



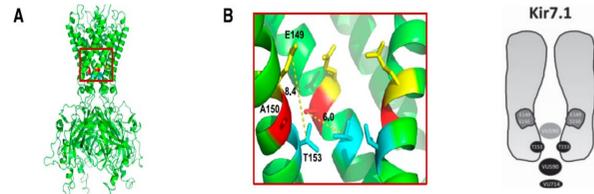
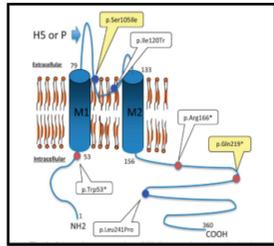
Side-Chain Polarity of Amino Acids within the Kir7.1 Channel Pore Lining Determine Permeability and Function

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Introduction

- Homotetrameric protein
- Maintains membrane potential
- Kir7.1 is present in the kidney, intestine, uterus, and the retina
- Mutations in *KCNJ13* gene (Kir7.1) lead to blindness
- **Hypothesis: A single amino acid change from a hydrophilic Threonine to a hydrophobic Isoleucine will structurally alter the channel pore and therefore alter function.**



Kharade et al. 2017 and Denton and Kharade 2017

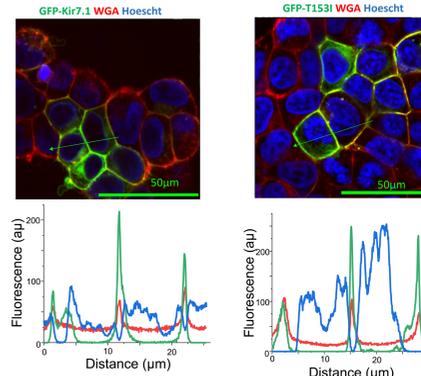
Methods

- Mass Spec: UW-Biotechnology Center utilized LC/MS with Trypsin/GluC and Mascot analysis.
- Transfection: HEK293 cells in culture were transfected with GFP wildtype (WT) Kir7.1, T153I, T153G, T153A, T153L, T153C, or T153S plasmid DNA.
- Live-cell Imaging: Nikon-C2 confocal system, mapped GFP fused protein. Hoescht stain for nucleus and WGA-Alexa 594 for membrane were utilized.
- Electrophysiology: Extracellular Ringer's or Rb⁺ determined channel function. Extracellular K⁺ gradient determined chord conductance. Data analyzed using the Clampfit program.
- Bioinformatics: MPex and Protean 3D were utilized for hydrophobicity and pore structure respectively.

Results

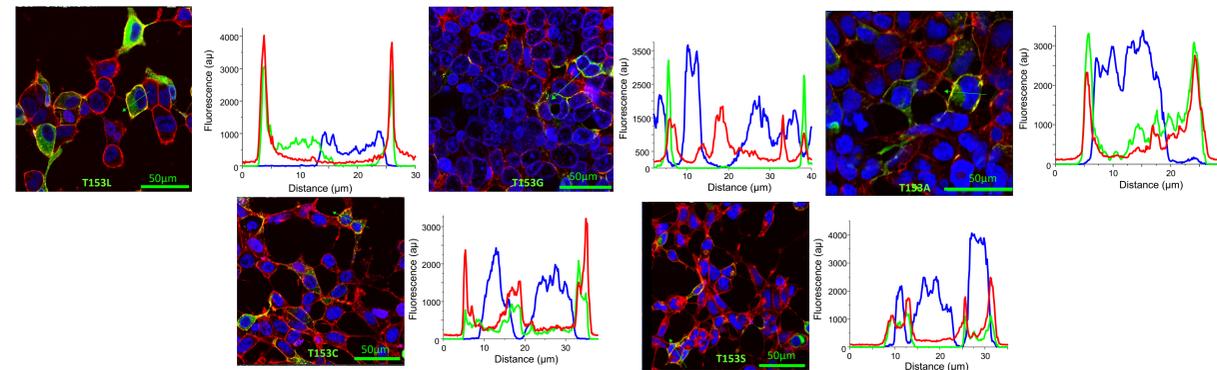
Translation and Localization of Kir7.1 WT and T153I

| Analysis Type | Kir7.1 % Match | Kir7.1 Quality Score | T153I % Match | T153I Quality Score |
|---------------|----------------|----------------------|---------------|---------------------|
| Mascot | 42% | 1137 | 34% | 1958 |
| GFP Kir | | | | |
| Trypsin/GluC | | | | |



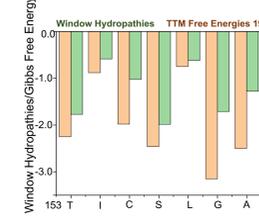
GFP T153I produces a full-length protein product which is localized to the cell membrane.

