Channelopathy of SK2 channel in Long QT Syndrome

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Outline

- Background
- Material and method
- Result
- Discussion and summary
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- Result
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# Background

## Drugs associated with LQTs

<table>
<thead>
<tr>
<th>Antiarrhythmics</th>
<th>Antibiotics</th>
<th>Antinauseants</th>
<th>Antineoplastic</th>
<th>Calcium channel blockers</th>
<th>Gastric promotility</th>
<th>Opiates</th>
<th>Antihistamines</th>
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</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>Chloroquine</td>
<td>Domperidone</td>
<td>Arsenic trioxide</td>
<td>Bepridil</td>
<td>Cisapride</td>
<td>Methadone</td>
<td>Terfenadine</td>
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<td>Disopyramide</td>
<td>Clarithromycin</td>
<td>Droperidol</td>
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<td>Lidoflazinea</td>
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<td>Levomethadyl</td>
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<td>Dofetilide</td>
<td>Erythromycin</td>
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<td>Ibutilide</td>
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<td>Sotalol</td>
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</tbody>
</table>

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*Note: Some drugs may have additional side effects or interactions.*
**Background**

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**Propulsid (Cisapride) Tablets**

- Each tablet contains: Cisapride as the monohydrate equivalent to 20mg of cisapride.

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**Dosage:**

- For information for use, see accompanying product literature. Dispense entire contents in container provided, along with attached Medication Guide.
- Store at 15°-25°C (59°-77°F).
- Protect from light and moisture.

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**Rx only**

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**Janssen Pharmaceutica**

- Janssen Pharmaceutica Inc.
- Titusville, NJ 08560
- U.S. Patent No. 4,962,115
Background

- **Mechanism of Drug induced Long QT**
  - Ikr (hHRG, KCNH2) blockade

Promiscuity to various drugs

Simulation of Ikr blockade

Table 3. Drugs Reported To Provoke Ventricular Tachycardia in the Presence of a Specific Mutation in One of the Long QT Syndrome Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Channel</th>
<th>Drug</th>
<th>Use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>$I_{Ks}$</td>
<td>Mefloquine</td>
<td>Antimalarial</td>
<td>147</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>$I_{Ks}$</td>
<td>Disopyramide</td>
<td>Antiarrhythmic</td>
<td>147</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>$I_{Ks}$</td>
<td>Terfenadine</td>
<td>Antihistamine</td>
<td>147</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>$I_{Ks}$</td>
<td>Cisapride</td>
<td>Cholinergic antagonist</td>
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<td>SCN5A</td>
<td>$I_{Na}$</td>
<td>Halofantrine</td>
<td>Antimalarial</td>
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<tr>
<td>MiRP1</td>
<td>$I_{Kr}$</td>
<td>Clarithromycin</td>
<td>Antibiotic</td>
<td>16</td>
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<tr>
<td>MiRP1</td>
<td>$I_{Kr}$</td>
<td>Quinidine</td>
<td>Antiarrhythmic</td>
<td>9</td>
</tr>
<tr>
<td>MiRP1</td>
<td>$I_{Kr}$</td>
<td>Sulfamethoxazole</td>
<td>Antibiotic</td>
<td>9</td>
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<tr>
<td>MiRP1</td>
<td>$I_{Kr}$</td>
<td>Procainamide</td>
<td>Antiarrhythmic</td>
<td>9</td>
</tr>
<tr>
<td>MiRP1</td>
<td>$I_{Kr}$</td>
<td>Oxatomide</td>
<td>Antihistamine</td>
<td>9</td>
</tr>
</tbody>
</table>
Pharmacokinetics of Ondansetron (Zofran®)

- Peak plasma levels of 20 to 30 ng/mL (82.2 nM) at around 1 1/2 hours after an 8 mg oral dose of ondansetron.
- An 8 mg infusion of ondansetron reached peak plasma levels of 80 to 100 ng/mL (274 nM).
- Repeat dosing of an 8 mg tablet every 8 hours for 6 days increased the peak plasma value to 40 ng/mL (109.6 nM).

Selective 5-HT3 receptor antagonist

KAREN et al. J. Pharm. 1996, 48:774-781
Effect of Ondansetron (Zofran®) on other K channels

Yuri A et al. THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS 2000
Background

- Identification of Ca\(^{2+}\) activated potassium channel
Background

- **Role of $I_{KAS}$ in ventricular repolarization**

Xu and Chiamvimonvat et al. J. Biol. Chem. 2003, 278:49085-49094
Background

- **Role of $I_{KAS}$ in repolarization reserve**

Background

- Blockade of $I_{KAS}$ can induce TdT

Background

- Role of $I_{KAS}$ in masking cardiac memory induced by V pacing

Circulation. 2015;132:1377-1386
Outline

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- Discussion and summary
Method: identification of variant

