Unraveling Persistent Pain Conditions With Genetic Biomarkers

FDA Workshop: Assessment of Analgesic Treatment of Chronic Pain

May 30, 2012

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Center for Neurosensory Disorders
University of North Carolina

Disclosure: Algynomics Inc. Cofounder and equity shareholder

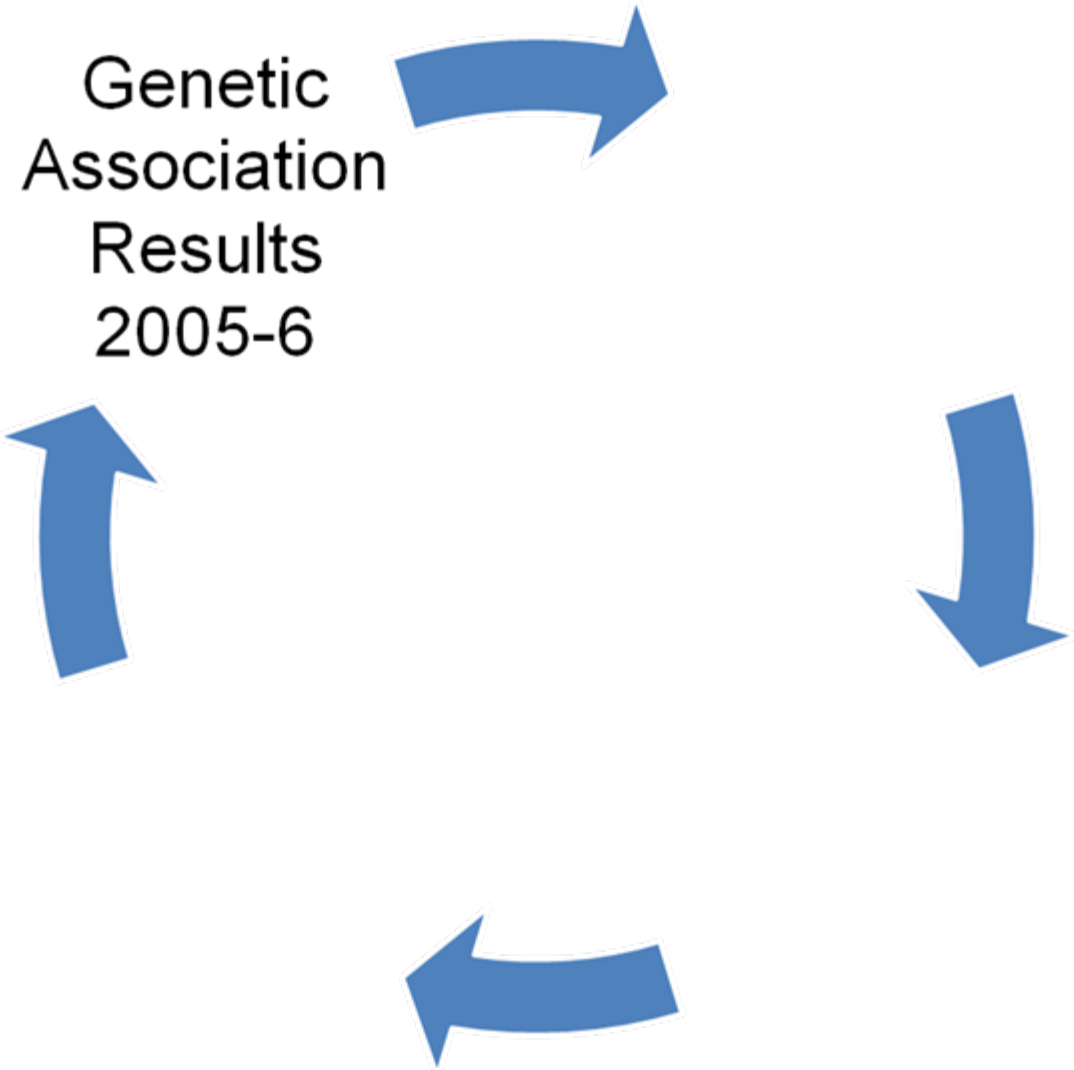
Why Do We Care About Human Pain Biomarkers?

- Diagnostic and prognostic markers enabling individualized decisions regarding efficacy and risk associated with:
  - Pharmacotherapies
  - Behavioral therapies
  - Invasive procedures
- Discovery of biological pathways underlying traits and diseases
- Drug discovery – target identification

Functional Class	Gene	Condition
Transporters	*SLC6A3, or DAT1*	PTSD
	SLC6A4, or 5-HTT, or SERT	FMS, CFS, D-IBS, migraine, TMJD
	ABCB1, or MDR1	**Efficacy of μ-opioid analgesia**
Metabolic genes, enzymes, and transcription regulators	***COMT***	**TMJD , opioid analgesia, migraine, FMS**
	CYP2D6	**Opioid analgesia**
	GCH1	LBP
	MTHFR	MA
	ACE	MA
	SPTLC1, or SPT1	HSAN I
	HSN2	HSAN II
	IKBKAP, or IKAP	HSAN III
	NGFB	HSAN V
Receptors	*NTRK1, or TRKA*	HSAN IV
	ADRA2A, ADRA2C	C-IBS
	ADRB2	TMJD
	DRD2	MA, PTSD
	DRD4	MO, FMS
	MC1R	**Opioid analgesia**
	OPRM1, or MOR1 and MOR1K	**μ-opioid analgesia**
	5-HTR2A	MA,TMJD
Cytokines	*IL1A, IL1B, and IL1RN*	LBP
	IL1RN	VVS
	IL1B	VVS, BMS
	IL6	LBP
	IL10	IBS
	TNF	IBS
	LTA	MO
Ion channels	*ATP1A2*	BM, FHM type 2
	KCNS1	LBP, Neuropathic
	CACNA1A	MA, FHM type 1
	CACNA2D3	LBP
	SCN1A	FHM type 3
	SCN9A IL6, or IFNB2	PE, PEPD, CAIP, CIDP

Diatchenko et al.. Trends in Genetics 23:605-13, 2007 and Kim et al. Journal of Pain 10:663-693, 2009

The Translational Research Clock



Diatchenko

Human Molecular Genetics, 2005, Vol. 14, No. 1 **135–143**
doi:10.1093/hmg/ddi013
Advance Access published on November 10, 2004

Genetic basis for individual variations in pain perception and the development of a chronic pain condition

Luda Diatchenko[1,8,*], Gary D. Slade[2], Andrea G. Nackley[1], Konakporn Bhalang[3], Asgeir Sigurdsson[1], Inna Belfer[4,7], David Goldman[4], Ke Xu[4], Svetlana A. Shabalina[5], Dmitry Shagin[6], Mitchell B. Max[7], Sergei S. Makarov[8] and William Maixner[1]

American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 141B:449–462 (2006)

Three Major Haplotypes of the β_2 Adrenergic Receptor Define Psychological Profile, Blood Pressure, and the Risk for Development of a Common Musculoskeletal Pain Disorder

Luda Diatchenko,[1*] Amy D. Anderson,[2] Gary D. Slade,[3] Roger B. Fillingim,[4] Svetlana A. Shabalina,[5] Tomas J. Higgins,[1] Swetha Sama,[1] Inna Belfer,[6,7] David Goldman,[6] Mitchell B. Max,[7] Bruce S. Weir,[2] and William Maixner[1]

