SCN1B, KCNE3, SCN3B

Andersen Syndrome
- KCNJ2

Timothy Syndrome
- CACNA1C

Familial Cardiac Conduction Diseases
- SCN5A

Short QT Syndrome
- KCNQ1, KCNH2, KCNJ2

Catecholaminergic Polymorphic VT
- RYR2, CASQ2, TRND

Familial and Lone Atrial Fibrillation
- KCNQ1, KCNE2, KCNA5, SCN5A, SCN10A, NPPA
Congenital Long QT Syndrome

Clinical Features

- Congenital prolongation of the rate-corrected QT interval
  
  \[ QTc \geq 440 \text{ msec in symptomatic individuals} \]
  
  \[ QTc \geq 460 \text{ in asymptomatic individuals} \]

- Recurrent syncope
- Cardiac arrest or torsade de pointes
- Family history of unexplained and premature sudden death
- Incomplete penetrance and variable expressivity

- Congenital deafness (recessive Jervell, Lange-Nielson)

- Molecular diagnostics - technically challenging (13 genes)
Congenital Long QT Syndrome (LQTS)

Normal ECG

Congenital LQTS

QT < 440 ms

QT > 440 ms
Congenital Long QT Syndrome

*Torsades de pointes*
## Genetic Heterogeneity in Congenital LQTS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Current</th>
<th>Frequency</th>
<th>Trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQT1</td>
<td>$\downarrow I_{Ks}$</td>
<td>45%</td>
<td>Exercise, emotion</td>
</tr>
<tr>
<td>LQT2</td>
<td>$\downarrow I_{Kr}$</td>
<td>40%</td>
<td>Sudden auditory stimuli</td>
</tr>
<tr>
<td>LQT3</td>
<td>$\uparrow I_{Na}$</td>
<td>10%</td>
<td>Sleep</td>
</tr>
<tr>
<td>LQT4</td>
<td>Ank2 complex</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>LQT5</td>
<td>$\downarrow I_{Ks}$</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>LQT6</td>
<td>$\downarrow I_{Kr}$</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>LQT7</td>
<td>$\downarrow I_{K1}$</td>
<td>Rare</td>
<td></td>
</tr>
<tr>
<td>LQT8</td>
<td>$\uparrow I_{Ca}$</td>
<td>Rare</td>
<td></td>
</tr>
</tbody>
</table>

Intracellular: $I_{Na}$, $I_{Ks}$, $I_{Kr}$, $I_{K1}$, $I_{Ca}$

Extracellular: $Na^+$, $Ca^{2+}$, $K^+$
KCNQ1 and KCNE1 Generate $I_{Ks}$

Slow delayed rectifier current in heterologous cells
## Mutations in Genetic Arrhythmias

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutations in HGMD</th>
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<tbody>
<tr>
<td><em>KCNQ1</em></td>
<td>602</td>
</tr>
<tr>
<td><em>KCNH2</em></td>
<td>910</td>
</tr>
<tr>
<td><em>SCN5A</em></td>
<td>802</td>
</tr>
<tr>
<td><em>RYR2</em></td>
<td>308</td>
</tr>
<tr>
<td><em>CACNA1C</em></td>
<td>57</td>
</tr>
</tbody>
</table>
Genetic Testing for LQTS

- Commercial genetic testing for LQTS has become standard-of-care
- Most variants discovered in LQTS cases are missense
- Most new variants have never been seen before (‘private mutations’)
- Most variants have uncertain functional consequences
- Diagnostic labs report many ‘variants of uncertain significance’
- No reliable methods to distinguish benign from pathogenic variants
Classification of Mutations

Variants without clear pathogenicity confound genetic diagnosis

Strategies for Variant Annotation

- Computational \((in \text{ silico})\)
- Experimental \((in \text{ vitro})\)
- Experimental \((in \text{ vivo})\)
Strong evidence for pathogenicity:

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
   Example:  Val→Leu caused by either G>C or G>T in the same codon  
   Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
   Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.

PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
Functional Annotation of Ion Channels Variants

Patch clamp electrophysiology is the gold standard

Conventional patch clamp:
- 16 measurements/person/day
- days-weeks per mutation
Functional Annotation of Ion Channels Variants

*Patch clamp electrophysiology is the gold standard*

**Automated patch clamp:**
- 768 measurements per hour
- multiple variants per day

SyncroPatch 768PE
Functional Analysis of $KCNQ1$ Variants by Automated Electrophysiology

Overcoming need for stable cell lines

Electroporation

Automated patch-clamp
Electroporation of KCNQ1 and KCNE1 evaluated by flow cytometry

Viability: 84.9%  Percent Co-transfected: 79.1%
Automated Electrophysiology of $I_{KS}$
(Transient transfection of CHO with KCNQ1/KCNE1)
Automated Electrophysiology of $I_{KS}$
(Transient transfection of CHO with KCNQ1/KCNE1)

Manual Patch

SyncroPatch

Normalized to peak current measured at +60 mV
Automated Electrophysiology of $I_{KS}$
(Transient transfection of CHO with KCNQ1/KCNE1)

Manual Patch $n = 18$

- $V_{1/2} = 22.5 \pm 0.8$ mV

SyncroPatch $n = 153$

- $V_{1/2} = 27.8 \pm 1.4$ mV
Automated Electrophysiology of $I_{KS}$
(Transient transfection of CHO with KCNQ1/KCNE1)
Automated vs Manual Electrophysiology

- $I_{var} - I_{WT}$, %
- $\Delta V_{1/2}$, mV
- $\Delta k$

<table>
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<tr>
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<tbody>
<tr>
<td>WT</td>
<td>C122Y</td>
<td>WT</td>
<td>C122Y</td>
<td>WT</td>
<td>C122Y</td>
</tr>
<tr>
<td>I132L</td>
<td></td>
<td>I132L</td>
<td></td>
<td>I132L</td>
<td></td>
</tr>
<tr>
<td>K196T</td>
<td></td>
<td>K196T</td>
<td></td>
<td>K196T</td>
<td></td>
</tr>
<tr>
<td>R174C</td>
<td></td>
<td>R174C</td>
<td></td>
<td>R174C</td>
<td></td>
</tr>
<tr>
<td>G179S</td>
<td></td>
<td>G179S</td>
<td></td>
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# Criteria to Classify KCNQ1 Variant Function

*Developed from a curated list of high-quality publications on 107 variants with functional characterization*

<table>
<thead>
<tr>
<th>Values Relative to WT $I_{Ks}$</th>
<th>Normal</th>
<th>Near Normal</th>
<th>Mild Loss of Function</th>
<th>Severe Loss of Function</th>
<th>Severe Gain of Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{peak}}$ at $+60 \text{ mV}$</td>
<td>90-110%</td>
<td>75-90 &amp; 110-125%</td>
<td>25-75%</td>
<td>&lt;25%</td>
<td>&gt;150%</td>
</tr>
<tr>
<td>Activation $V_{1/2}$ (mV)</td>
<td>&lt;5 mV depolarization/ hyperpolarization</td>
<td>10-5 mV depolarization/ hyperpolarization</td>
<td>10-20 mV depolarization</td>
<td>&gt;20 mV depolarization</td>
<td>&gt;15 mV hyperpolarization plus 120-150% $I_{\text{peak}}$</td>
</tr>
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ClinVar Assertions for 36 KCNQ1 Variants

Original classifications

- Pathogenic
- Likely Pathogenic
- Likely benign
- VUS
- Conflicting Interpretation
- No assertion

16/36 (44%) have clear classification
Reclassifying 36 KCNQ1 Variants

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<th>Original</th>
<th>Functional</th>
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<td>Likely pathogenic</td>
<td>Severe LOF</td>
</tr>
<tr>
<td>VUS</td>
<td>Severe LOF</td>
</tr>
<tr>
<td>No assertion</td>
<td>Severe LOF</td>
</tr>
<tr>
<td>No assertion</td>
<td>Severe GOF</td>
</tr>
<tr>
<td>No assertion</td>
<td>Normal</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Normal</td>
</tr>
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Pathogenic | Likely Pathogenic | Variant of Unknown Significance | Likely Benign | Benign
Reclassifying 36 KCNQ1 Variants

