SOCIETÀ ITALIANA GERONTOLOGIA E GERIATRIA

CONGRESSO NAZIONALE

52° Firenze, Palazzo dei Congressi

28 novembre - 2 dicembre 2007

Paese vecchio, assistenza nuova: il caso Italia

SOCIETÀ ITALIANA DI GERONTOLOGIA E GERIATRIA

Farmacogenetica della terapia antidolorifica

Giovanni Gambassi

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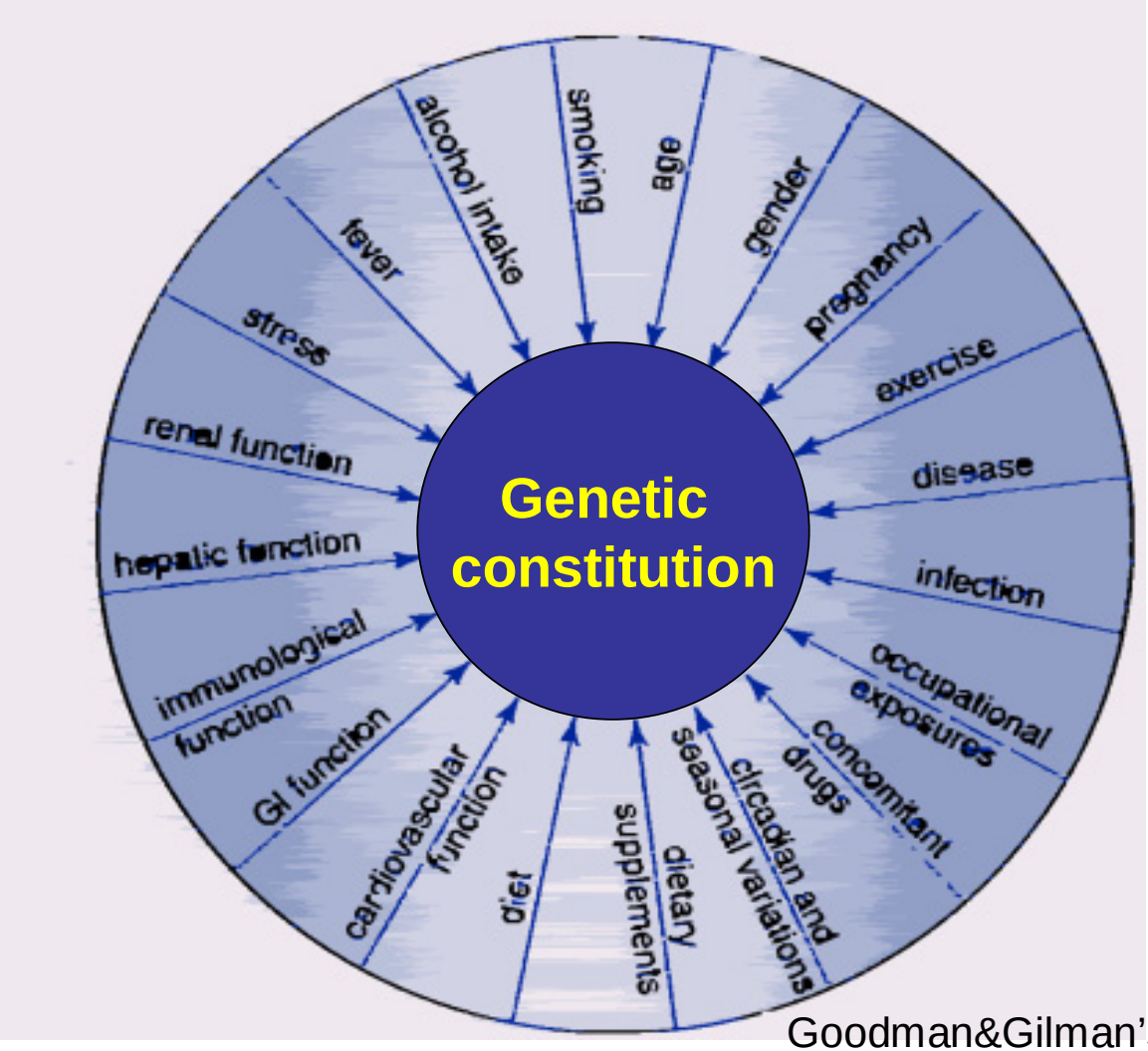
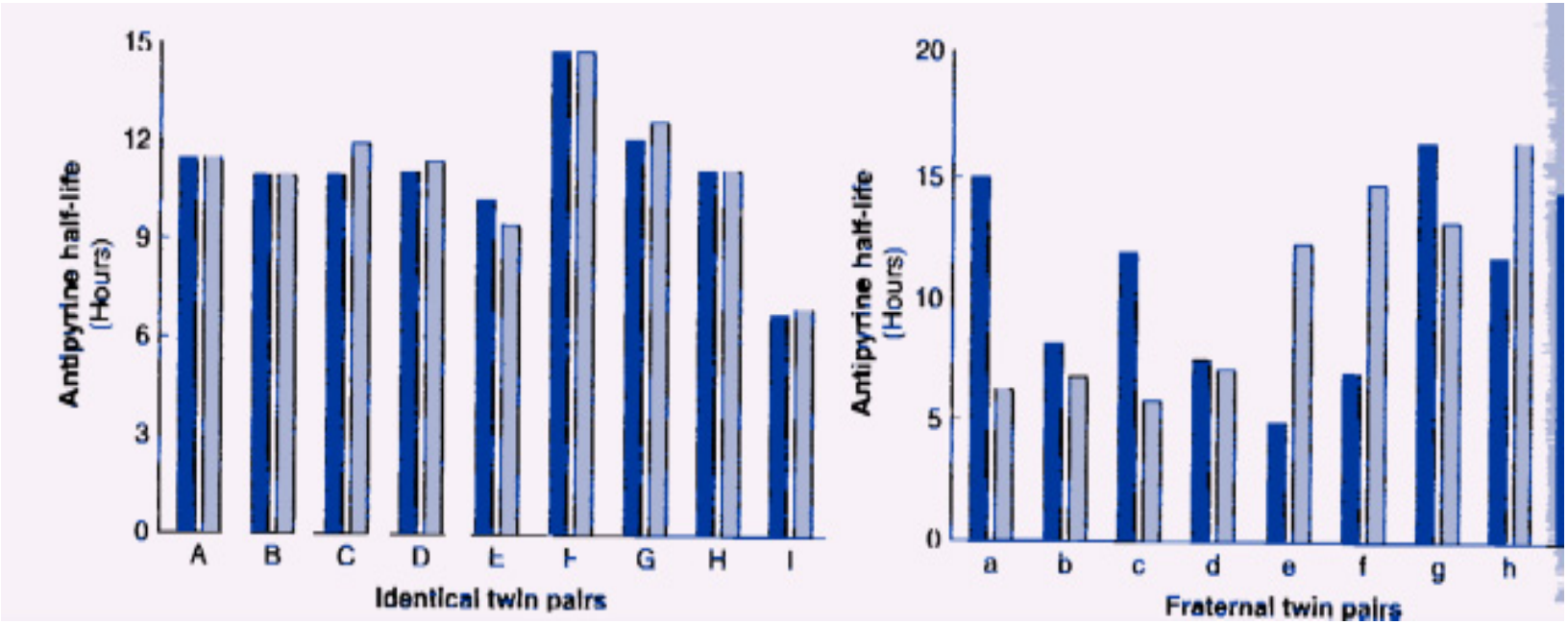
Varianti della risposta ai farmaci



