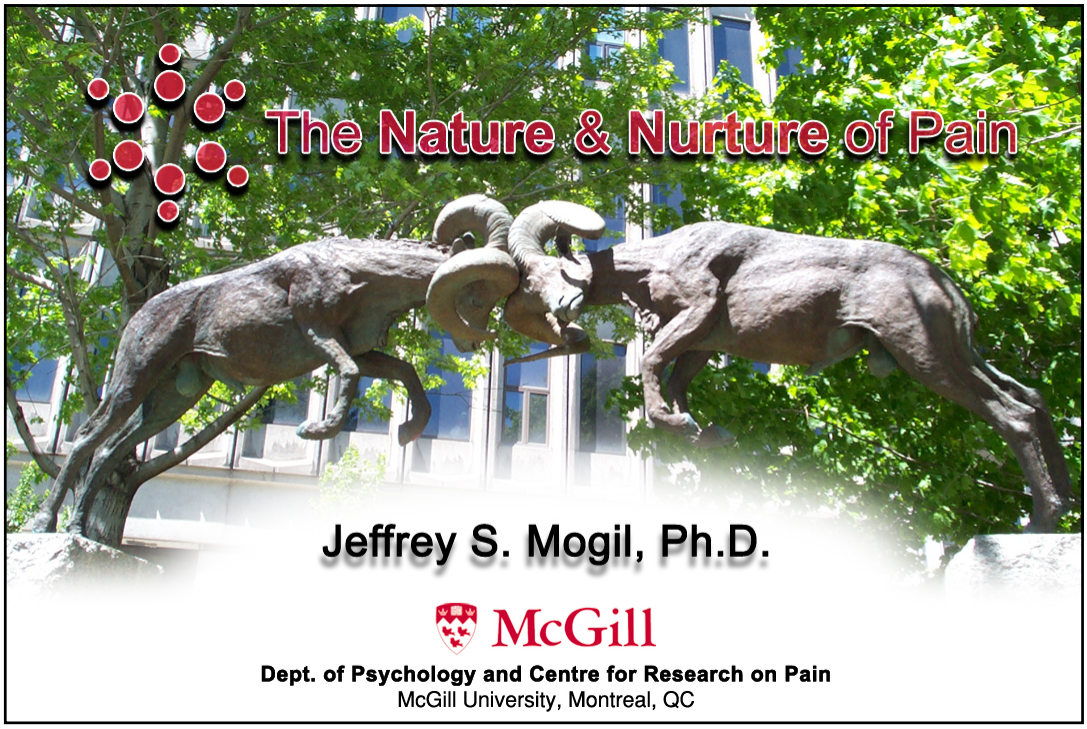

The Two "Genetics"

1. "Genetics" as the study of biology on the level of the gene.
   - -genetics à la Watson & Crick
   - -defining the molecular "building blocks" of pain
2. "Genetics" as the study of variability, of inherited individual differences.
   - -genetics à la Gregor Mendel

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Why Do Genetics?

1) identifying new targets for drug development
   -an *unbiased* target discovery approach
   -a way to do biology without drugs/antibodies

2) explaining individual differences

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Current Genetic Techniques

Technique	Molecule	Advantages	Disadvantages
In rodents:			
Transgenics	DNA	• simplicity	• interpretation difficult • single-gene focus
QTL Mapping	DNA	• causality • multigenic	• time-consuming • not guaranteed to work • blind to environment
Microarray	mRNA	• multigenic • genes or environment	• no causality • genes or environment?
Knockdown	mRNA	• simplicity	• hard to interpret null effects • single-gene focus
In humans:			
Linkage	DNA	• doable in humans!	• single-gene traits only
Association	DNA		• hard to replicate
Microarray	mRNA		• limited to available tissues
Deep Sequencing	DNA		• very expensive

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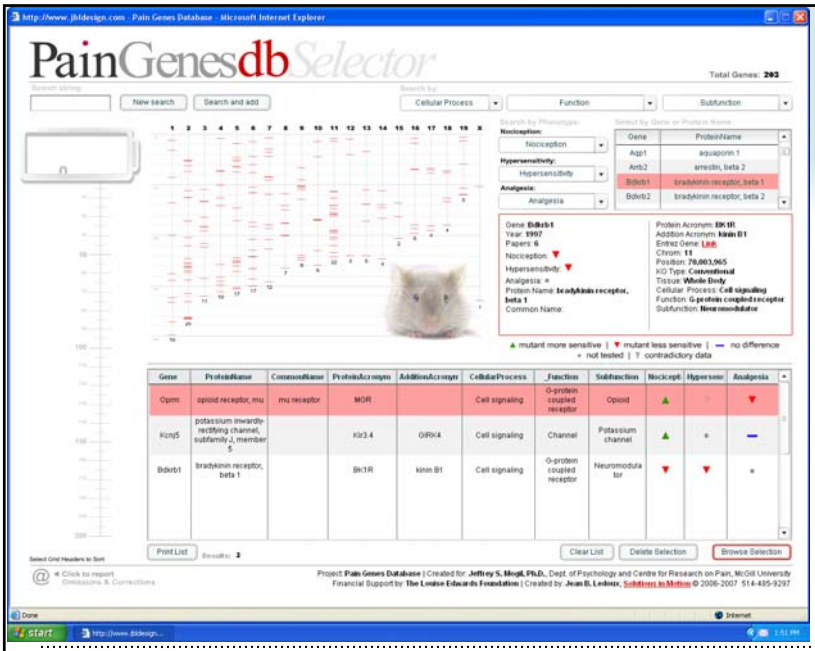

"Pain Genes" Knockout Mice with a Pain Phenotype

Total Genes: **322**
Total Papers: **755**

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"If it were not for the great variability among individuals medicine might as well be a science and not an art."