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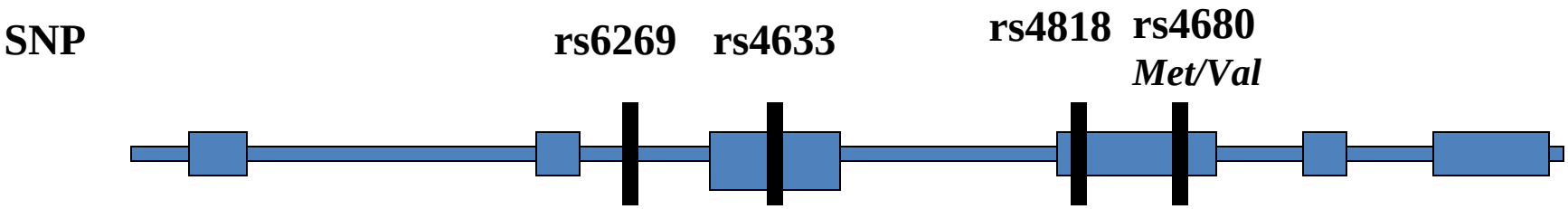
[2]*University of Washington, Department of Biostatistics, Seattle*
[3]*University of Adelaide, Australian Research Centre for Population Oral Health, Adelaide*
[4]*University of Florida College of Dentistry, Gainesville*
[5]*Computational Biology Branch, NCBI, NIH, Bethesda, Maryland*
[6]*Laboratory of Neurogenetics, NIAAA, NIH, Rockville, Maryland*
[7]*Pain & Neurosensory Mechanisms Branch, NIDCR, NIH, Bethesda, Maryland*

Confidential

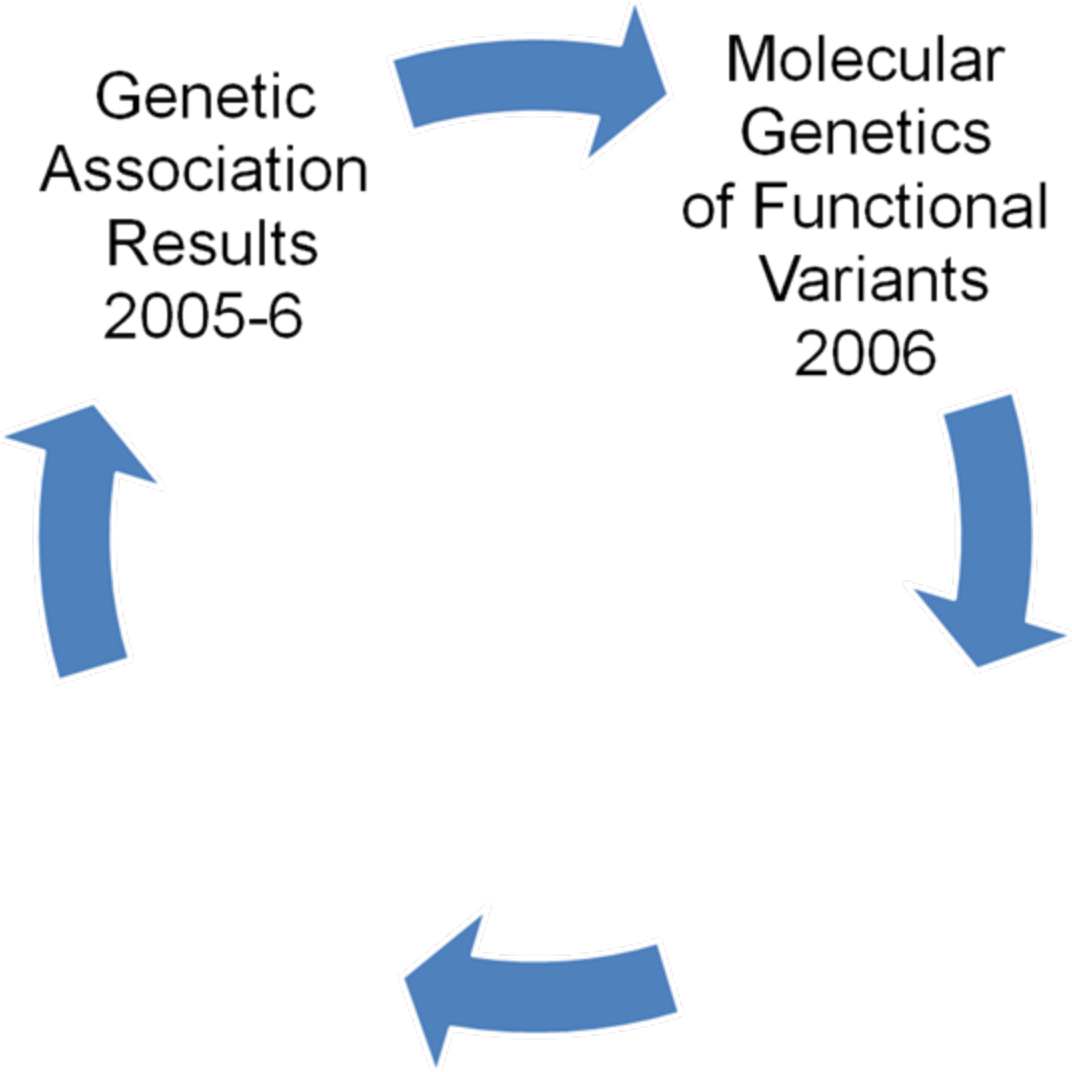
Three Primary *COMT* Haplotypes



Haplotype	Sequence	Frequency, %
Low Pain Sensitive (LPS)	G ----- C ---------- G -- G_{val}	37.5
Average Pain Sensitive (APS)	A ----- T ---------- C -- A_{met}	47.3
High Pain Sensitive (HPS)	A ----- C ---------- C -- G_{val}	10.3
Other		4.9

Diatchenko et al, Hum.Mol.Gent, 2005

The Translational Research Clock



Diatchenko

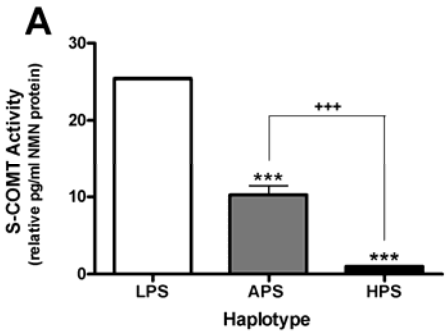
Science Vol 314:1930-1932, 2006

Human Catechol-*O*-Methyltransferase Haplotypes Modulate Protein Expression by Altering mRNA Secondary Structure

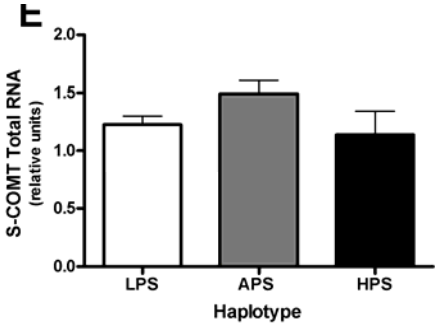
A. G. Nackley,[1] S. A. Shabalina,[2] I. E. Tchivileva,[1] K. Satterfield,[1] O. Korchynskyi,[3]
S. S. Makarov,[4] W. Maixner,[1] L. Diatchenko[1*]

rom www.sciencemag.org c

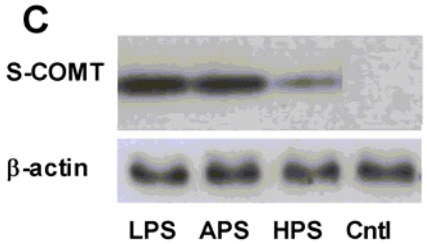
COMT Haplotype Dependent Functional Activity



