From Genes to Pain: Lessons from Rare Inherited Disorders -- and Extrapolation to the Rest of Us

Stephen G. Waxman, M.D., Ph.D.

Bridget Marie Flaherty Professor of Neurology, Neurobiology and Pharmacology; Director, Center for Neuroscience & Regeneration Research, Yale University School of Medicine and VA Connecticut
TM: Highly decorated police officer
Gun shot wound

- 29 y.o. college grad: GSW. Injury to radial n. Distinguished Service Medal
- Motor deficit, partial sensory loss in radial n. distribution.
- Severe burning pain. Exacerbated by tactile & thermal stimuli.
- Minimal or no response to NSAIDs, tricyclics, gabapentin, SSRIs, etc.
- Limited relief w/ opiates, carbamazepine (non-selective Na channel blocker). Doses limited by SEs (diplopia, confusion, sleepiness).
- Disabled. Followed by neurologist, pain specialist, psychiatrist

- Neuropathic pain: an unmet need (DM, PHN, chemotx, trauma)
Voltage-gated Na Channels

Na channels (1800 a.a. polypeptides) are diverse.
Nine genes, different properties, selective expression

Increased or altered Na channel expression or function can contribute to pain disorders, both rare and common

Some Na channel subtypes represent opportune therapeutic targets
Different Na channel subtypes: Different functional properties

- Fast TTX-S
- Slow TTX-R
- Persistent TTX-R

Current-voltage relationship

Steady-state inactivation
DRG neurons express multiple Na channel isoforms

\[ \text{Na}_v^{1.1} \quad \text{Na}_v^{1.2} \quad \text{Na}_v^{1.3} \quad \text{Na}_v^{1.5} \]

\[ \text{Na}_v^{1.6} \quad \text{Na}_v^{1.7} \quad \text{Na}_v^{1.8} \quad \text{Na}_v^{1.9} \]
Hyper-excitability of pain-signaling nerve cells produces pain after nerve injury and SCI

- Are there “peripheral” subtypes of Na channels that are essential for pain signaling in peripheral neurons, but do not play major roles in brain?

- Is it possible to target Na channels without Central or Cardiac SEs?
Hyperexcitability in Injured DRG Neurons: Potential Molecular Targets

Preferentially Expressed in Peripheral Neurons:

**Nav 1.7** \( (\text{gene } SCN9A) \)  
Boosts subthreshold depolarizations; may facilitate DH terminal invasion; sets gain in nociceptors

**Nav 1.8** \( (SCN10A) \)  
AP upstroke; high-frequency firing in response to depolarization

**Nav 1.9** \( (SCN11A) \)  
Depolarizes RMP; amplifies response to depolarizing stimuli

Up-regulated After Axonal Injury:

**Nav 1.3** \( (SCN3A) \)  
Persistent current; rapid repriming; amplifies small inputs
WHY SEARCH THROUGHOUT THE WORLD TO FIND A RARE GENETIC DISORDER?

Rare genetic disorders are “experiments of nature” that can:

• Define molecular mechanisms in humans, and

• Identify therapeutic targets

that are relevant to common disorders
**Na\(_v\) 1.7** Pref. expressed in nociceptive DRG & Symp. ganglion neurons

- Slow closed-state inactivation
- Amplifies small depolarizations near RMP at peripheral terminals; may facilitate invasion and/or transmitter release in DH
- Sets gain on nociceptors

**Na\(_v\) 1.8** Sensory Neuron Specific

- Remains available when depolarized
- Generates 60-80% \(I_{inward}\) during AP upstroke
- High-frequency firing in response to depolarization
A Model Disease: Primary Erythromelalgia
The “Man on Fire” Syndrome

- Severe burning pain, triggered by mild warmth.
- Erythema
- Autosomal dominant, 100% penetrant
- Refractory to existing pharmacotherapies
- Mutation found first in families in China, analyzed in New Haven. Families now identified in Europe, U.S.A., Canada
- Cause: Gain-of-Function of Na\textsubscript{v}1.7
A Model Disease: Primary (Inherited) Erythromelalgia

The “Man on Fire” Syndrome

Searing, burning pain, “like hot lava poured into my body”
Molecular Pathophysiology of a Human Hereditary Pain Syndrome: Inherited Erythromelagia

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Lynda Tyrrell, MSc
Ted Cummins, PhD
Tony Rush, PhD
Angelika Lampert, MD
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Xiaoyang Cheng, PhD
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Bonnie Wallace, PhD (UK)
Andreas O’Reilly, PhD (UK)