-Sir William Osler, 1892

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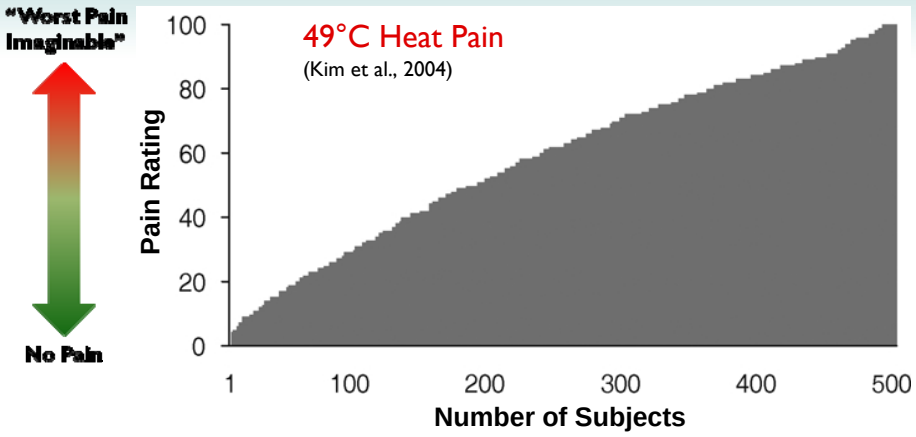
Variability in Human Pain Sensitivity: Experimental

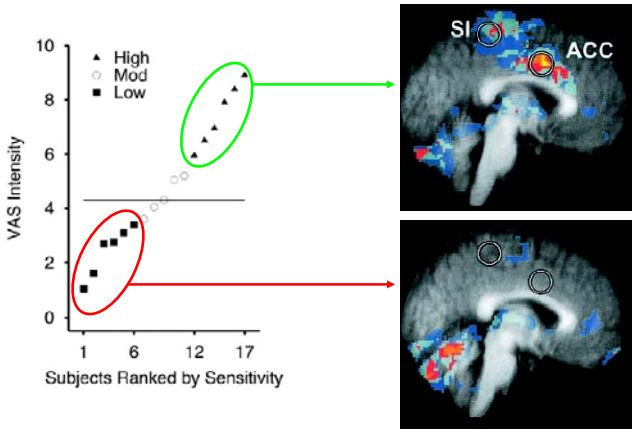


Is Variability in Human Pain Sensitivity *Real*?

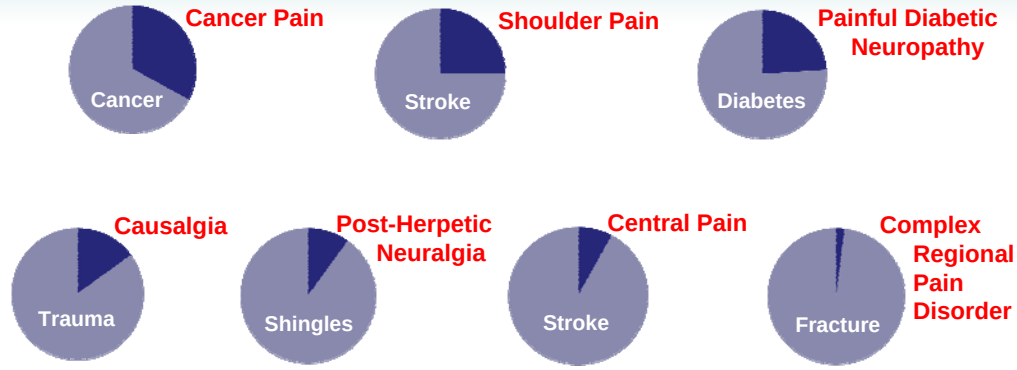
Neural correlates of interindividual differences in the subjective experience of pain

Robert C. Coghill*†‡, John G. McHaffie*, and Ye-Fen Yen§

Proc. Natl. Acad. Sci. USA, 100:8538, 2003



Variability in Human Pain Sensitivity: Clinical



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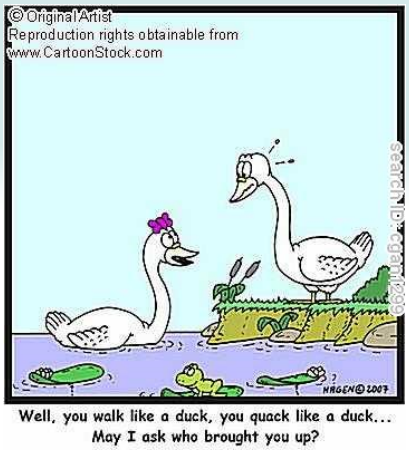

Trait Variability

GENETIC VARIABILITY ("nature") ("GxE interaction") **ENVIRONMENTAL VARIABILITY** ("nurture")