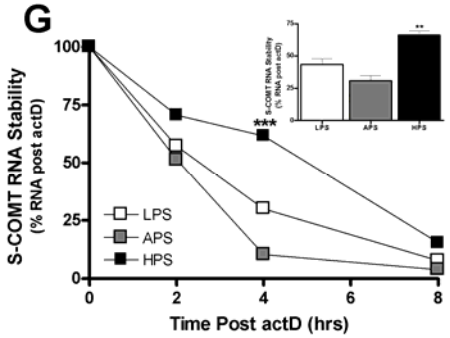
Enzymatic Activity



RNA Levels



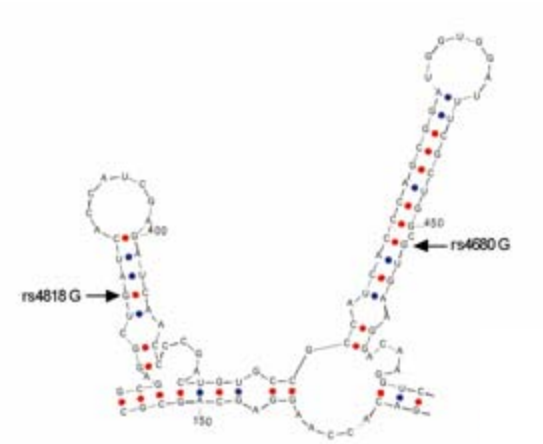
Protein levels



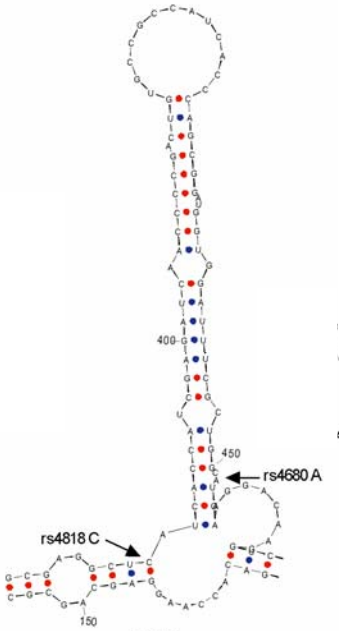
RNA stability

Nackley et al. Science 2006

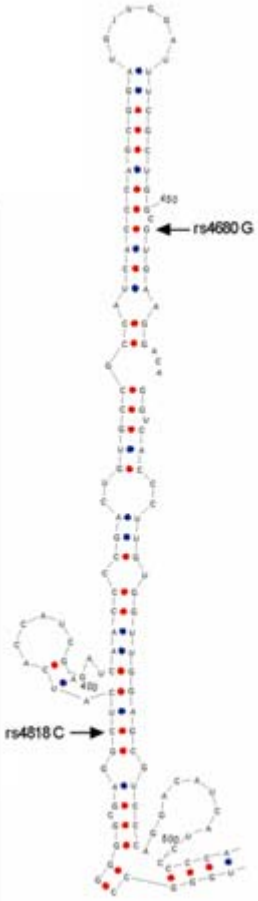
COMT Haplotype-Specific mRNAs



LPS
ΔG = -20.0 kcal/mol

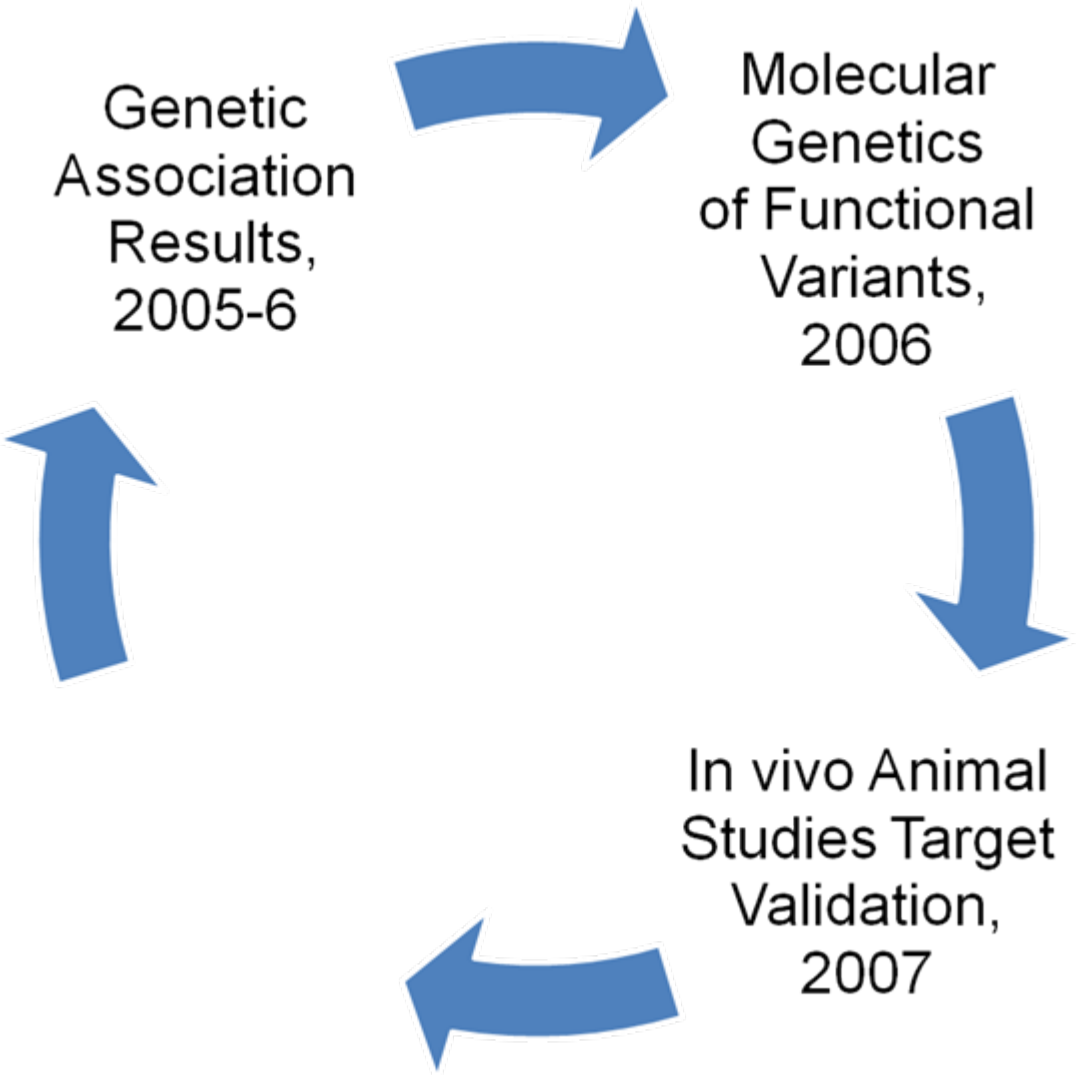


APS
Δ G = -28.9 kcal/mol



HPS
Δ G = -36.7 kcal/mol

The Translational Research Clock



Diatchenko

Pain 128 (2007) 199–208

PAIN

www.elsevier.com/locate/pain

Research papers

Catechol-*O*-methyltransferase inhibition increases pain sensitivity through activation of both β_2- and β_3-adrenergic receptors

Andrea Gail Nackley [a,*], Kai Soo Tan [b], Karamarie Fecho [c], Patrick Flood [b], Luda Diatchenko [a], William Maixner [a]

