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**What is my message?**



- Computational genetic analyses in mice enable translatable genetic discoveries
  - Narcotic Drug Addiction
  - Neuropathic Pain
- Integrative approaches for analysis of complex pathobiology
  - Acetaminophen Toxicity
  - Narcotic Drug Responses

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**Computational Tools Accelerate the Pace of Genetic Discovery**

**QTL Analysis**

Produce, Genotype and Phenotype 200–1000 F2

Each F2 is unique

Analyze 10-70 Mb

3-5 scientists  
3-10 years



*"You can't possibly do that!"*

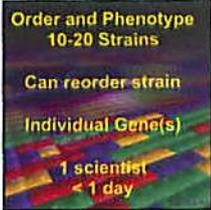
**Computational Analysis**

Order and Phenotype 10-20 Strains

Can reorder strain

Individual Gene(s)

1 scientist  
< 1 day



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## Mouse SNP Database: Catalogue of Genetic Variation

- NIH funded Database  
240,000 SNPs  
19 strains  
3200 genes
- 'Haplotypic' patterns  
enable computational  
mapping
- <http://mouseSNP.roche.com>



Jianmei Wang

Guochun Liao

Science 306: 690, 2004

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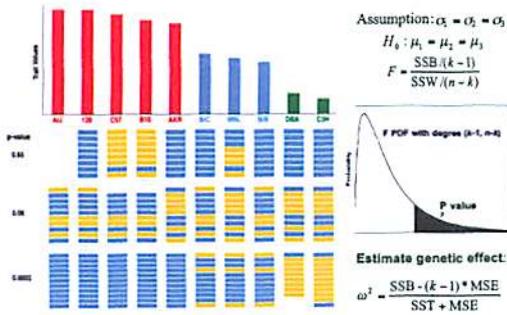
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## Haplotype-Based Mapping

Quantitative Trait: One-way ANOVA




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## Computational Genetic Mapping

	Gene	Journal	Year
<b>In silico Genetics</b>	AHR	Science	2001
<b>Gene Expression</b>	H2-Ea	Science	2004
<b>Pharmacogenetics</b>			
Warfarin	Cyp2c29	Nat. Biotech	2006
Irinotecan	Ugt1a1	PNAS	2007
PKA Kinase Inhibitor	Acx1	Pharmacol J	2010
<b>Infection Susceptibility</b>			
Aspergillosis	Plg	PLoS Path.	2008
Respiratory Syncytial Virus	H2	J. Immunol.	2010
Candida	CTs	Infec. Immun.	2011
<b>Narcotic Drug-Addiction</b>			
Mechanical Hyperalgesia	Adrb2	Anesthesiology	2006
Thermal Hyperalgesia	Abcb1b	PGG	2006
Analgesia	Kery9	PGG	2008
Withdrawal	H13a	PGG	2009
<b>Inflammatory Pain</b>			
Early Formalin Response	Ap1b3	Pain	2009
Late Formalin Response	Mapk8	Mol. Pain	2010
Neuropathic Pain	P2rx7	Submitted	2011
<b>Wound Biology</b>	Nalpb	AAA (part 1&2)	2010

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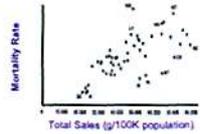
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## Narcotic Drug Addiction: An Iatrogenic Public Health Problem

### Prescription Narcotic Drugs

- 90% of chronic pain patients
- Abused by 11 M people per month

Vicodin  
120M per year



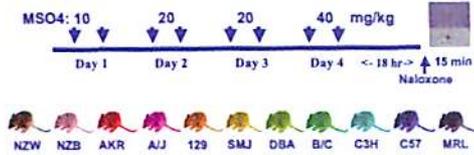
### Sub-optimal Management

- Controlled substances
- No new approach in 30 yrs

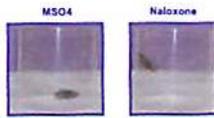


Am J Prev Med 31: 506, 2008

## Murine Model: Narcotic Dependence



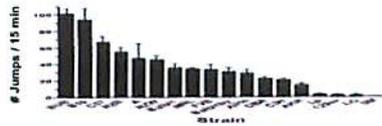
- Jumpiness = Physical Dependence
- Measure in 18 Strains
- Computational Genetics