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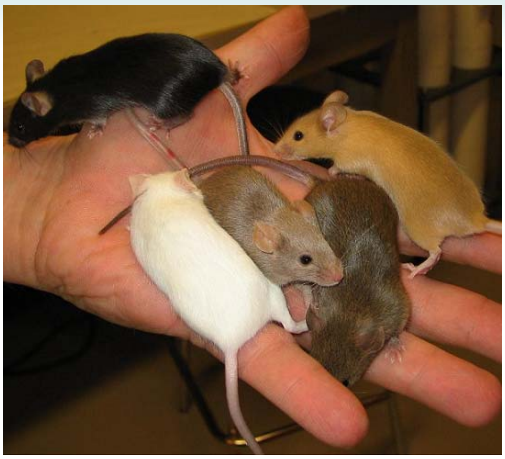
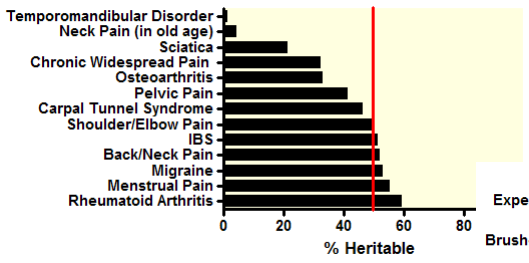
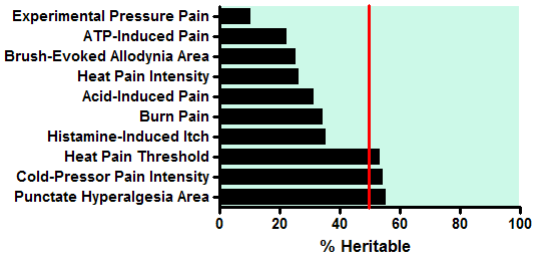
Estimating Heritability

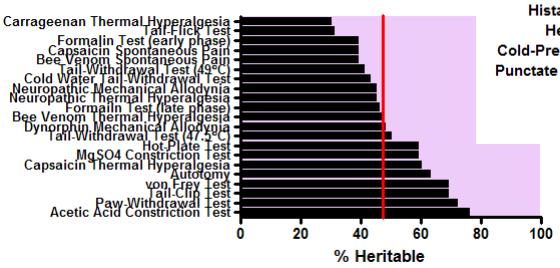
$V_{Trait} = V_{Genetic} + V_{Environmental}$



Heritability of Pain Traits

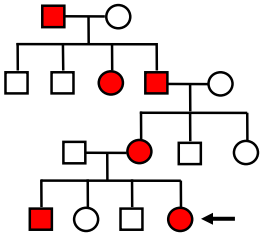




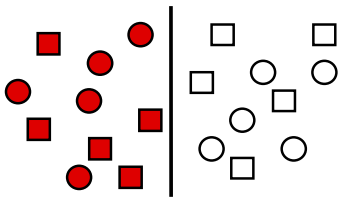


Finding "Pain Variability Genes"

Linkage Mapping



Association Studies



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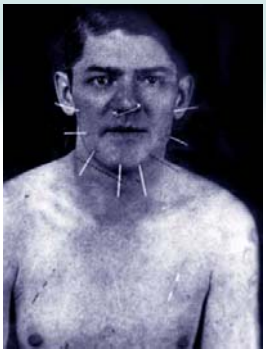
Genes Responsible for Monogenic Pain Disorders



"The Human Pincushion" (congenital insensitivity to pain with anhidrosis; HSN Type IV)

HSN Type I	9q22.1	*SPTLC1*	sphingolipid synthesis
HSN Type II	12p13	*HSN2*	(function unknown)
HSN Type III	9p31	*IKBKAP*	transcription factor
HSN Type IV	1q21	*NTRK1*	neurotrophin receptor
HSN Type V	1p13.1	*NGFB*	neurotrophin
"	2q24	*SCN9A*	sodium ($Na_v1.7$) channel
FEPS	8q12	*TRPA1*	cation (TRPA1) channel
PE	2q24	*SCN9A*	sodium ($Na_v1.7$) channel
PEPD	2q24	*SCN9A*	sodium ($Na_v1.7$) channel
FHM Type I	19p13	*CACNA1A1*	calcium channel subunit
FHM Type II	1q21	*ATP1A2*	ion pump subunit
FHM Type III	2q24	*SCN1A*	sodium ($Na_v1.1$) channel

FEPS: familial episodic pain syndrome; HSN: hereditary sensory neuropathy;
PE: primary erythromelalgia; PEPD: paroxysmal extreme pain disorder; FHM: familial hereditary migraine

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Genes Reported to be Associated with Pain States