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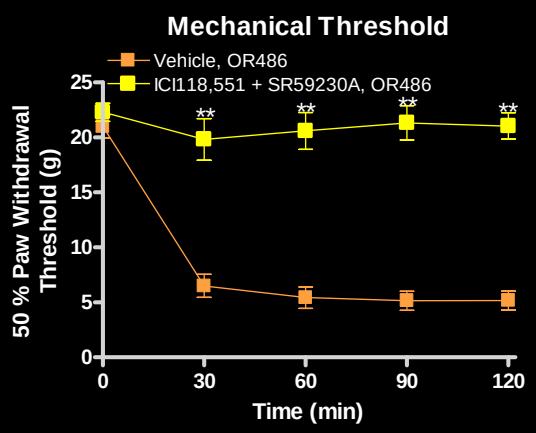
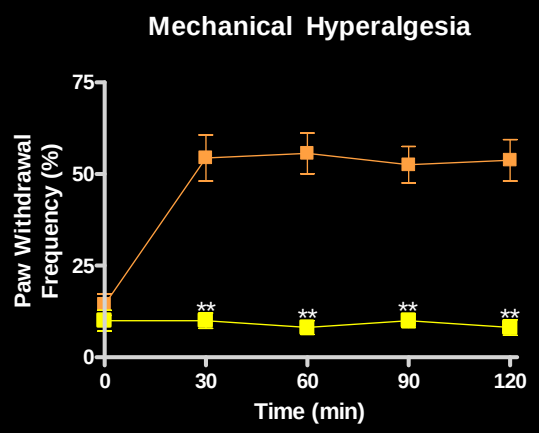
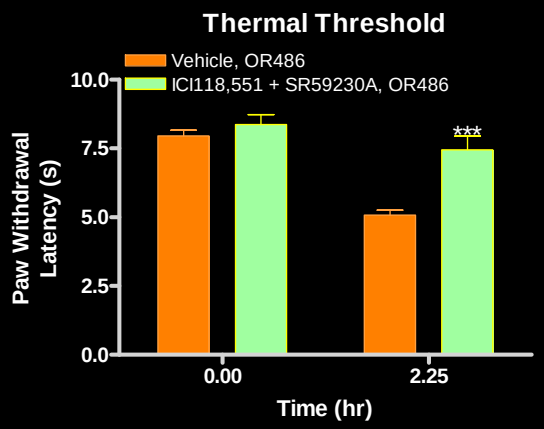
[b] *Comprehensive Center for Inflammatory Disorders, School of Dentistry, University of North Carolina, Chapel Hill, NC 27599-7455, USA*
[c] *Division of Pain Medicine, Department of Anesthesiology, School of Medicine, University of North Carolina, Chapel Hill, NC 27599-7010, USA*

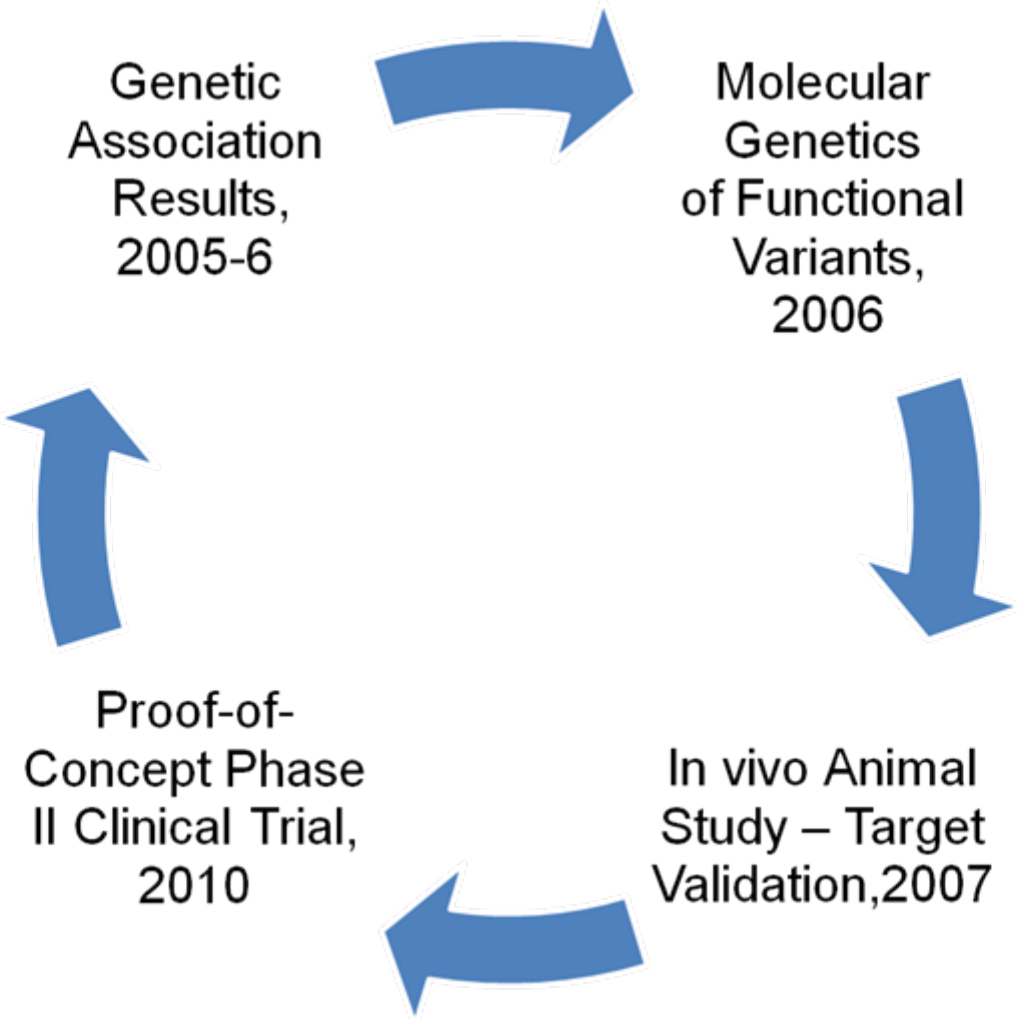
Allodynia/Hyperalgesia Induced by COMT Inhibition is Blocked by β_2+β_3 Adrenergic Receptor Antagonists







The Translational Research Clock – Closing the Circle



Diatchenko

Pharmacogenetics and Genomics 20:239–248, 2010

Effect of catechol-*O*-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study

Inna E. Tchivileva, Pei Feng Lim, Shad B. Smith, Gary D. Slade, Luda Diatchenko, Samuel A. McLean and William Maixner

Pharmacogenetics and Genomics 20:239–248, 2010

Effect of catechol-*O*-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study

Inna E. Tchivileva, Pei Feng Lim, Shad B. Smith, Gary D. Slade, Luda Diatchenko, Samuel A. McLean and William Maixner