Steve Waxman, MD, PhD
Mutations in Exon 15 of SCN9A in Two Chinese families

Mutations in Exon 15 of SCN9A

ATC
Isoleucine (I 848)

ACT
Threonine (T)

CTC
Leucine (L 858)

CAC
Histidine (H)

Scn9A

Promoter
Exons
Exon 15

NH2
L1
L2
L3
L4
COOH

Na1.7


Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythermalgia
Y Yang*, Y Wang*, S Li, Z Xu, H Li, L Ma, J Fan, D Bu, B Liu, Z Fan, G Wu, J Jin, B Ding, X Zhu, Y Shen

I1848T and L858H Mutations Shift Activation, Slow Deactivation, and Enhance Response to Small, Slow Depolarizations to produce a Gain-of-Function in Na\textsubscript{v}1.7

Cummins et al, J.Neurosci. 2004
**A863P:**

*de novo* Na$_v$1.7 mutation in sporadic erythromelalgia

F1449V: Na\textsubscript{\text{v}}1.7 Gain-of-Function Mutation in a Large Family with Erythromelalgia

Dib-Hajj et al, Brain, 2005
Homology model: F1449V destabilizes closed-state conformation of Na\(_{V}1.7\)

(a)

Wild-type

F1449V

Lampert et al, J. Biol. Chem. 2008

Dib-Hajj et al, Brain, 2005
Nav1.7 Loss-of-function: “An SCN9A channelopathy causes congenital inability to experience pain”


“All six individuals had never felt any pain, at any time, in any part of the body”

Painless fractures, burns, childbirth, tooth extractions
Lessons from the “Erythro” in Erythromelalgia:

1. A single ion channel mutation can have **opposing effects in different types of neurons**

2. Nav1.7 gain-of-function does not act alone in making DRG neurons hyperexcitable. It works **together with Nav1.8**

Tony Rush, PhD  
Ted Cummins, PhD  
Dmytro Vasylyev, PhD  
Sulayman Dib-Hajj, PhD  
Joel Black, PhD  
Steve Waxman, MD, PhD
The L858H gain-of-function Na\textsubscript{v}1.7 mutation makes spinal sensory (DRG) neurons hyperexcitable.
The L858H gain-of-function $\text{Nav}1.7$ mutation makes sympathetic ganglion (SCG) neurons *hypoexcitable*.
DRG neurons express $\text{Na}_V\,1.8$ (SNS). Sympathetic ganglion (SCG) neurons do not.

Inactivated by Depolarization

Remains Available, Supports Repetitive Firing
DRG neurons express $\text{Nav}_1.8$ (SNS).
Sympathetic ganglion (SCG) neurons do not.

Hypothesis: Presence of $\text{Nav}1.8$ in DRG, & absence in SCG, endows these neurons with different responses to depolarization produced by $\text{Nav}1.7$ gain-of-function.
Co-expression of $\text{Na}_\text{v}1.8$ together with L858H mutant $\text{Na}_\text{v}1.7$ channels rescues electrogenic properties in SCG neurons.

SCG neuron expressing WT $\text{Na}_\text{v}1.7$

L858H mutant $\text{Na}_\text{v}1.7$ without $\text{Na}_\text{v}1.8$

Rush et al, PNAS, 2006
Co-expression of Na\(_{\text{V}}\)1.8 together with L858H mutant Na\(_{\text{V}}\)1.7 channels rescues electrogenic properties in SCG neurons.

**SCG neuron expressing**

WT Na\(_{\text{V}}\)1.7

L858H mutant Na\(_{\text{V}}\)1.7 without Na\(_{\text{V}}\)1.8

L858H mutant Na\(_{\text{V}}\)1.7 with Na\(_{\text{V}}\)1.8

The Firing Phenotype of an ion channel mutation can vary, depending on the cell background, of other channels, in which it is expressed.

**Na\(_{\text{V}}\)1.8 acts as a Molecular Switch:** Presence or absence of Na\(_{\text{V}}\)1.8 determines whether Na\(_{\text{V}}\)1.7 EM mutation produces hyper- or hypoexcitability.
What about the “rest of us”? 