ADRB2	temporomandibular disorder	Diatchenko, 2006
CACNG2	chronic postoperative pain	Nissenbaum, 2010
COMT	experimental pain	Zubieta, 2003; Diatchenko, 2005/6; Kim, 2006
	temporomandibular disorder	Diatchenko, 2005
	fibromyalgia	Gursoy, 2003
ESR1	TMJ osteoarthritis	Kang, 2007
FAAH	experimental pain	Kim, 2006
GCH1	low back pain	Tegeder, 2006
	experimental pain	Tegeder, 2006, Kim, 2007
HLA (many)	CRPS	Kemler, 1999; Mailis, 1994; van Hilten, 2000
	postherpetic neuralgia	Sato, 2002
HTR2A	fibromyalgia	Bondy, 1998
	irritable bowel syndrome	Pata, 2004
HTT	temporomandibular disorder	Herken, 2001; Cohen, 2002
IL1	low back pain	Solovieva, 2004
IL1RN	low back pain	Solovieva, 2004; Foster, 2004
	vulvar vestibulitis	Jeremias, 2000
IL6	sciatica	Noponen-Hietala, 2005
	rheumatoid arthritis	Oen, 2005
IL10	pelvic pain	Shoskes, 2002
KCNS1	experimental pain, lumbar root pain	Costigan , 2010
MAOB	postoperative pain	Sery, 2006
MC1R	experimental pain	Mogil, 2005
	vulvar vestibulitis	Foster, 2004
OPRD	experimental pain	Kim, 2004
OPRM	experimental pain	Fillingim, 2005
	chronic, non-cancer pain	Janicki, 2006
SCN9A	osteoarthritis, sciatica, postamputation pain	Reimann, 2010
SLC6A4	fibromyalgia	Offenbaecher, 1999
TRPA1	experimental pain	Kim, 2006
TRPV1	experimental pain	Kim, 2004

The Problem with Association Studies…

NATURE|Vol 447|7 June 2007 nature

Replicating genotype–phenotype associations

NCI-NHGRI Working Group on Replication in Association Studies

"…So far, comprehensive reviews of the published literature, most of which reports work based on the candidate-gene approach, have demonstrated a plethora of questionable genotype-phenotype associations, replication of which has often failed in independent studies."

Why Most Published Research Findings Are False

John P. A. Ioannidis

PLoS Medicine | www.plosmedicine.org 0696 August 2005 | Volume 2 | Issue 8 | e124

What About Human Pain Genetics?

COMT: -*val*158*met* associated with pain (Zubieta et al., 2003)
-a haplotype associated with pain, but *not* val158met (Diatchenko et al., 2005)
-no association with neuropathic pain (Armero et al., 2005)
-no association with post-operative pain (Kim et al., 2006)
-*val*158*met* associated with fibromyalgia (Cohen et al., 2009)
-no association with chronic pain (Hocking et al., 2010)
-a *different* haplotype associated with low back pain (Dai et al., 2010)

MC1R: -variants associated with *decreased* pain sensitivity (Mogil et al., 2005)
-variants associated with *increased* pain sensitivity (Liem et al., 2005)

GCH1: -a haplotype associated with pain (Tegeder et al., 2006)
-a haplotype associated with pain, but *only* after sensitization (Tegeder et al., 2008)
-no association with post-operative pain (Kim et al., 2007)
-a haplotype associated *only* with capsaicin pain (Campbell et al., 2009)
-a haplotype delays cancer pain (Campbell et al., 2009)

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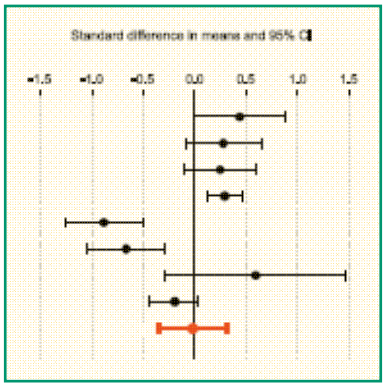
A Meta-Analysis of *OPRM1* and Pain

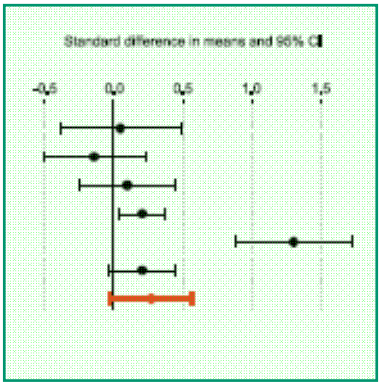
Meta-analysis of the relevance of the *OPRM1* 118A>G genetic variant for pain treatment

Carmen Walter, Jörn Lötsch *

Opioid Requirements (G vs. AA)



Pain Scores (G vs. AA)



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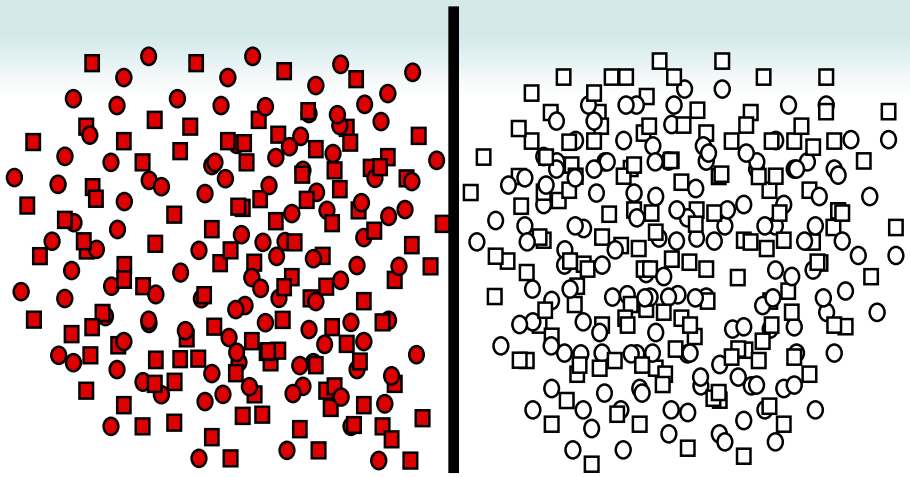
Genome-Wide Association Studies (GWAS)