Searched 2,306,335 records in INPC electronic database QTc > 500ms and received QT prolongation drug (Table 1)

Matched with blood sample stores in Indiana biobank (n = 29)

Manual review of ECG
normal QTc at baseline, QTc prolongation after drug - 7 days after antibiotics, 60 days after non-antibiotics (n = 11)

Genetic screening with Sanger sequencing
Compared with 11 matched controls
Patient profile: 52 year old Female

- Hx of HTN, GERD
- No Hx of Syncope
- No Family Hx of syncope
- Complain of chronic abdominal pain and nausea
- Treated with ondansetron
- Serum potassium level: 3.9-4.4 meq/L

A. Heart rate = 81 bpm, QT = 408 ms, QTc = 473 ms

B. Heart rate = 74 bpm, QT = 542 ms, QTc = 601 ms
Patient profile: 52-YEAR-OLD Female

- Observed variants in this patient
  - heterozygous for the *KCNN2* c.1509C>G variant (p.F503L in NM_021614.3)
  - heterozygous for the *KCNE1* p.S38G variant (mild modifier)
  - p.P2835S variant in *ANK2* (rs3733617)
  - heterozygous for Carnitine palmitoyltransferase II (*CPT II*) deficiency: Adult polyglucosan body disease
  - homozygous for a *GBE1* with apparently damaging variant: peripheral neuropathy and spasticity, dementia
**KCNN2 variant**

<table>
<thead>
<tr>
<th>Reference aa</th>
<th>Patient aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference (F)</td>
<td></td>
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<tr>
<td>Reference (R)</td>
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<tr>
<td>Patient (F)</td>
<td></td>
</tr>
<tr>
<td>Patient (R)</td>
<td></td>
</tr>
<tr>
<td>Reference (R)</td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of KCNN2 protein structure with labels for S1 to S6 and function sites](image)
Method

Transient transfection of HEK-293 cells

HEK-293 cells with 60% confluency

Transfection With WT KCNN2 - GFP

Transfection With KCNN2 variant (p.F503L) - GFP

Culture for 48hrs

Whole cell patch clamp for comparison of current
Method

- Transfected HEK-293 cells
Method

- Patch clamp
Method

- Whole cell patch setting
  - Axopatch 200B amplifier/Digidata 1440A acquisition system, using pCLAMP-10.4 software (Molecular Devices/Axon, Sunnyvale, CA)
  - Pipette resistance: 2-3 MΩ
  - Room temperature
  - Bath solution (mmol/L): NaCl 140, KCl 4, MgCl₂ 1, HEPES 10, CaCl₂ 1.5, Glucose 10, Ph 7.4 (adjusted by NaOH)
  - Pipette solution (mmol/L): K-Gluconate 144, Mg, MgCl₂ 1.15, HEPES 10, MgATP 2, Glucose 10, Free Ca²⁺ 500nM, Ph 7.25 (adjusted by KOH)
  - Calculated K reversal potential: -94.2mV
Example of whole cell recording
Example of whole cell recording

Apamin (500nM)
Method: OMAP

- Hydrostatic pressure
- O₂/CO₂
- Tyrode’s reservoir
- Warmer and air trapper
- Heater
- Chamber
Method: OMAP
Method: OMAP
Method : OMAP
Method : OMAP

Tyrode’s (K 4.5meq/L)+Blebbstatin 10uM

Tyrode’s (K 2.4meq/L)+Blebbstatin 10uM

Tyrode’s (K 4.5meq/L)+Blebbstatin 10uM+ondansetron 100nM
Method: OMAP

Tyrode’s (K 4.0) + Blebbistatin 10uM

Tyrode’s (K 4.0) + Blebbistatin 10uM + ATXII 10nM

Tyrode’s (K 4.0) + Blebbistatin 10uM + ATXII 10nM + Zofran 100nM + Apamin 100nM

Tyrode’s (K 4.0) + Blebbistatin 10uM + ATXII 10nM + Apamin 100nM + Zofran 100nM

Tyrode’s (K 4.0) + Blebbistatin 10uM + ATXII 10nM + Zofran 100nM + Apamin 100nM

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Tyrode’s (K 4.0) + Blebbistatin 10uM + ATXII 10nM + Zofran 100nM + Apamin 100nM
Outline

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Result: $I_{KAS}$ in non-transfected HEK-293 cells

Ondansetron at 1 mM (A) or apamin at 100 nM (B) do not alter endogenous currents in non-transfected HEK293 cells.
Result: $I_{\text{KAS}}$ in transfected HEK-293 cells

Representative recordings of apamin-sensitive whole-cell currents obtained from voltage-clamped HEK293 cells.