Goodman&Gilman's, 2006 Edition

Genetica e farmacocinetica



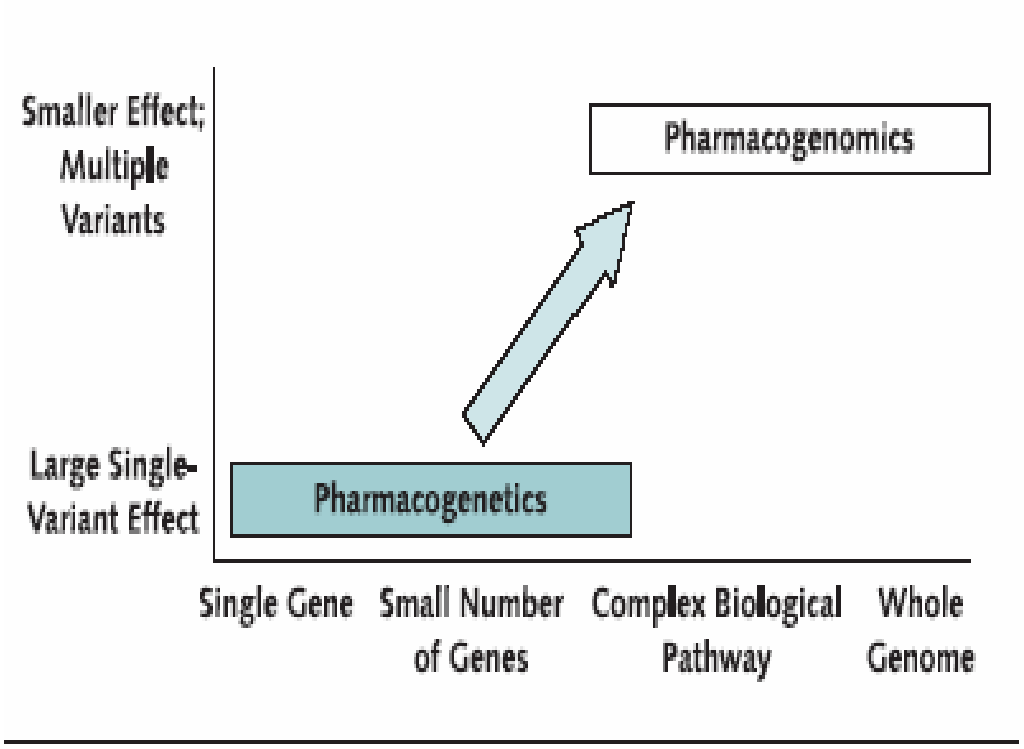
Goodman&Gilman's, 2006 Edition

Definizioni

- Farmacogenetica: Studio dei polimorfismi genetici che possono causare risposte variabili ed inattese a farmaci sia dipendenti da parametri farmacodinamici che farmacocinetici.

- Farmacogenomica: Area di ricerca che partendo dal genoma identifica nuovi bersagli terapeutici e/o ottimizza la scelta e la dose del farmaco per ogni individuo sulla base di modificazioni genetiche.

Current Concepts



Roden DM et al. Ann Intern Med 2006; 145:749-57

FARMACOGENETICA

- Polimorfismi genetici coinvolti nelle alterate risposte ai farmaci (mancata o solo parziale efficacia, comparsa di gravi effetti collaterali)

- Si definisce "**polimorfismo**" una variante che si verifica nel 1-2% della popolazione.

