Disclosures

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4) GSK.

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Astra Zeneca
Glenmark Pharmaceuticals
Lundbeck
Bayer healthcare
Mundipharma
Regeneron
Afferent Pharma

Why does tissue injury cause pain?
We have a good understanding of why injury cause pain

**TRANSDUCTION**
- Heat
  - TRPV1
  - TRPV2
  - TRPV3
  - TRPV4
  - TRPV4
- Cold
  - TRPM8
- Damage mediators
  - Prostaglandins
  - NGF
  - NT
  - etc.
- Neuropeptides and other SHIT
  - Substance P
  - CGRP

**MODULATION**
- Glutamate
- ATP
- Neuropeptides
- BDNF
- Inhibitory controls
  - MOR
  - CB1/2
  - GABA
- Microglial Activation
  - ATP
  - TRPV1
- Inflammatory mediators & targets
  - Prostaglandins
  - NGF
  - NT
  - etc.
- Neuropeptides and other SHIT
  - Substance P
  - CGRP

**TRANSMISSION**
- Nav1.7, Nav1.8
- Nav1.6 (Nav1.3)
- Threshold

Why is the pain experience so variable?

Experimental pain in healthy human subjects varies enormously

![Graph showing the variation in heat pain threshold and tolerance among 2000 twins tested.](image-url)

**Heat Pain Threshold (°C)**
- 35 to 50

**Heat Pain Tolerance (°C)**
- Mean ± 2 x std dev

>2000 twins tested
Pain and pathology are only weakly correlated

Prevalence of pain

Magnitude of pain

Pain can persist when pathology resolves

What explains this variability?
What explains pain variability? Genetic factors

Single gene mutations can result in abnormal pain states, but are very rare.
Phenotypes are dramatic and don’t explain pain normal variability.

Loss of function
Gain of function

Normal Sural nerve
HSN IV patient

Known genes: NGF/TRKA; Na1.7; Na1.8; PRDM12

Known genes: Na1.7; Na1.8; Na1.9; TrpA1

Genetic influences on Pain – Twin Studies

- Heat Pain Threshold (HPT)
- Pain during creation of 45°C thermal burn
- Primary & secondary hyperalgesia after burn
- Pain during iontophoresis of Acid + ATP
- Itch after iontophoresis of Histamine

Monzygotic (MZ)
Dizygotic (DZ)

Twin Studies

Heritability of pain traits – twin studies

Norbury et al., 2007
Heritability of pain traits from twin studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subject details</th>
<th>Cohort</th>
<th>Heritability</th>
<th>Phenotype examined</th>
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<tbody>
<tr>
<td>MacGregor et al., 2004</td>
<td>Female twins (181 MZ, 351 DZ)</td>
<td>Twins UK</td>
<td>0.52-0.68</td>
<td>Lower back pain, Neck pain</td>
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<td>Norbury et al., 2007</td>
<td>Female twins (51 MZ, 47 DZ)</td>
<td>Twins UK</td>
<td>0.22-0.59</td>
<td>Quantitative sensory testing</td>
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<td>Battie et al., 2007</td>
<td>Male twins (147 MZ, 153 DZ)</td>
<td>Finnish twin cohort</td>
<td>0.3-0.46</td>
<td>Lower back pain</td>
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<td>Nielsen et al., 2008</td>
<td>Mixed twins (53 MZ, 39 DZ)</td>
<td>Norwegian twin registry</td>
<td>0.6</td>
<td>Cold-pressor pain, Heat contact pain</td>
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<td>Altman et al., 2011</td>
<td>Female twins (1,867 MZ, 1,293 DZ)</td>
<td>Swedish twin registry</td>
<td>0.3</td>
<td>Bladder pain syndrome</td>
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<td>Williams et al., 2010</td>
<td>Mixed twins (991 MZ, 1,074 DZ)</td>
<td>Twins UK</td>
<td>0.46</td>
<td>Pain at different body sites</td>
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<td>Markkula et al., 2009</td>
<td>Mixed twins (12,500)</td>
<td>Finnish twin cohort</td>
<td>0.51</td>
<td>Fibromyalgia</td>
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<td>Kato, Sullivan and Pedersen, 2010</td>
<td>Mixed twins (28,531 pairs)</td>
<td>Swedish twin registry</td>
<td>♂ 0.09, ♀ 0.13</td>
<td>Chronic widespread pain</td>
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<td>Hocking et al., 2012</td>
<td>2,195 extended families</td>
<td>Scottish family health study</td>
<td>0.16, 0.3</td>
<td>Any chronic pain, Severe chronic pain</td>
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<td>Livshits et al., 2011</td>
<td>Female twins (371 MZ, 698 DZ)</td>
<td>Twins UK</td>
<td>0.43-0.68</td>
<td>Lower back pain</td>
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<td>Hartvigsen et al., 2009</td>
<td>Mixed twins (6,700 MZ, 8,500 DZ)</td>
<td>Danish twin registry</td>
<td>0.33-0.39</td>
<td>Back pain (lumbar, thoracic and neck)</td>
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<td>Polderman et al., 2015</td>
<td>Mixed twins (9,019)</td>
<td>Meta-analysis</td>
<td>0.42 (0.36-0.47)</td>
<td>Migraine</td>
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</table>

What genes contribute to pain heritability?
GWAS – comparing the genomes of different individuals

Human genome ~ 3 billion base pairs. SNPs on average every 300 nucleotides. Any 2 individuals likely to differ by <0.5%.