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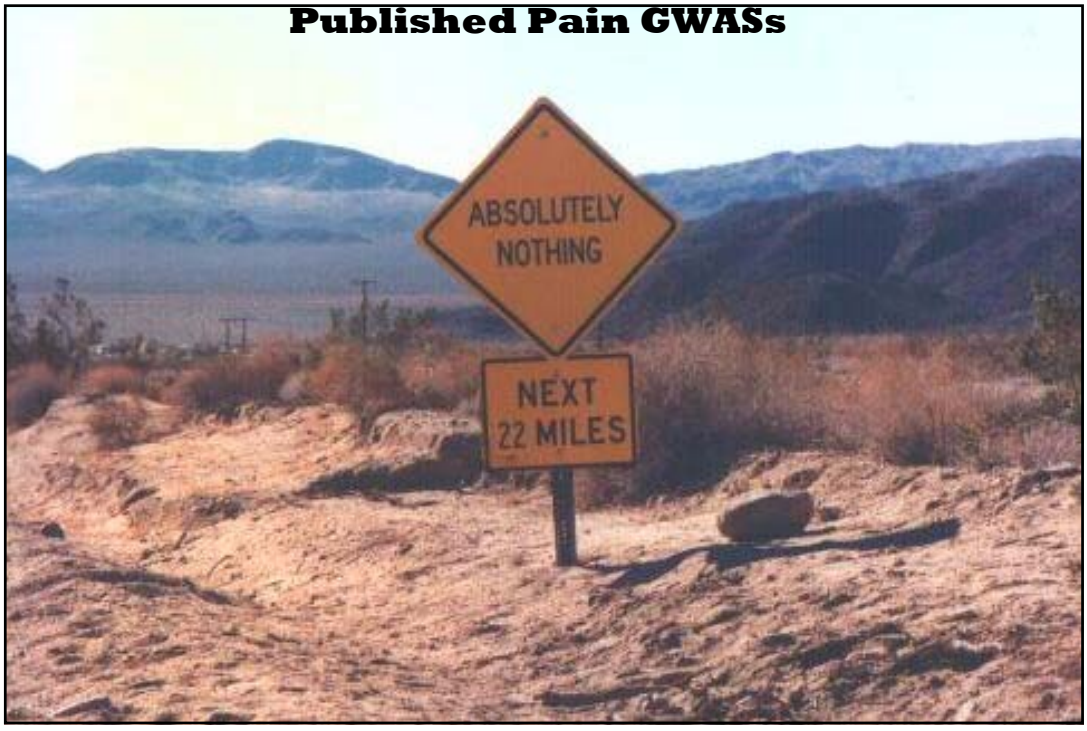
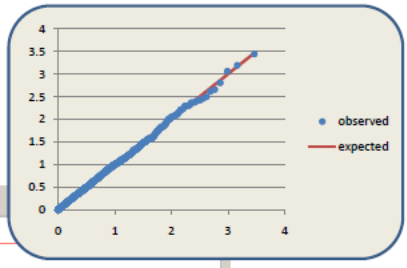
Published Pain GWASs

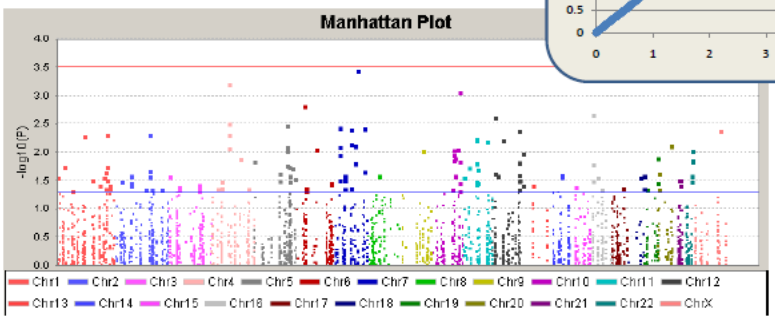


The Closest Approximation in Pain: The OPPERA Study (W. Maixner, PI)

177 TMD cases vs 1443 controls

CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	OR	P
7	rs728273	IFRD1	99.57%	0.41	0.69	0.64	0.00036
4	rs1563826	EREG	100.00%	0.21	0.51	0.60	0.00063
10	rs12415832	GRK5	99.88%	0.021	0.051	2.54	0.00086





Do GWASs "Work"?

YES

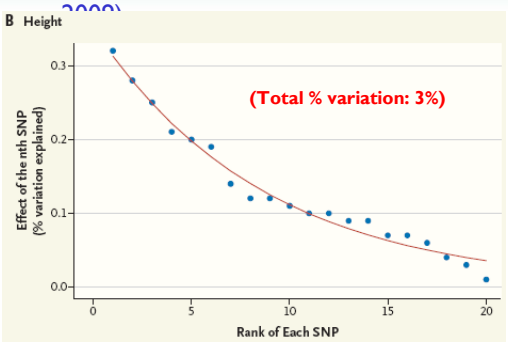
J.N. Hirschhorn (*N. Engl.J. Med.*, 2009)

…in 2005, two friends and well-known geneticists, Francis Collins and Thomas Gelehrter, made a public bet: Gelehrter predicted that no more than three new common variants would be reproducibly associated with common diseases by the time the American Society of Human Genetics (ASHG) held its meeting in the autumn of 2008. During the past 2 years, however, genomewide association studies have identified more than 250 genetic loci in which common genetic variants occur that are reproducibly associated with polygenic traits... Collins was the clear winner, by a margin of more than 200 new associated variants.