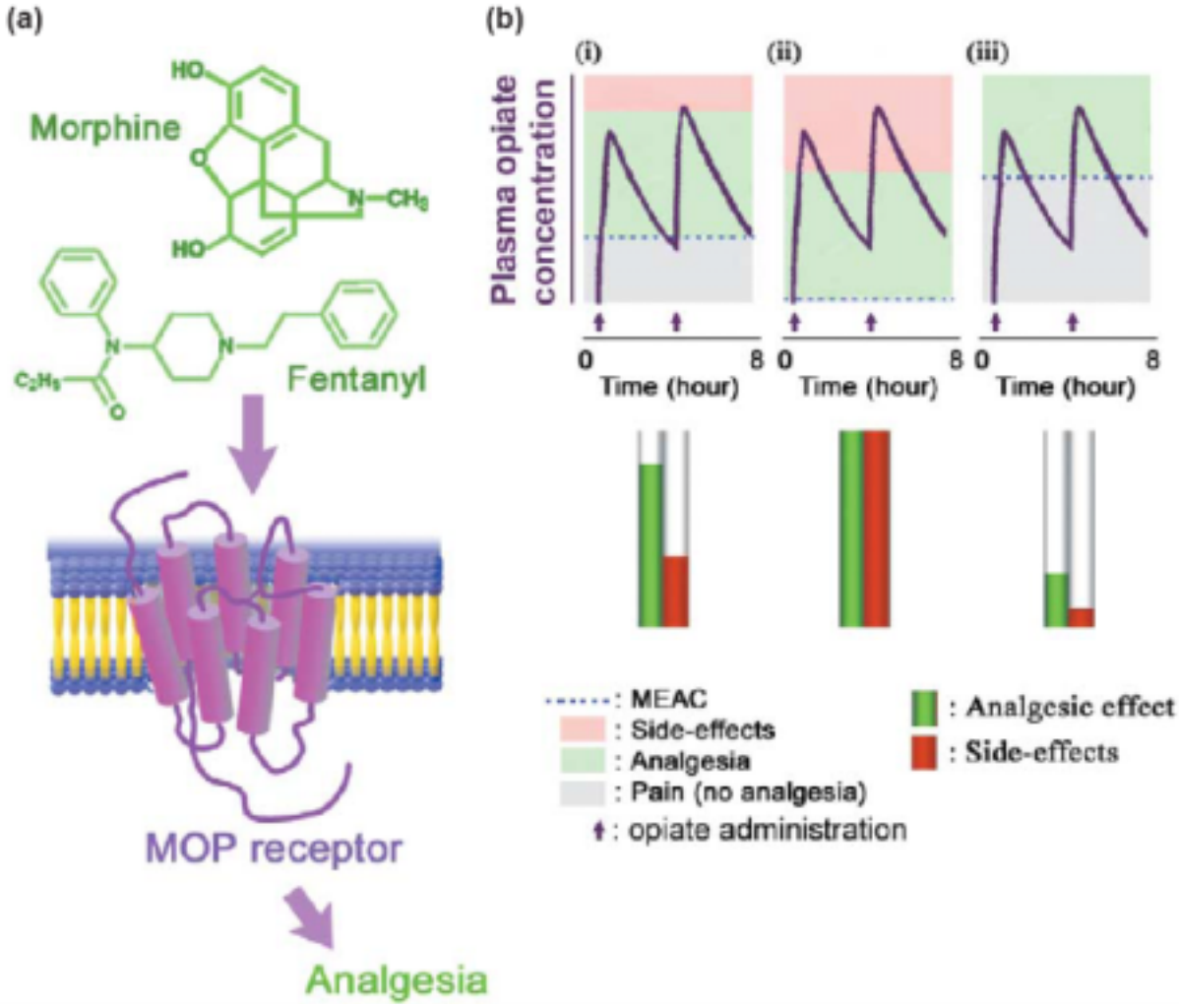
- Si chiamano "**mutazioni**" quelle varianti, più rare, che si verificano in meno del 1% della popolazione.