Pilot Case Crossover Trial of Propranolol in TMD Patients

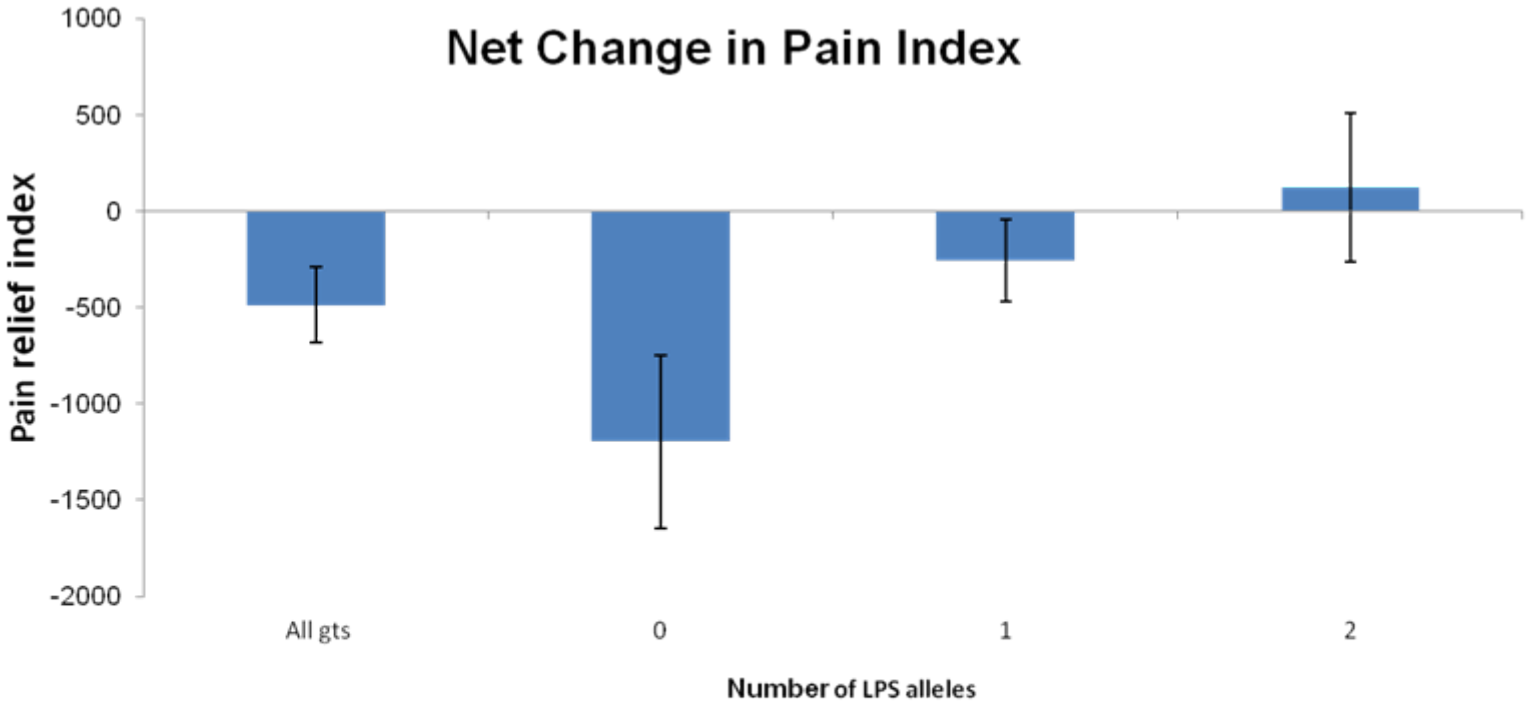
- 40 patients with chronic TMJD
- Two period, double-blind, placebo-controlled, randomized case crossover study
- Propranolol (20mg BID) vs. placebo for 7 days

Reported Improvement in Pain

Measure	Placebo	Propranolol	P value	X^2 Square
Pain During Past Week	13 (33%)	27 (67%)	0.01	8.5

Tchivileva et al. Pharmacogenetics and Genomics, 20: 239-248, 2010

Blocking βARs with Propranolol Produces Analgesia in TMD Patients



Tchivileva et al. Pharmacogenetics and Genomics, 20: 239-248, 2010

Human Molecular Genetics, 2009, Vol. 18, No. 6 **1037–1051**
doi:10.1093/hmg/ddn439
Advance Access published on December 22, 2008

Expansion of the human μ-opioid receptor gene architecture: novel functional variants

Svetlana A. Shabalina[1,*], Dmitri V. Zaykin[2], Pavel Gris[3], Aleksey Y. Ogurtsov[1], Josee Gauthier[3], Kyoko Shibata[2], Inna E. Tchivileva[3], Inna Belfer[4,5,6], Bikashkumar Mishra[4,5], Carly Kiselycznyk[4,5], Margaret R. Wallace[7], Roland Staud[8], Nikolay A. Spiridonov[9], Mitchell B. Max[4,6,†], David Goldman[5], Roger B. Fillingim[8], William Maixner[3] and Luda Diatchenko[3,*]

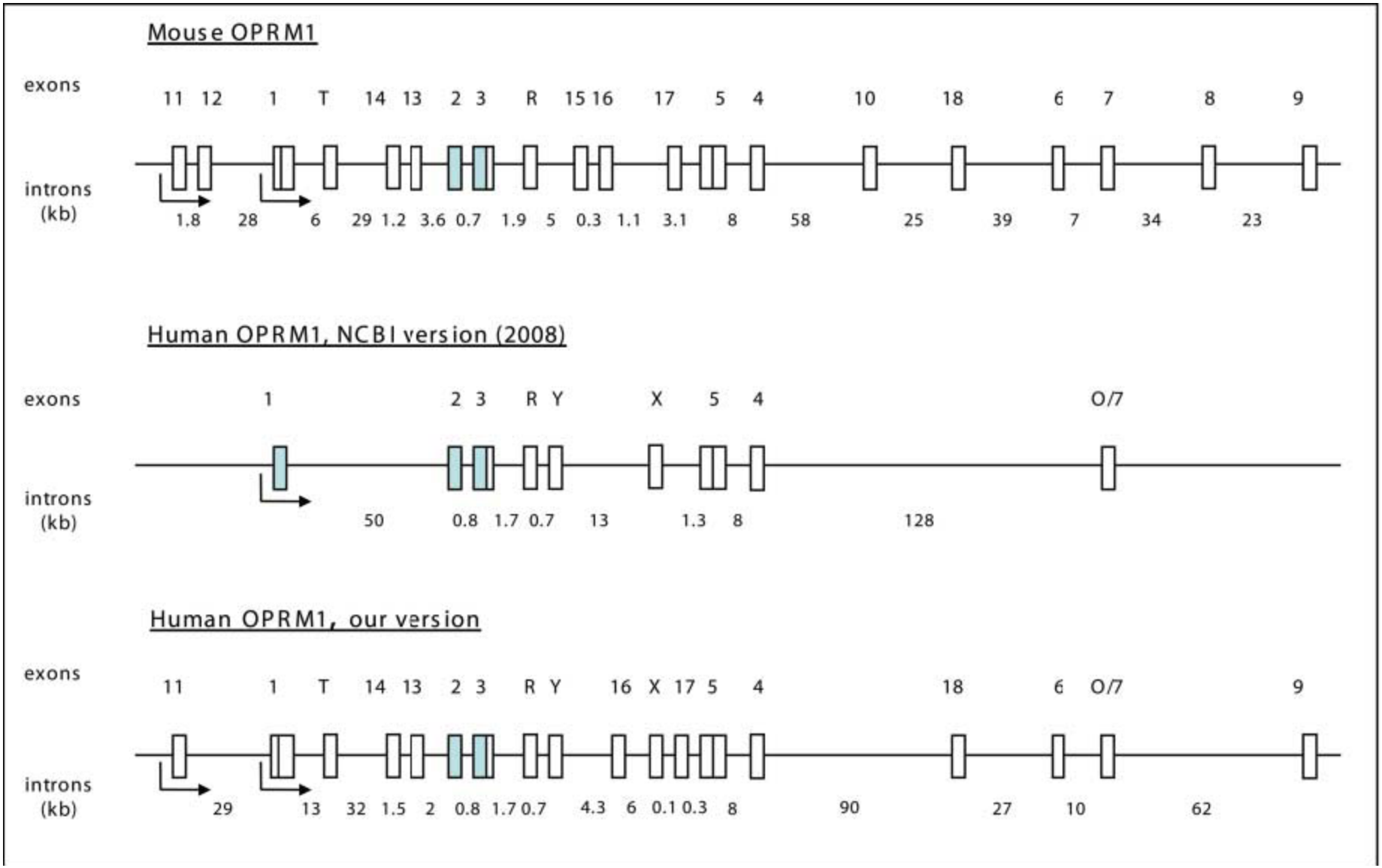
Morphine-Induced Changes in Pain Ratings as a Function of *OPRM1* rs563649 Genotype