$Na_v^{1.7}$ and $Na_v^{1.8}$ accumulate in injured axon tips in Painful Human Neuromas

A common SNP in Nav1.7 biases susceptibility to pain after injury:

**R1150W**: W allele: 18% contr. chromosomes (28% population heterozygous W, 4% homozygous)

V400M Na\textsubscript{V}1.7 Pharmacoresponsive Mutation: Carbamazepine Normalizes Activation and Inactivation

Fischer, TZ et al Ann Neurol 2009
Atomic-level structural modeling and energetic coupling analysis predict mutant sensitivity to CBZ

Data Summary:

<table>
<thead>
<tr>
<th></th>
<th>$V_{1/2}$</th>
<th>$\Delta J^*$</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>22.42</td>
<td>6.83</td>
<td>energically coupled</td>
</tr>
<tr>
<td>V400M</td>
<td>32.23</td>
<td>7.88</td>
<td></td>
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<tr>
<td>S241T</td>
<td>37.67</td>
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<tr>
<td>VM/ST</td>
<td>43.39</td>
<td>5.99</td>
<td></td>
</tr>
<tr>
<td>F1449V</td>
<td>33.94</td>
<td>6.83</td>
<td>energically dependent</td>
</tr>
<tr>
<td>VM/FV</td>
<td>48.67</td>
<td>7.46</td>
<td></td>
</tr>
</tbody>
</table>

Note: $F = 0.023$

Yang et al, 2012
Extrapolating to common pain disorders:

Na\textsubscript{v}1.7, Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9 mutations in painful neuropathy

Clinical:
Janneke Hoeijmakers, MD
Giuseppe Lauria, MD

Molecular Biology:
Sulayman Dib-Hajj, PhD
Lynda Tyrrell, MA
Palak Shah, MA
Larry Macala, MA
Peng Zhao, PhD

Physiology / Biophysics:
Chongyang Han, PhD
Mark Estacion, PhD
Dmytro Vasylev, PhD
Jianying Huang, PhD
Xiaoyang Cheng, PhD
Hyesook Ahn, PhD
Jin Choi, PhD

Catherina Faber, MD, PhD
Ingemar Merkies, MD, PhD
Steve Waxman, MD, PhD
Small Fiber Neuropathy

- Dysfunction/degeneration of small myelinated & unmyelinated nerve fibers:
  - Burning pain, usually distal (feet > hands)
  - Autonomic sx (orthostatic hypotension, impotence, palpitations etc)

- **Dx**: Confirmed by biopsy: loss of IENF

- **Etiologies**: Diabetes, IGT, amyloidosis, sarcoid, anti-PL Ab syndromes, HIV, ChemoTx

  - ~50%: Idiopathic or Cryptogenic
    - (no apparent cause)
IEM Mutations:
Robust biophysical changes in channel.
Early (infantile / early childhood) onset,
100% penetrance,
Strong phenotype

SFN Mutations:
Subtle biophysical changes in channel.
Adult onset. Variable expressivity
Nav1.7 (SCN9A) mutations: 8 of 28 pts with biopsy-confirmed SFN

Patients referred with a clinical diagnosis of Small Fiber Neuropathy (SFN)  
N=248

- No underlying causes identified  
  N=63 (25.4%)

- Underlying causes identified  
  N=185 (74.6%)*

  - Lost to follow-up or refused further participation  
    N=19

  - Excluded

Patients with idiopathic SFN  
N=44

- IENFD + QST normal  
  N=3

- IENFD or QST abnormal  
  N=13

- IENFD + QST abnormal  
  N=28

  - No mutation found in SCN9A  
    N=20

  - Mutation found in SCN9A  
    N=8

- Excluded

Excluded

* Excluded

Faber et al, Ann. Neurol., 2011
Variable expressivity of SFN: Proximal Itch vs Distal Pain

Devigili et al, Pain, 2014
Nav1.8 (SCN10A) missense mutations:

Nav1.8: Sensory Neuron Specific, Resistant to Inactivation, Supports Repetitive Firing

7 mutations in Nav1.8 in 9 individuals w/out Nav1.7 mutations within cohort of 104 pts with painful, predominantly small-fiber neuropathy

3 Nav1.8 mutations profiled by VC and CC:
  2 showed gain-of-function at channel and cellular level

Pathogenic gain-of-function mutations in Nav1.8 in at least 3, and at most 7, of 104 pts with painful predominantly small-fiber neuropathy

Faber et al, PNAS, 2012
The image contains a page with various graphs and data plots related to the Na_1.8 / I1706V channel. The page includes statistical data, histograms, and graphs comparing WT and I1706V neurons.

**A1** shows the waveform of WT and I1706V neurons.

**A2** presents a graph comparing the fraction of peak current (%). The x-axis represents voltage (mV), and the y-axis represents G/G_max. The graph includes data points for WT and I1706V, showing differences in peak current fraction.