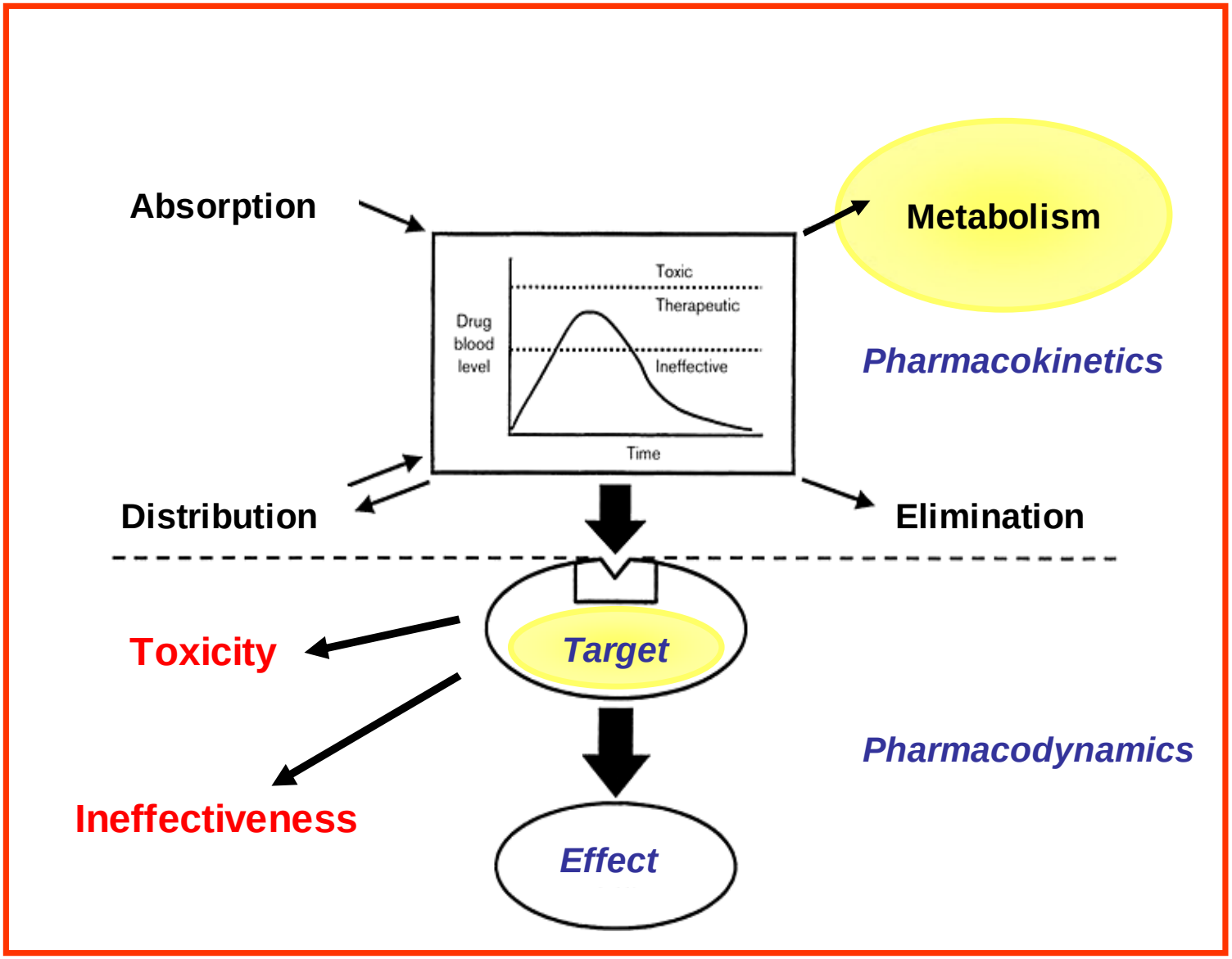
1. Pharmacogenetics of opioids

Introduction

Background

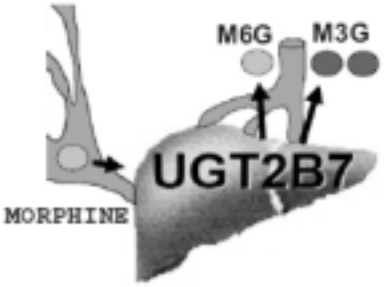
- L’efficacia analgesica degli oppioidi può essere molto variabile da persona a persona
- La dose analgesica efficace di un oppioide può essere molto variabile da persona a persona
- Nella medesima persona esiste una estrema variabilità nell’efficacia analgesica di diversi oppioidi
- La cross-tolleranza è incompleta e la conversione da un oppioide ad un altro può essere molto difficile


(a)
Morphine
HO
O
N–CH3
HO
Fentanyl
N
N
C2H5
O
MOP receptor
Analgesia
(b)
(i)
(ii)
(iii)
Plasma opiate concentration
0
8
Time (hour)
: MEAC
: Side-effects
: Analgesia
: Pain (no analgesia)
: opiate administration
: Analgesic effect
: Side-effects


Absorption
Metabolism
Toxic
Therapeutic
Drug blood level
Ineffective
Time
Pharmacokinetics
Distribution
Elimination
Toxicity
Target
Pharmacodynamics
Ineffectiveness
Effect