Reversal potential: -93.6 mV
(estimated: -94.2 mV by Nernst equation)
Result: $I_{KAS}$ in transfected HEK-293 cells

- Current levels at 0 mV measured with the patches shown in A as a function of time.
Result: Comparison of $I_{\text{KAS}}$ density

Box plots comparing densities of apamin-sensitive currents measured at 0mV in HEK293 cells expressing wild-type KCNN2 or F498KCNN2.
Result: Comparison of [Ca] sensitivity

representative current traces with inside-out patches from a wild-type (A) and a F498L KCNN2 (B) transfected HEK293 cell.
Result: Comparison of [Ca] sensitivity

Current levels at 30 mV were measured at each concentration, normalized to the maximal value, and plotted as function of Ca\(^{2+}\) concentration. The concentration-response relationships were fitted with the Hill equation.

### Wild type
- EC50: 609.9nM

### F503L
- EC50: 483.19nM
One trace recorded is shown before and during exposure to increasing concentrations of ondansetron (100, 500 and 1000 nM) followed by application of 100 nM apamin in the continued presence of 1000 nM ondansetron.

- a - 100nM
- b - 500nM
- c - 1µM
- d - apamin 100nM
Result: effect of ondansetron on SK current

- Current amplitude at 0 mV measured every from the same cells was plotted as a function of time.
Result: effect of ondansetron on SK current

- whole-cell current responses to 100 nM apamin alone followed by increasing concentrations of ondansetron in the continued presence of 100 nM apamin

- a - 100 nM apamin
- b - 10 µM ondansetron
Result: effect of ondansetron on SK current

- Current amplitude at 0 mV measured every from the same cells was plotted as a function of time.
Result: effect of ondansetron on SK current

- Bar graphs comparing the magnitude of $I_{KAS}$-inhibition as a function of ondansetron concentration.

$$IC_{50} = 185 \text{nM/L}$$
Result: OMAP

Tyrode's (K 4.5 meq/L) + Blebbstatin 10uM

200ms

150ms

Tyrode's (K 4.5 meq/L) + Blebbstatin 10uM + ATX2 10nM

200ms

150ms

Tyrode's (K 4.5 meq/L) + Blebbstatin 10uM + ATX2 10nM + Apamin 100nM

200ms

150ms
Ondansetron unmasks $I_{\text{KAS}}$ upregulation in mouse hearts treated with the $I_{\text{Na-L}}$ activator ATX-II.

- a. Blebb, b. ATXII 15nM+Blebb, c. ATXII 15nM+Blebb+ondanetron 100nM
- d. ATXII 15nM+Blebb+ondanetron 100nM+apamin 100nM
Result: OMAP

- a. Blebb, b. ATXII 15nM + Blebb, c. ATXII 15nM + Blebb + ondansetron 100nM
- d. ATXII 15nM + Blebb + ondansetron 100nM + apamin 100nM
Ondansetron unmasks $I_{KAS}$ upregulation in mouse hearts treated with the $I_{Na-L}$ activator ATX-II.

a. Blebb, b. ATXII 15nM+Blebb, c. ATXII 15nM+Blebb+apamin 100nM
d. ATXII 15nM+Blebb+apamin 100nM+ondansetron 100nM
Result: OMAP

- a. Blebb, b. ATXII 15nM+Blebb, c. ATXII 15nM+Blebb+apamin 100nM
  d. ATXII 15nM+Blebb+apamin 100nM+ondansetron 100nM
Result: OMAP

- a. Blebb, b. ATXII 15nM+Blebb, c. ATXII 15nM+Blebb+apamin 100nM
- d. ATXII 15nM+Blebb+apamin 100nM+ondansetron 100nM
Result: OMAP

- a. Blebb, b. Blebb+vehicle1, c. Blebb+vehicle1+vehicle2
- d. Blebb+vehicle1+vehicle2+vehicle3
Result: Effect of ATXII on SK current

- ATX-II does not alter $I_{KAS}$ in KCNN2-transfected HEK293 cells.

Baseline, a

a – ATXII 15nM
b – Apamin 100nM
Result: Effect of ATXII on SK current

- ATX-II does not alter $I_{KAS}$ in KCNN2-transfected HEK293 cells.
Outline

- Background and hypothesis
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- Summary
A genetic variant of \textit{KCNN2} was identified in a patient with drug-induced long QT syndrome. p.F503L \textit{KCNN2} variant is associated with significantly increased \( I_{KAS} \). Ondansetron blocks \( I_{KAS} \) at clinical serum concentrations. Activation of cardiac \( I_{KAS} \) preserves repolarization reserve under conditions of increased \( I_{Na-L} \). Ondansetron, at therapeutic concentrations, compromises this \( I_{KAS} \)-mediated rescue mechanism, which might lead to diLQT in susceptible patients.