**A3** displays the ramp voltage (mV) over time, with a comparison between WT and I1706V.

**A4** and **A5** show data for RMP (mV), voltage threshold (mV), and current threshold (pA) for WT and I1706V neurons.

**A6** and **A7** illustrate the effects on spontaneously firing neurons, with histograms and APs/500 ms.

**A8** demonstrates the relationship between stimulus (pA) and APs/500ms, with data for WT and I1706V marked with significance levels (* * * * * * *).

Huang J et al., 2013.
Nav1.9: Non-inactivating, broad overlap (window) current, TTX-R
Expressed specifically in DRG, TRG and other sensory neurons
Nav1.9 regulates RMP and excitability of DRG neurons
Nav1.9 (SCN11A) missense mutations:

11 missense variants of Nav1.9 within cohort of 344 pts w/out Nav1.7 or Nav1.8 mutations, with painful, predominantly small-fiber neuropathy

4 Nav1.9 mutations in conserved, membrane-spanning regions of the channel. 3 mutations shown to be gain-of-function by voltage-clamp and current-clamp thus far:

Pathogenic gain-of-function mutations in Nav1.9 in at least 3, and at most 4, of 344 pts with painful predominantly small-fiber neuropathy

Huang et al, Brain, 2014
**Na$_{1.9}$/I$_{381T}$**

**A1**

WT

I$_{381T}$

**A2**

WT

I$_{381T}$

**A3**

WT

I$_{381T}$

**A4**

WT

I$_{381T}$

**A5**

WT

I$_{381T}$

**A6**

Spontaneously firing neurons

**A7**

WT

I$_{381T}$

Huang J et al., 2014.
Where next? Early clinical studies on small n’s of genomically characterized human subjects have been carried out.

Next Steps: larger n’s; longer-term studies; can we extrapolate to larger pain indications?

Ultimate goal: a new, more effective class of pain medications, with minimal central side effects, without addictive potential
L858H mutation: Persistent current (window current) depolarizes RMP

Vasylyev et al, 2014
Hypothesis: Gain-of-function in Na channels drives injurious Reverse Na/Ca exchange that contributes to axonal degeneration in Small Fiber Neuropathy

Small-Diam. Nerve Fibers:
- High Input Impedance
- Short Length Constant (Electrotonic and Diffusional)
- Large Effects of Relative Small Numbers of Na Channels

Stys, Waxman, Ransom J. Neurosci

Waxman, Black, Kocsis & Ritchie, PNAS
Nav1.7 SFN-associated mutation I228M

Hs Na\textsubscript{v}, 1.1  NVSALRTFRVLRALKTISV\textsubscript{P}
Hs Na\textsubscript{v}, 1.2  NVSALRTFRVLRALKTISV\textsubscript{P}
Hs Na\textsubscript{v}, 1.3  NVSALRTFRVLRALKTISV\textsubscript{P}
Hs Na\textsubscript{v}, 1.4  NISALRTFRVLRALKTVI\textsubscript{P}
Hs Na\textsubscript{v}, 1.5  NVSALRTFRVLRALKTISV\textsubscript{S}
Hs Na\textsubscript{v}, 1.6  NVSALRTFRVLRALKTISV\textsubscript{P}
Hs Na\textsubscript{v}, 1.7  NVSALRTFRVLRALKTISV\textsubscript{P}
Hs Na\textsubscript{v}, 1.8  GISGLRTFRVLRALKTVS\textsubscript{P}
Hs Na\textsubscript{v}, 1.9  KLLPLRTFRVFRALKTVS\textsubscript{P}
Hs Na\textsubscript{v}, 1.7\textsubscript{I228M}  NVSALRTFRVLRALKTISV\textsubscript{M}

A

WT

I228M

B

\( I_{\text{I228M}} / I_{\text{WT}} \)

\( V_{1/2,\text{fast inact}} \rightarrow -81.2\pm2.2\text{mV} \)

\( V_{1/2,\text{act}} \rightarrow -26.1\pm2.5\text{mV} \)

\( G_{\text{max}} \text{ (mV)} \)

C

\( I_{\text{max}} / \text{V} \)

\( V_{1/2,\text{slow inact}} \rightarrow -56.2\pm1.2\text{mV} \)

\( \text{APs / 500 msec} \)

D

\( \text{Stimulus (pA)} \)

0.9 ± 0.5 Hz

\( \text{F/17; 29%; (0.22; 0%)} \)
\[ I = \overline{g}_{Na} m^3 h (V - E_{Na}) + \overline{g}_K n^4 (V - E_K) + \overline{g}_{\text{leak}} (V - E_{\text{leak}}) \]

Hodgkin and Huxley, 1952
‘When Hodgkin and I finished writing the 1952 papers, each of us moved to other lines of work because we could not see how to make progress on the mechanism of channel action. Any idea of analyzing the channels by patch clamp or molecular biology would have seemed to us to be... science fiction.’