"The main goal of these studies is not prediction of individual risk but rather discovery of biological pathways underlying polygenic diseases and traits."

NO

D.B. Goldstein (*N. Engl.J. Med.*, 2009)



"If common variants are responsible…most genes are "height genes" or "type 2 diabetes genes"…in pointing at everything, genetics would point at nothing."

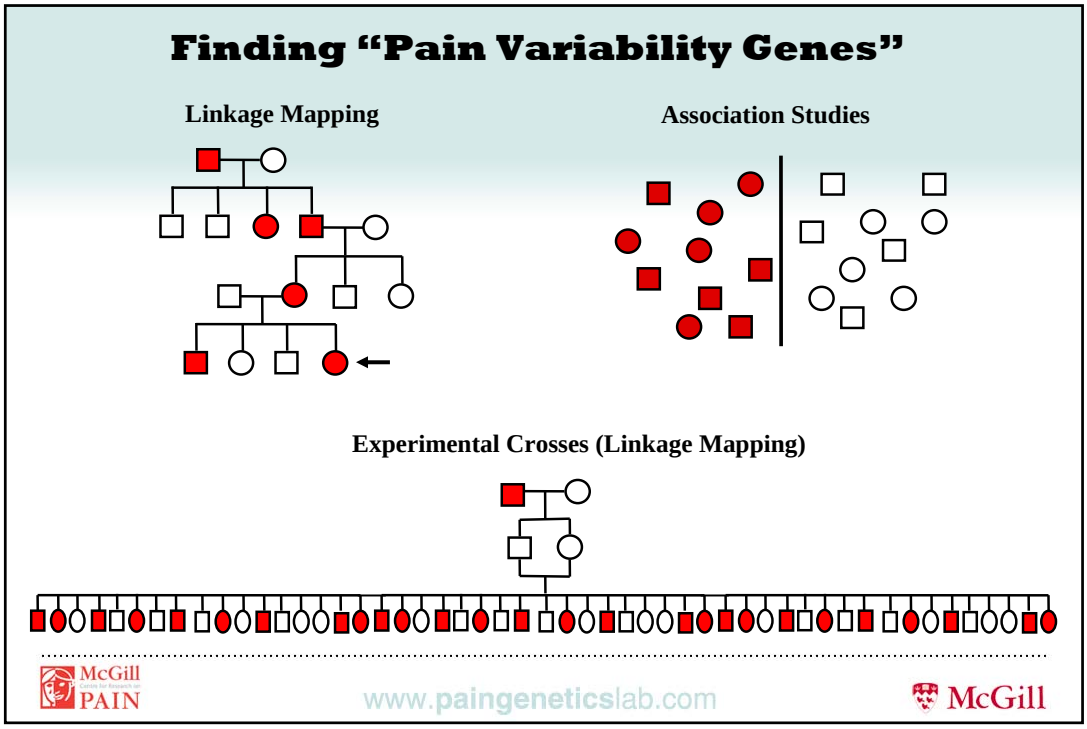
But wait…

- what about *APOE*?
- what about *BRCA1* and *BRCA2*?
- what about *CFH* and macular degeneration?
- what about hearing loss genes?

Q. Is chronic pain more like type-2 diabetes, or more like macular degeneration?

The Future: Genome-Wide Resequencing

- if the common disease-common variant hypothesis is wrong, and the common disease-rare variant hypothesis is correct, then the solution is to find the rare variants
- this can be done by sequencing many people with the disease, at great (but decreasing; the "$1000 genome") cost
- the truth is…pain is not "important" enough to spend the money (!)


Finding "Pain Variability Genes"
Linkage Mapping
Association Studies
Experimental Crosses (Linkage Mapping)
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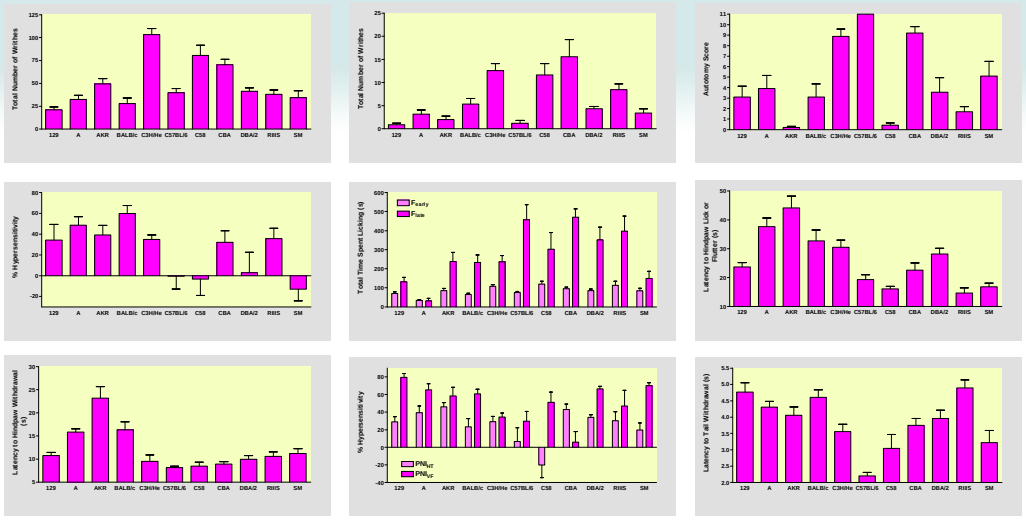
129P3
A
AKR
BALB/c
C3H/He
C57BL/6
C57BL/10
C58
CBA
DBA/2
RIIIS
SM