Pao Abjo VAH, D. Clark, D. Liang

Pharmacogenetics and Genomics 19: 193, 2009

## Computational Genetics: Morphine Dependence



P-value	Neurotype	Chr.	Position (Mb)	#SNPs	Symbol
4.46E-04		5	43,358-43,859	76	163a
4.46E-04		9	49,660-49,667	10	163a
4.00E-05		2	189,348-189,247	9	N/A
4.96E-05		6	109,305-109,318	7	Rpr1
7.14E-05		8	87,624-87,680	8	Cadherin1
6.00E-13		11	167,813-167,890	27	Carrng3
1.00E-23		14	93,385-93,670	63	Mmp14
6.20E-24		13	79,179-79,824	10	Mafk
6.00E-29		1	6,110-6,113	3	Alplv1h
6.00E-29		6	108,318-108,432	6	Rpr1

## 5-HT Receptors

- Serotonin (5HT)
  - Mood, cognition, circadian rhythm, pain...
  - Synthesis: Raphe, midbrain & pons
- 5HT3 Receptor: (one of 7 families)
  - Ligand-gated ion channel: 5 subunits
  - Antagonists: close Agonists: open
  - Modulate transmitter release: DA, GABA, 5HT
  - Cortex, amygdala, nucleus accumbens



- 5HT3 Antagonist
- Chemotherapy-induced nausea
- Well tolerated with few side effects



Ondansetron (Zofran)  
FDA approved: 01/91  
8 mg bid or tid

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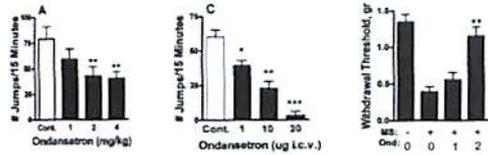
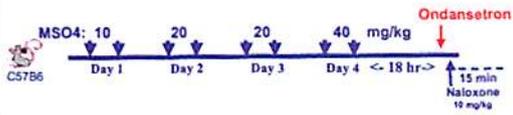
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## 5-HT3a Receptor & Narcotic Dependence??



David Clark

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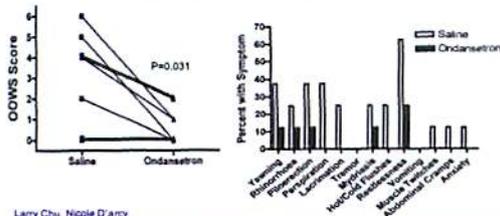
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## Acute Experimental Morphine Withdrawal in Human Subjects

Randomized, double-blinded, placebo-controlled, crossover study



Larry Chu, Nicole D'arcy

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## 5HT3a & Narcotic Drug Addiction

- Genetic Analysis -> Novel Solution for dependence & withdrawal
- 5HT3 Antagonists effective in mouse & man
- 'Medical Vaccination' to remedy an iatrogenic problem



Pharmacogenetics and Genomics 19:153, 2009

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## Genetic Analysis: Incisional Wound Biology

Full Thickness Skin Incision:  
16 Strains, 24 hr

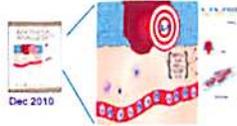


Analyze 16 Cytokines  
mRNA Profile: 39K Genes

Haplotype-Based Genetic Analysis

Select candidate gene(s)

Experimental Analysis  
-in vitro & in vivo mouse  
-Human surgical incisions  
and sunburns



New model: IL-1 regulates the production of multiple inflammatory mediators

Perioperative IL-1 inhibition can reduce post-surgical pain and inflammation

Clinical Trial begun at Stanford 2011:  
Will perioperative administration of an IL-1 inhibitor reduce incisional pain?