Single AA Mutants at Position 153 are Localized to the Membrane



Hydrophobicity and Pore Size Analysis

- Polarity and pore size play a role in Kir7.1 K⁺ permeability
- A polar side chain at aa position 153 is required for Kir7.1 function
- A pore radius near 3.2 Å^o is indicative of function

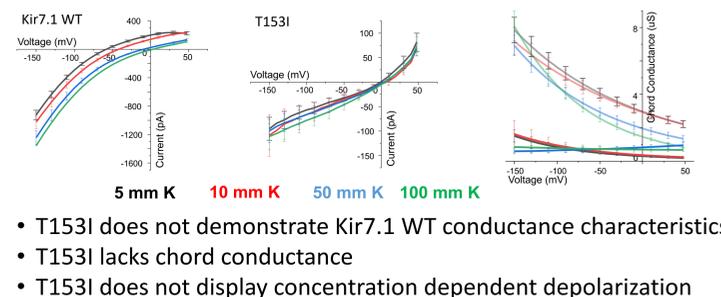
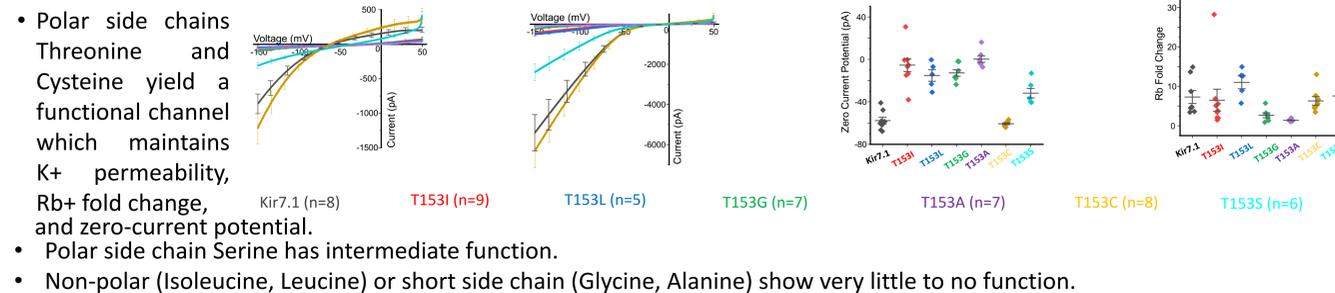


| Channel | Pore Size | Radius (d) |
|---------|----------------------|------------------------------|
| WT | 124.29Å ² | 3.145(6.29) Å ^o |
| T153I | 113.85Å ² | 3.01 (6.02) Å ^o |
| T153C | 127.08Å ² | 3.18 (6.36) Å ^o |
| T153S | 126.28Å ² | 3.17 (6.34) Å ^o |
| T153L | 56.21Å ² | 2.115 (4.23) Å ^o |
| T153G | 403.28Å ² | 5.665 (11.33) Å ^o |
| T153A | 237.24Å ² | 4.345 (8.69) Å ^o |

Main Finding

- T153 is within the inner pore.
- T153I produces a full-length protein product which localizes to the membrane.
- Channel function is altered by a single aa change at position 153.
- Bioinformatics show that single aa changes alter hydrophobicity and pore region structure both contributing to functional alterations.
- Taken together this study supports the importance of the inner pore region for the permeability of the Kir7.1 channel.

Functional Effects of AA Changes in Inner Pore



| Kir Channel | Slope (mV/mM K) |
|-------------|-----------------|
| Kir7.1 WT | 0.6066 |
| T153I | -0.0044 |

Acknowledgments

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