Strain Differences in Pain Sensitivity



Mogil et al., *Pain*, 1999

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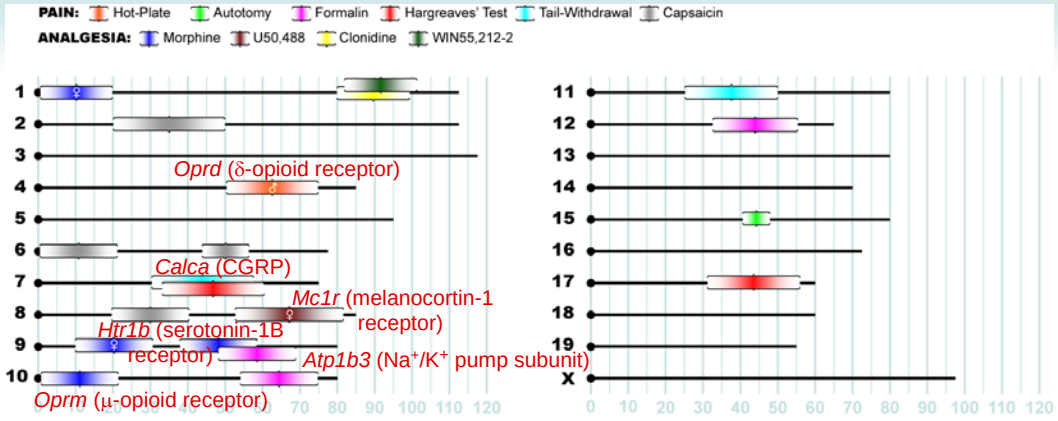
Published Pain-Relevant Genomic Linkages (Quantitative Trait Loci)



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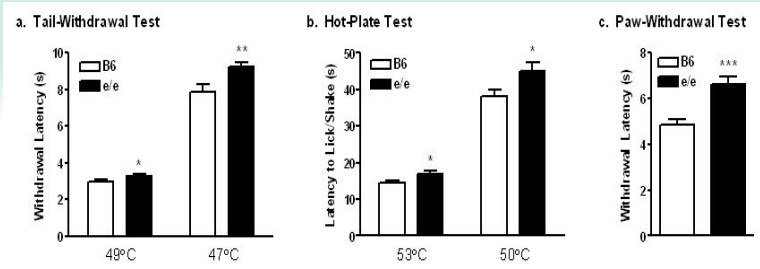
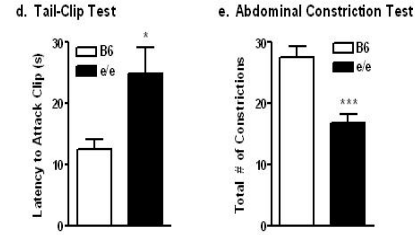
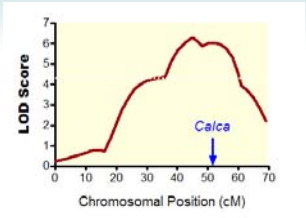
MC1R (melanocortin-1 receptor) and Pain: Mice and Humans

2+ Variants 0/1 Variants

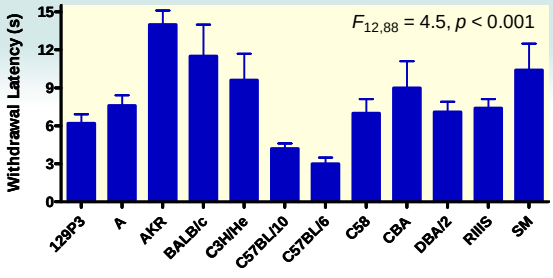




Calca (CGRP) and Thermal Pain: Mice and Humans



Mogil et al., *PNAS*, 2006



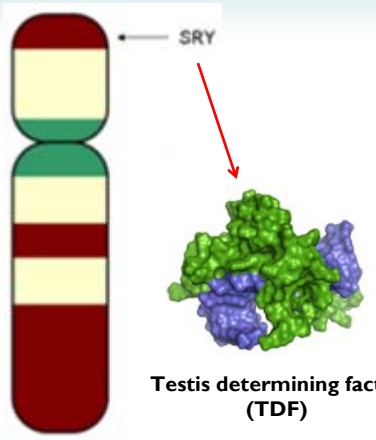
1246 cases and controls with RC4 (thermal sensitivity) factor score

OPPERA Study (in preparation)