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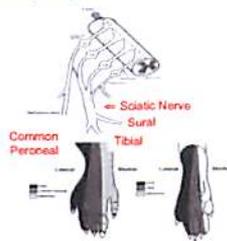
## Spared Nerve Injury (SNI) a rodent model of chronic pain

- Partial sciatic nerve injury is a model for neuropathic pain

- Ligate 2 branches of Sciatic Nerve
- Sural nerve is 'spared'

- Rapid (<24 hr), prolonged (> 6 month) changes in the Sural N. distribution:

- Mechanical hypersensitivity
- Hyperalgesia
- Cold allodynia
- Heat hyperalgesia



Pain 87:149, 2000

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## P2rx7 & Chronic Pain

- P2rx7 allelic differences alter
  - Experimental pain response in mice
  - Chronic pain in humans
- P2rx7 pore formation (but not channel function) is key
  - Peptides active in spinal cord-DRG
- Enable 'Personalized Pain Medicine'
  - Rx: target pore formation
  - Dx: individuals with responsive alleles




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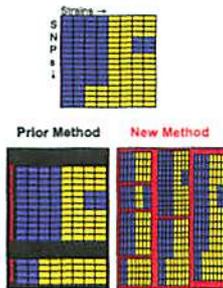
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## 'Next Generation' Computational Genetic Mapping

Whole Genome SNP dB  
Use genome sequence  
~12M SNPs per strain

New Block Construction Method  
Reduces false negatives

Dynamic Block Construction  
30K-fold faster  
Customized & 'real time'



Infection and Immunity, In Press

David Dill and Ming Zheng

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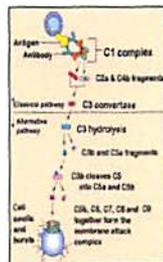
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## Murine Models of Candida Albicans Infection

- Comensal fungus
  - 96% of fungal infections in immunocompromised pts
  - 47% mortality in ICU patients
- Mimics human infection
  - Brain & kidney are target organs
  - Death results from kidney failure
- Complement has a well defined role
  - C5 deficient mice have poor survival
  - 50% of strains are C5 deficient

...but, C5 is not the whole story!



Infection and Immunity in press 2011

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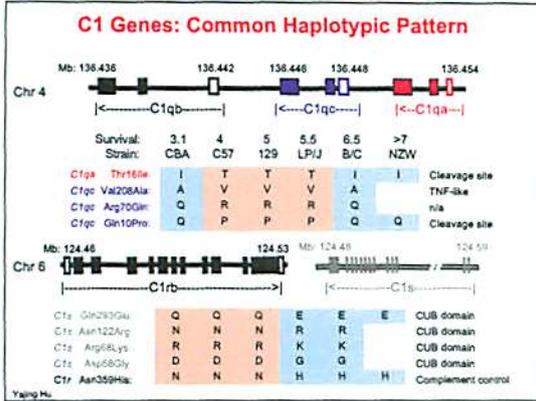
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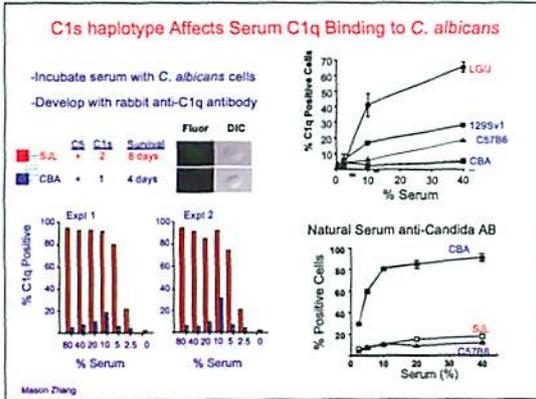
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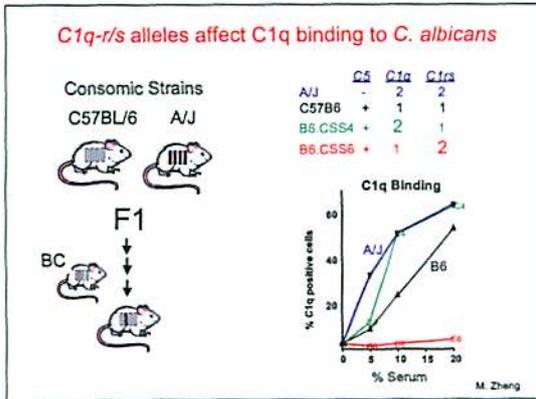
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