Andrew Huxley, in *The Axon*, 1995
Mitochondrial damage injures neurites of DRG neurons. Block of Na channels or of reverse NCX is protective.

Axonal Injury in SFN:

Age-dependence (adult onset), length-dependence, and variable expressivity of SFN suggest a multi-hit mechanism of axonal injury:

- Additional genomic variants
- Environmental factors
- Epigenetic factors
- Mitochondrial dysfunction (stochastic, age- and/or length-related)
Fire, Fantoms and Fugu: Sodium Channels from Squid to Clinic

Stephen G. Waxman, M.D., Ph.D.

Bridget Marie Flaherty Professor of Neurology, Neurobiology and Pharmacology; Director, Center for Neuroscience & Regeneration Research, Yale University School of Medicine and VA Connecticut Healthcare, New Haven, Connecticut
Nerve injury triggers up-regulated expression of sodium channels which makes DRG neurons and their axon tips hyperexcitable.

**Graphs and Images:**

- **Graph A:** A graph showing the recovery interval (ms) on the x-axis and the fraction recovered on the y-axis. The graph includes two curves: axotomized TTX-S in yellow and uninjured TTX-S in magenta. The recovery intervals are marked as approximately 14 ms and 61 ms.

- **Graph B:** A graph showing relative current on the y-axis and membrane potential (mV) on the x-axis. The graph includes three curves: Axot. DRG in brown, DRG + Nav1.3-TTXr in blue, and 500 msec in green.

- **Image A:** A gel image showing various molecular bands.

- **Image B:** A microscopic image showing cells.

- **Image C:** A microscopic image showing cells with a stained pattern.

- **Image D:** A microscopic image showing cells.

- **Image E:** A microscopic image showing cells with a stained pattern.

- **Image F:** A microscopic image showing a tissue section with a stained pattern.
shRNA knockdown of Nav1.3 attenuates nerve injury-induced neuropathic pain

Samad et al, 2013
Chasing Men on Fire: Sodium Channels and Neurological Disorders

Stephen G. Waxman, M.D., Ph.D.

Bridget Marie Flaherty Professor of Neurology, Neurobiology and Pharmacology; Director, Center for Neuroscience & Regeneration Research, Yale University School of Medicine and VA Connecticut Healthcare, New Haven, Connecticut
Hyperexcitability in Injured DRG Neurons: Potential Molecular Targets

**Preferentially Expressed in DRG Neurons:**

**Nav 1.7 (PN1)**
- Boosts subthreshold depolarizations; sets gain in nociceptors

**Nav 1.8 (SNS)**
- AP upstroke; high-frequency firing in response to depolarization

**Nav 1.9 (NaN)**
- Depolarizes RMP; amplifies response to depolarizing stimuli

**Up-regulated After Axonal Injury:**

**Nav 1.3**
- Persistent current; rapid repriming; amplifies small inputs
Nav1.7 is necessary for heat pain after burn injury

Shields et al, 2012
Neuropathic Pain and Phantom Pain

“I was wounded on July 1st 1862. Walking a mile I had my right arm amputated, never quite losing consciousness.

I never think of my right arm with thoughts of using it. The fingers remain in my mind, however, half-closed.

As with everybody who has lost a limb, pain occurs often…”

From a letter to S. Weir Mitchell, MD
Written by a veteran of the Civil War
Yale University School of Medicine

Center for Neuroscience & Regeneration Research of Yale University, VA Medical Center, West Haven, CT

A Collaboration of the Paralyzed Veterans of America and United Spinal Association with Yale University

Harnessing the molecular revolution to preserve and restore function after injury to the brain and spinal cord

Established 1986
Na\textsubscript{v}1.6, Na\textsubscript{v}1.7, Na\textsubscript{v}1.8 and Na\textsubscript{v}1.9: co-expressed with NCX2 in Intracutaneous Nerve Terminals

Persson et al, 2010

Vasylyev & Waxman, J. Neurophysiol
Nav1.9 (SCN11A) missense mutations:

11 missense variants of Nav1.9 within cohort of 344 pts w/out Nav1.7 or Nav1.8 mutations, with painful, predominantly small-fiber neuropathy

4 Nav1.9 mutations in conserved, membrane-spanning regions of the channel. 2 mutations shown to be gain-of-function at voltage-clamp and current-clamp level thus far:

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Huang et al, *Brain*, 2014