	CC (n=75)	TT or CT (n=16)	F-Value
Pain Z-score	-0.54 (6.1)	1.67 (5.7)	P<0.05
Pain Intensity Change Score*	36.6(51.4)	8.0 (39.8)	F(1,89)=4.37, p=.039
Pain Unpleasantness Change Score*	36.6 (51.8)	5.8 (35.6)	F(1,89)=5.13, p=.026

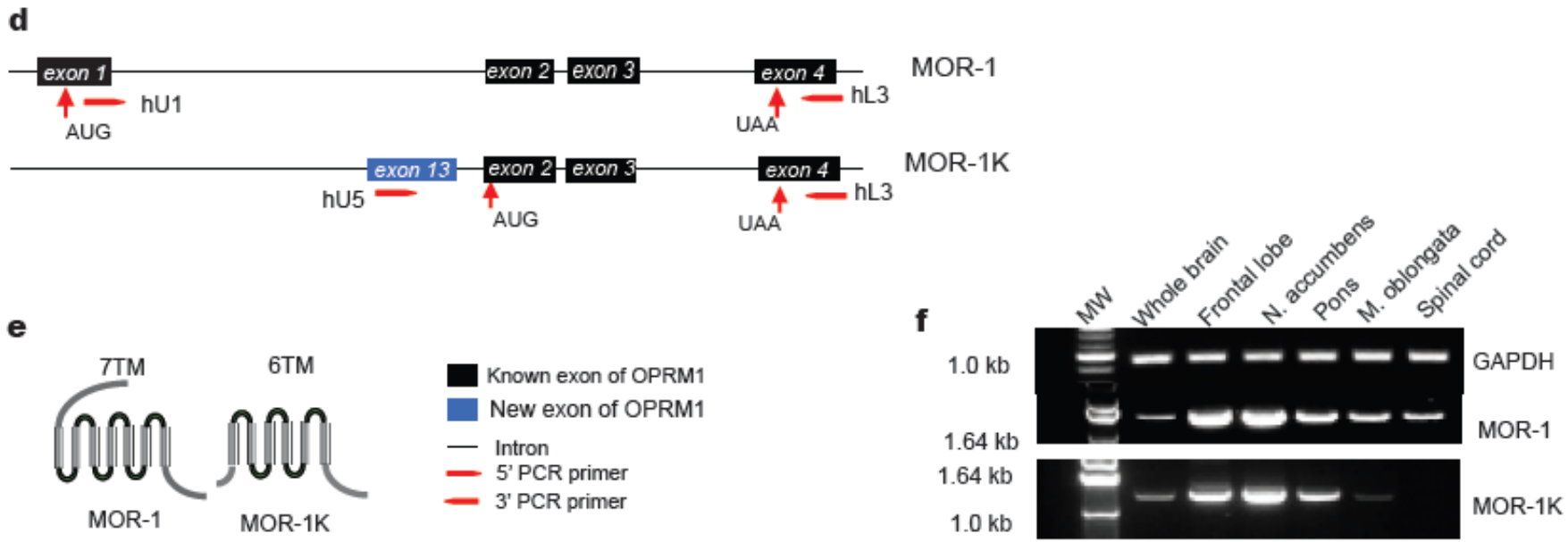
*after administration of .08 mg/kg of intravenous morphine

Shabalina et al, *Human Mol Genet*, 2009

The Structure of Human and Mouse OPRM1 gene.



Exonic Composition and Expression Patterns of *MOR-1* and *MOR-1K* Isoforms

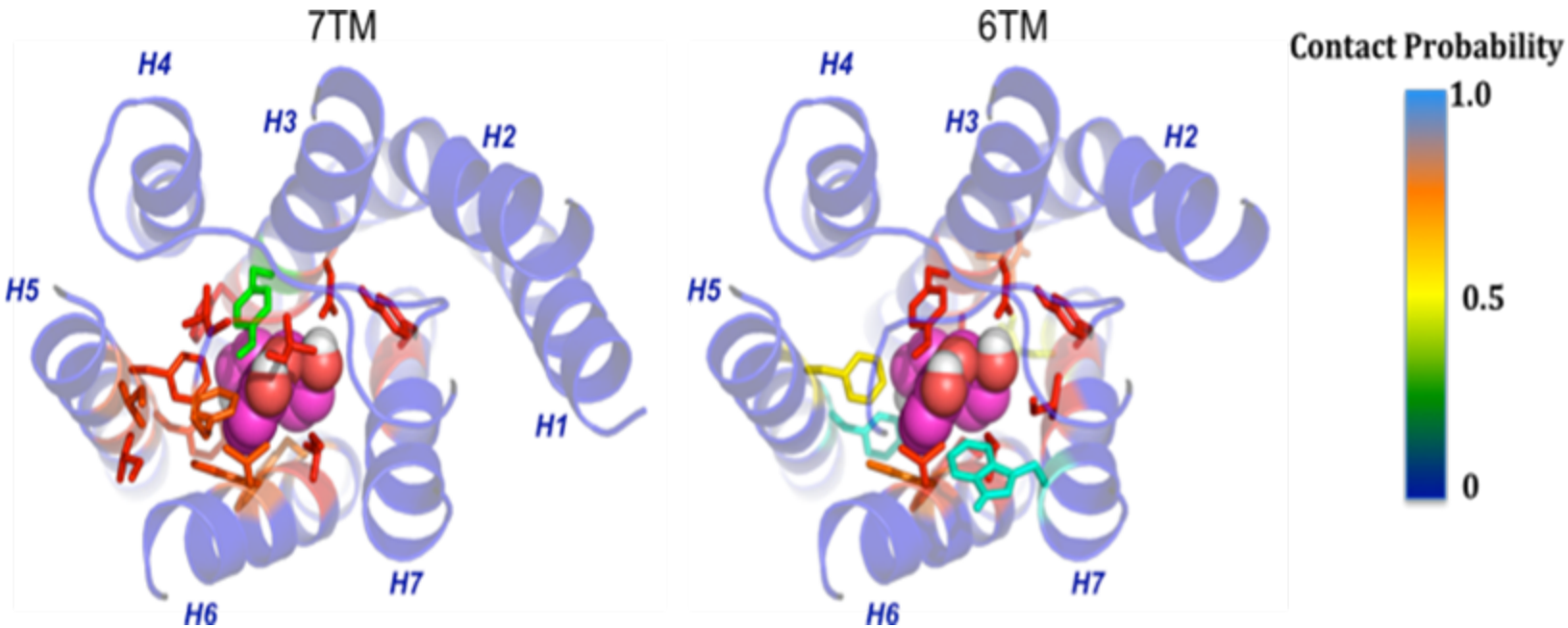


The alternative spliced variant of human OPRM1 containing exon 13:

- It is expressed in many CNS regions.
- It is not expressed by peripheral leukocytes .
- The human version of OPRM1 has a larger exon 13 that codes for a splice variant that is larger than the mouse orthologue.

Gris P, Gauthier J, Cheng P, Gibson DG, Gris D, Laur O, Pierson J, Wentworth S, Nackley AG, Maixner W, Diatchenko L. Mol Pain. 2010 Jun 2;6:33.