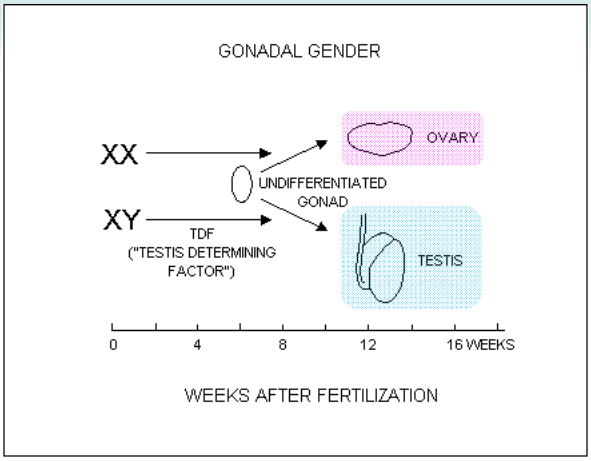
CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	BETA	P
11	rs1553005	CALCA	99.81%	0.31	0.34	0.13	0.00055
4	rs1024323	GRK4	100.00%	0.38	0.59	-0.12	0.00074
3	rs2236953	CACNA2D2	99.32%	0.13	0.03	-0.18	0.00096

One Other Gene We *Know* is Involved in Pain

Chromosome Y



Testis determining factor (TDF)



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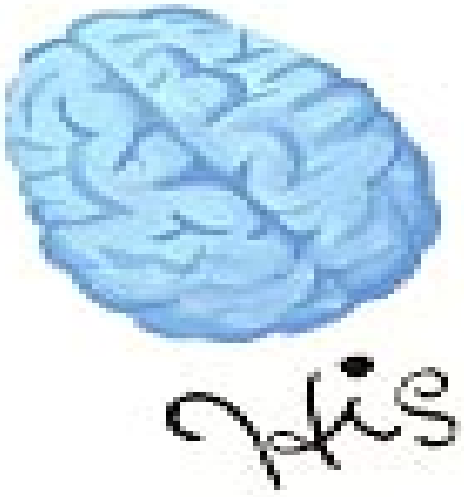
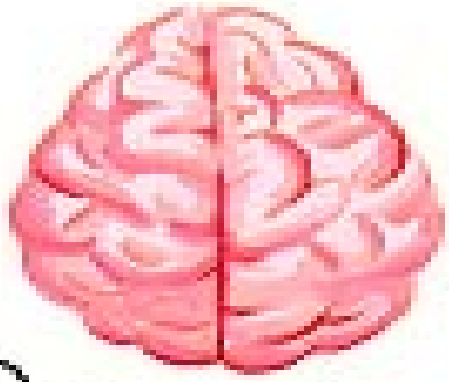
Qualitative Sex Differences in Pain Mechanisms

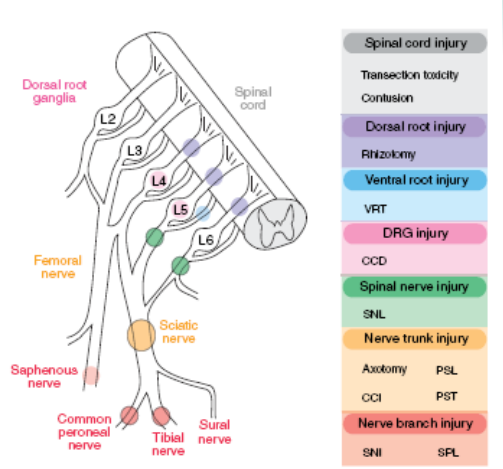


His



Hers

Strain Survey (31 Strains!) of Neuropathic Mechanical Allodynia



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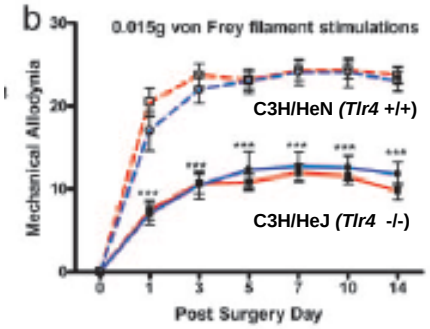
Sex-Specific Effect of *Tlr4* (Toll-like receptor 4) Genetic Dysfunction on Mechanical Allodynia

PNAS

The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy

Flobert Y. Tanga*†‡, Nancy Nutile-McMenemy*‡, and Joyce A. DeLeo*†‡§



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Male-Specific and Testosterone-Dependent Involvement of *Spinal* TLR4 in Chronic Pain

Conclusions

• variability in pain sensitivity, susceptibility to developing chronic pain disorders, and analgesic response is robust in humans and rodents

• slowly but surely, pain-relevant genes are being identified and confirmed in mice and humans

• the large total number of pain-relevant genes suggests, however, that the process will take quite some time, and that incorporation of genetic data into pain treatment is premature

• the genetic technique nonetheless has considerable heuristic value, especially as a guide to and refinement of drug development efforts (e.g., TLR4)

Thanks to…

At Illinois:
Dr. Sonya (Wilson) Lehto
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Dr. Andrew Rankin
Dr. William Lariviere
Dr. Karine Bon
Dr. Wan You

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(University of Florida)
Dr. William Maixner
(University of North Carolina)
Dr. Albert Dahan
(Leiden University)
Dr. Kenneth Craig
(University of British Columbia)
Dr. Wendy Sternberg
(Haverford College)
Dr. Ben Kest
(CUNY/CSI)

At McGill:
Dr. Shad Smith
Jennifer Ritchie
Susana Sotocinal
Dr. Kumar Nemmani
Jean-Sebastien Austin
Dr. Mona Lisa Chanda
Dr. Dale J. Langford
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