<table>
<thead>
<tr>
<th>Original</th>
<th>Functional</th>
<th>Reclassification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likely pathogenic</td>
<td>Severe LOF</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>VUS</td>
<td>Severe LOF</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td>No assertion</td>
<td>Severe LOF</td>
<td>Likely pathogenic</td>
</tr>
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<tr>
<td>No assertion</td>
<td>Normal</td>
<td>Likely benign</td>
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<tr>
<td>Likely benign</td>
<td>Normal</td>
<td>Benign</td>
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Variant of Unknown Significance
ClinVar Assertions for 36 KCNQ1 Variants

Original classifications

- Pathogenic
- Likely benign
- Likely Pathogenic
- Conflicting Interpretation
- VUS

16/36 (44%) have clear classification

Revised classifications

- Pathogenic
- Likely benign
- VUS

30/36 (83%) have clear classification
Automated Electrophysiology of Human Ion Channels

**Sodium channels**
- SCN1A: Epilepsy, Dravet syndrome
- SCN2A: Epilepsy, autism spectrum disorder
- SCN3A: Epilepsy
- SCN5A: Cardiac arrhythmia
- SCN8A*: Epileptic encephalopathy
- SCN9A*: Genetic pain disorders
- SCN10A*: Genetic pain disorders
- SCN11A*: Genetic pain disorders

**Potassium channels**
- KCNE1: Cardiac arrhythmia
- KCNJ2: Anderson syndrome
- KCNB1: Epileptic encephalopathy
- KCNT1*: Epileptic encephalopathy

* Work in progress
Sodium Channelopathies

NaV1.1
NaV1.2
NaV1.3
NaV1.4
NaV1.6
NaV1.7
NaV1.5
NaV1.8
NaV1.9

>2,100 variants/mutations in HGMD
SyncroPatch Recordings of Na\textsubscript{v}1.1
(stable expression in HEK293)

Holding potential -120 mV; single hole recordings
SyncroPatch Recordings of $\text{Na}_V 1.1$
(stable expression in HEK293)
SyncroPatch Recordings of $\text{Na}_V1.1$

Persistent Current

$\text{Na}_V1.1$-WT

$\text{Na}_V1.1$-R1648H
SyncroPatch Recordings of $Na_V1.1$

Persistent Current

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**Graph 1**

- **Time, ms**
  - 0, 40, 80, 120, 160, 200
  - **Na$_V$1.1-WT**
  - **Na$_V$1.1-R1648H**

**Graph 2**

- **P < 0.001**
- **R1648, n = 84**
- **WT, n = 41**
- **Na$_V$1.1-WT**
- **Na$_V$1.1-R1648H**
SCN1A Variants of Unknown Significance associated with Dravet syndrome
Transient expression in HEK293

Automated Electrophysiology of Na$_v$1.2*

Comparison of manual with automated patch clamp

*Na$_v$1.2 is the sodium channel encoded by SCN2A
Automated Electrophysiology of Na\textsubscript{V}1.2

Variants associated with epileptic encephalopathy or autism

<table>
<thead>
<tr>
<th>Variant</th>
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<tbody>
<tr>
<td>p.Glu430Ala</td>
<td>p.Arg188Trp</td>
</tr>
<tr>
<td>p.Tyr816Phe</td>
<td>p.Arg379His</td>
</tr>
<tr>
<td>p.Gly879Arg</td>
<td>p.Arg937His</td>
</tr>
<tr>
<td>p.Ala880Ser</td>
<td>p.Cys1386Arg</td>
</tr>
<tr>
<td>p.Gly882Glu</td>
<td>p.Thr1420Met</td>
</tr>
<tr>
<td>p.Glu999Lys</td>
<td>p.Lys1422Glu</td>
</tr>
<tr>
<td>p.Met1879Thr</td>
<td>p.Glu1880Lys</td>
</tr>
</tbody>
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Automated Electrophysiology of $\text{Na}_V1.2$

Variants associated with epileptic encephalopathy or autism
Automated Electrophysiology of Na\textsubscript{V}1.2

Variants associated with epileptic encephalopathy or autism
Creation of Neuronal Cell Line (ND7/no-Nav) with suppressed endogenous Na current
Creation of Neuronal Cell Line (ND7/no-Nav) with suppressed endogenous Na current
Pharmacological Profiling of Sodium Channel Mutations in Epilepsy
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