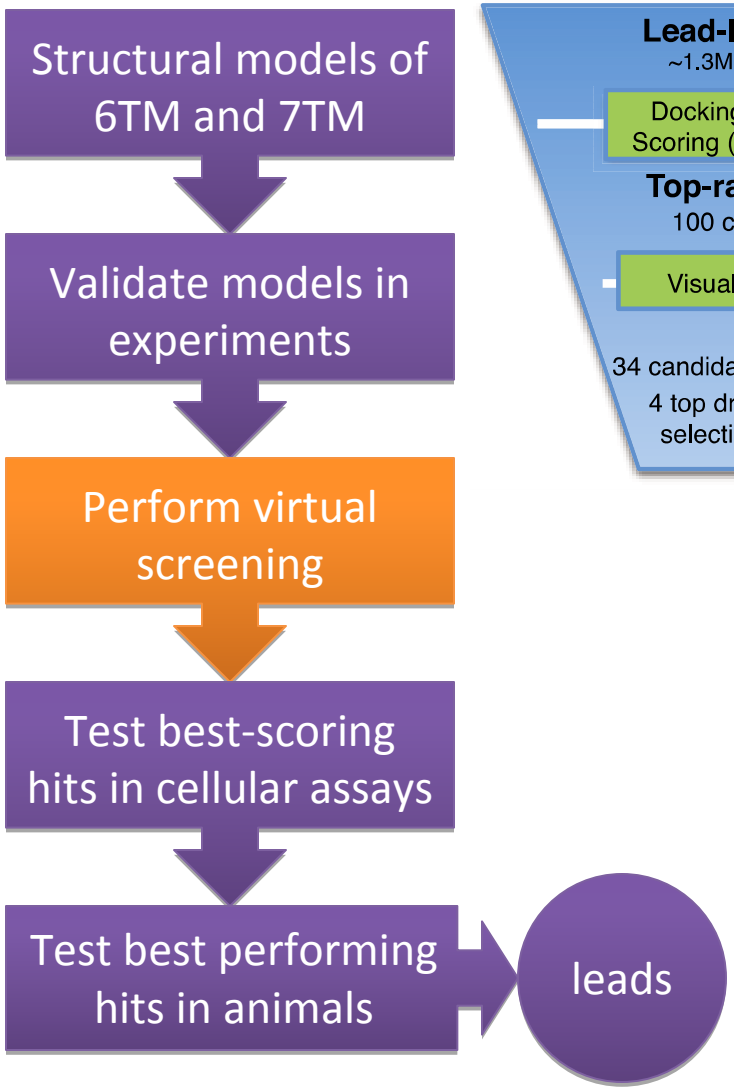
Binding Pockets of the 7TM and 6TM Isoforms

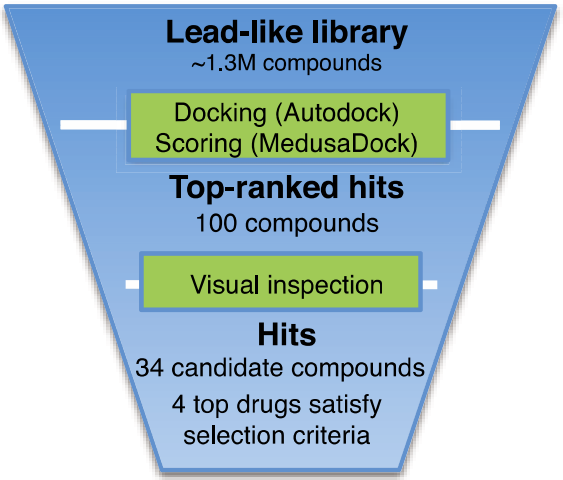


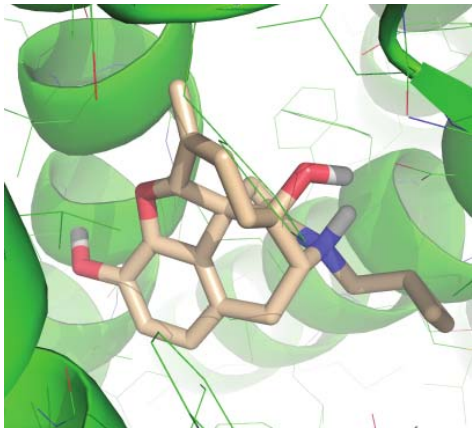
*Contact probability of 1 indicates that the specific residue is always within 4.5 Å of morphine, while 0 indicates that the residue is always beyond 4.5 Å of morphine.

Serohijos AW, Yin S, Ding F, Gauthier J, Gibson DG, Maixner W, Dokholyan NV, Diatchenko L. Structure. 2011 Nov 9;19(11):1683-90.

6TM – Opioid Program: Activities







Serohijos et al., *Structure*, 19: 1683-1690 (2011)

Truncated G protein-coupled mu opioid receptor MOR-1 splice variants are targets for highly potent opioid analgesics lacking side effects

Susruta Majumdar[a], Steven Grinnell[a], Valerie Le Rouzic[a], Maxim Burgman[a], Lisa Polikar[a], Michael Ansonoff[b], John Pintar[b], Ying-Xian Pan[a], and Gavril W. Pasternak[a,1]

[a]Molecular Pharmacology and Chemistry Program and Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; and [b]Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, Piscataway, NJ 08854

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved October 21, 2011 (received for review September 17, 2011)

Pain remains a pervasive problem throughout medicine, transcending all specialty boundaries. Despite the extraordinary insights into pain and its mechanisms over the past few decades, few advances have been made with analgesics. Most pain remains treated by opiates, which have significant side effects that limit their utility. We now describe a potent opiate analgesic lacking the traditional side effects associated with classical opiates, including respiratory depression, significant constipation, physical dependence, and, perhaps most important, reinforcing behavior, demonstrating that it is possible to dissociate side effects from analgesia. Evidence indicates that this agent acts through a truncated, six-transmembrane variant of the G protein-coupled mu opioid receptor MOR-1.

administration. In addition to its high potency, IBNtxA also displayed full efficacy, as indicated by most mice reaching cutoff values at higher doses. We also assessed IBNtxA analgesia by using a graded response [percentage maximal possible effect (% MPE)] (Fig. S2). Its ED_{50} value (0.34 mg/kg; 95% confidence limits: 0.23, 0.52) was very close to that with quantal responses (Fig. 3*A*). Furthermore, IBNtxA analgesia was not limited to the radiant heat tail-flick assay. IBNtxA was a potent analgesic in the hot plate assay (ED_{50} = 0.6 mg/kg) as well (Fig. S3).

Opioid receptor KO mice have been valuable in exploring opioid action. A number of mice targeting MOR-1 have been reported (11–14). All MOR-1 KO mice with a disruption of exon 1

A Future for Human Pain Phenotypic and Molecular Biomarkers

- Discovery of biological pathways underlying traits and diseases
- Drug discovery – target identification
- Diagnostic and prognostic markers enabling individualized decisions regarding efficacy and risk of :
    - Pharmacotherapies
    - Behavioral therapies
    - Invasive procedures

Regional Center for Neurosensory Disorders

- Eric Bair
- Kanokporn Bhalang
- **Luda Diatchenko**
- Greg Essick
- Richard Gracely
- Mark Hollins
- Kevin Kahn
- Pei Feng Lim
- Dylan Maixner
- Sam McLean
- Andrea Nackley-Neely
- Asgier Sigurdsson
- Gary Slade
- Shad Smith
- Kati Thieme
- Inna Tchivileva
- William Whitehead
- Denniz Zolnoun

Collaborators

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- Roger Fillingim, *University of Florida (Gainesville) ,*
- Dmitry Shagin - *Institute of Bioorganic Chemistry (Moscow, Russia)*
- Sergei Makarov – *Attagene Inc.*

National Institutes of Health

- David Goldman - *NIAAA*
- Inna Belfer - *NIDCR/NIAAA*
- Mitchell Max - *NIDCR*
- Ke Xu - *NIAAA*
- Sveta Shabalina – *NCBI*
- Dmitri Zaykin - NIEHS

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UNC School of Dentistry

Comprehensive Center for Inflammatory Disorders

Thurston Arthritis Center

Attagene Inc.

Algynomics Inc.