Test the statistical significance of the association. Does not imply disease causing.

GWAS in large numbers of migraineurs identifies 44 SNPs

60,000 migraineurs, 316,000 controls
Only 2 loci close to neuronal channels
6 associated with NO signalling
30% associated with vascular function
Odds Ratio of individual loci typically 5-10%
Most SNPs are in regulatory regions
Together only explain 10-20% of the heritability.
Rare variants in painful diabetic peripheral neuropathy: candidate gene approach (Nav1.7)

100 Diabetic Peripheral Neuropathy

Nav1.7 published variants [1]
Nav1.7 unpublished variants [6]

Pain sensitivity varies enormously in health and disease

In summary, rare variants play a role in explaining variability in pain tolerance and threshold in diabetic peripheral neuropathy. Further research into these variants could provide insights into pain mechanisms and potential therapeutic targets.
Beyond genetics:
Environmental risk factors

Pain ‘history’
(experimental pain
thresholds, prior pain)

Psychosocial factors
(expectation, catastrophising,
depression, anxiety)

Age
Gender

Early life stressors

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<thead>
<tr>
<th>Pre-op Pain</th>
<th>Age</th>
<th>Sex</th>
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<td>Knee</td>
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<td>Sinus</td>
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<td>Bladder</td>
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<td>Sling</td>
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Total ~23,000

Odds ratio to develop pain

Mechanisms of acquired vulnerability: altered brain connectivity

Role of endogenous pain modulation in chronic pain
Mechanisms and treatment

Cells have the same genes. Different genes are switched on or off in different
types of cell. These regulatory patterns traditionally considered stable in maturity.
Genetics

- Mutation
- Permanent Phenotypic inheritance

Epigenetics

- DNA methylation
- Histone Variants
- Plastic Stable
  Memory of transitional change
  Somatic variability and development
  Response to environmental cues

DNA methylation

- CpG Island
- Transcriptional start site
- Typical gene

Histone modifications.
Multiple proteins participate in chromatin modifications.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Fold Change</th>
<th>Adj P value</th>
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<tr>
<td>HDAC1</td>
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<td>HDAC4</td>
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Epigenetics: relevance to pain

1. Potential for novel therapeutic targets

2. Pain chronication & vulnerability

Many genes are dysregulated in chronic pain states

L4 DRG, 14 day spinal nerve injury

HDAC1
HDAC4

Ana Antunes-Martin
HDAC4 cKO mice have normal acute pain responses

Crow et al., FASEB 2015

HDAC4 cKO mice have reduced inflammatory pain

Crow et al., FASEB, 2015

Epigenetics: relevance to pain

1. Potential for novel therapeutic targets

2. Pain chronification & vulnerability
Activated glia contribute to some neuropathic pain states.


IL-1β, TNFα, IL-6, NO, PGs, BDNF

IBA1 immunostaining

persistent pain states are associated with altered epigenetic signatures

Sham
Neuropathic

Regions enriched for H3K4me1 = enhancers

In experimental neuropathy new enhancers emerge in the regulatory regions of some genes in spinal cord cells.

Denk et al. Cell Reports, 2016

Novel enhancers day 7
Dysregulated transcripts day 7

Enhancers remain when transcription reverts to normal

Denk et al., 2016
Methylation studies in heat-pain discordant monozygotic twins (Jordana Bell)

Phenotype ~2500 twins for thermal pain sensitivity

Identify 25 pairs of MZ twins with biggest difference in noxious heat sensitivity

Genome-wide sequencing after methylated DNA

Replication cohort of 50 unrelated individuals

Differential methylation of DNA is associated with pain at various loci

First example of an epigenetic 'mark' associated with pain sensitivity in humans. Nature Comms, 2014

Pubmed finds 145 articles on “epigenetics AND pain”. 66 are reviews
Pain vulnerability: implications for personalised medicine

Vulnerability/ Resilience = Genetic factors + Environmental factors

- **40%**
- **60%**

Individual genetic variants contribute very little risk.
Together, genetic variants explain only a fraction of known heritability.
If gene x gene interactions are important, they will be very difficult to identify.

Twins studies recognize shared and unique environmental factors.
Surprisingly, most twin studies find that shared environmental factors predict very little personal risk.
If gene x environment interactions are important, they will be very difficult to identify.