Heritability

Clinical Pain

- Low back pain 30-68%
- Fibromyalgia 51%
- Neck pain 34-58%
- Knee pain 44%
- Osteoarthritis 30-46%
- Pelvic pain 41%
- Headache
  - Males 39-44%
  - Females 49-58%

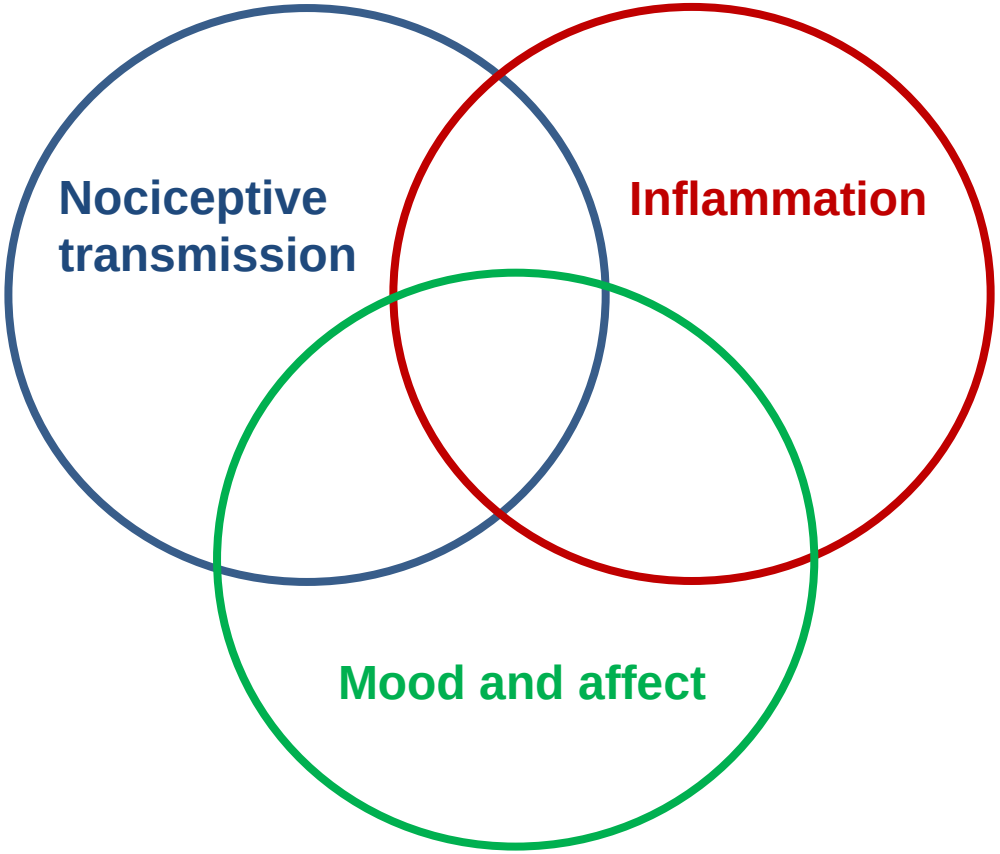
Experimental Pain

- Punctuate hyperalgesia 55%
- Heat pain threshold 53%
- Pain during burn induction 34%
- Itch after histamine iontophoresis 35%
- Pain during acid iontophoresis 31%
- Pain during ATP iontophoresis 22%
- Brush evoked allodynia area

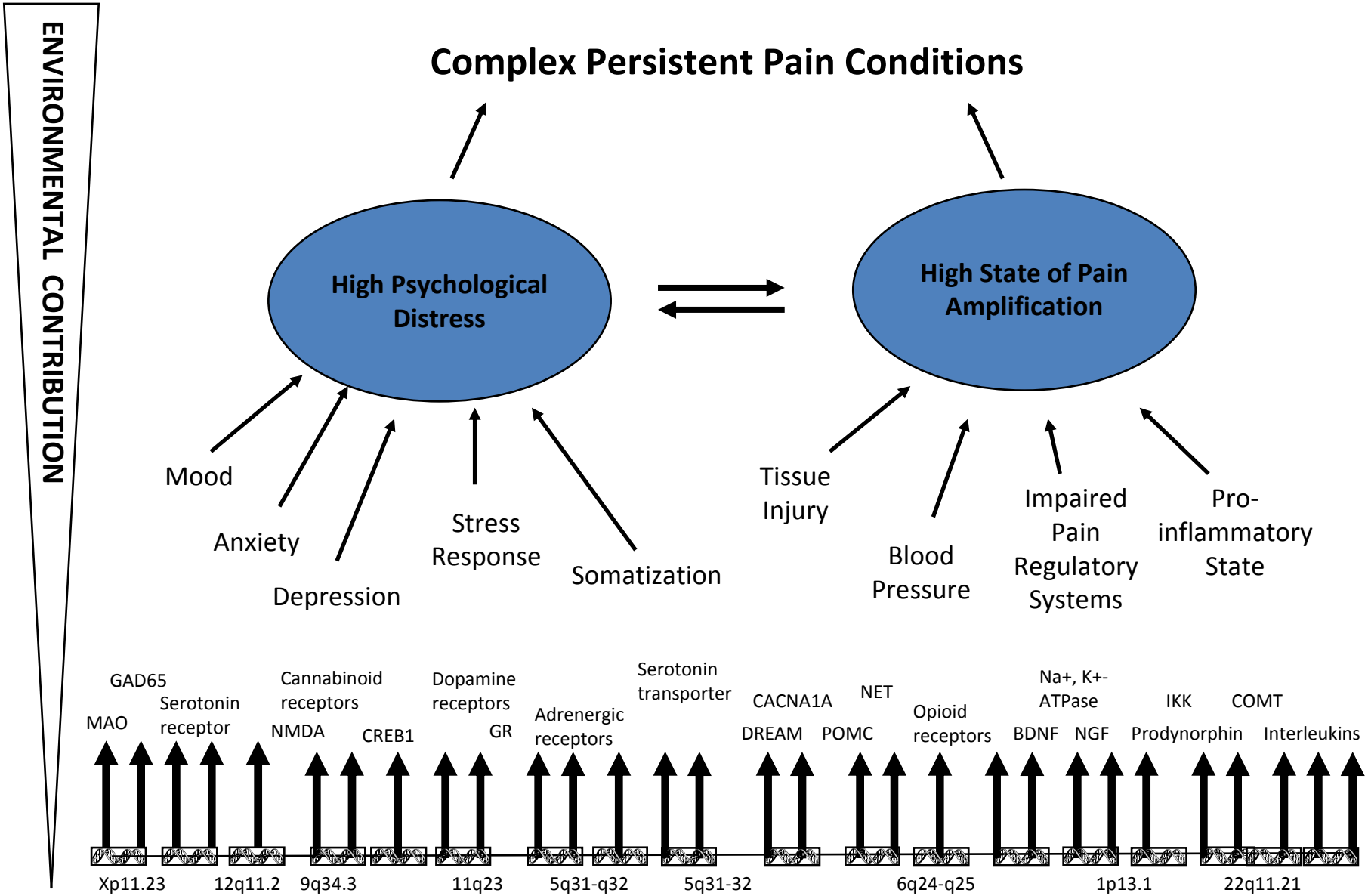
Pain Research Panel

Assessment of 3295 SNPs from 350 genes implicated in key pathways that regulate the perception of pain

- Affymetrix ParAllele microarray platform using Molecular Inversion Probe (MIP) technology
- 3 domains relevant to hypothesized risk pathways
- Genes coding for proteins that mediate or modify the therapeutic effects of pharmacological agents used to treat pain
- SNP choice selective for putatively functional loci
- LD coverage of entire gene at $r^2 > 0.8$
- 160 ancestry-informative markers



Hypothesized Risk Determinants



Diatchenko et al, Pain 123: 226-30, 2006