Genetic variability and opioid efficacy

METABOLISM

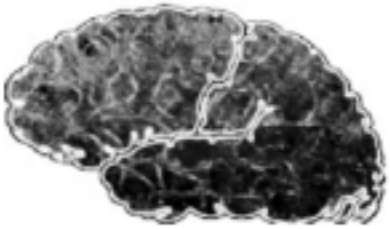


UGT2B7 gene

INTRACELLULAR MECHANISMS

STAT6 gene
ßARRESTIN GENE

BLOOD BRAIN BARRIER



MDR1 gene

BIOLOGICAL MODIFYING SYSTEMS

COMT gene
MC1R gene

OPIOID RECEPTOR

OPRM1 gene

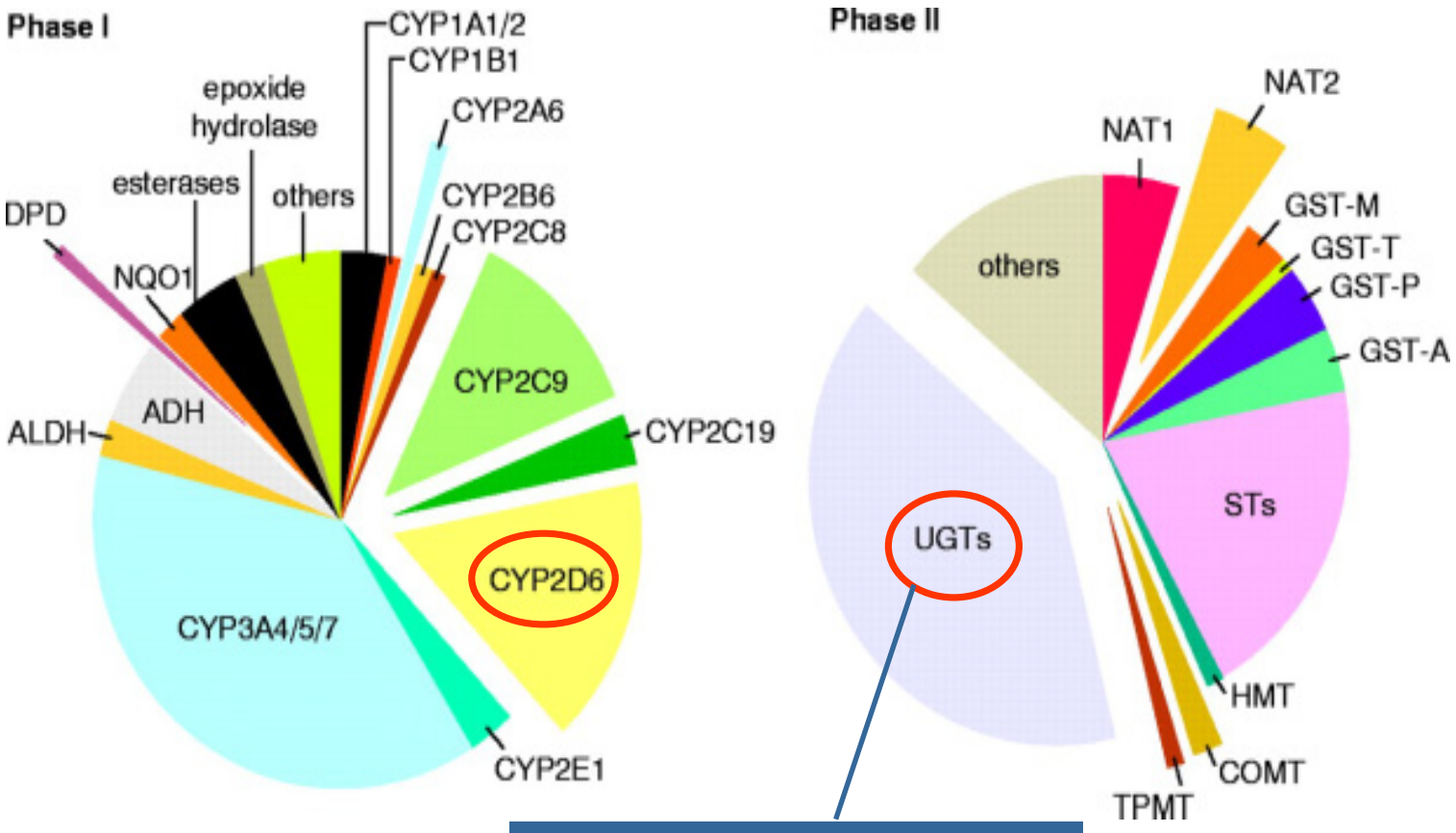
Current Anaesthesia & Critical Care (2007) 18, 149–156

ELSEVIER

2. Pharmacogenetics of opioids

Metabolism

Proportion of drugs metabolized by enzymes



UDP-glucuronosyltransferase

CYP2D6 & UGT2B7

- **CYP2D6**
  codeine to more active metabolite, morphine
  tramadol to active metabolite, O-desmethyl

- **UGT2B7**
  morphine to more active metabolite, M6G

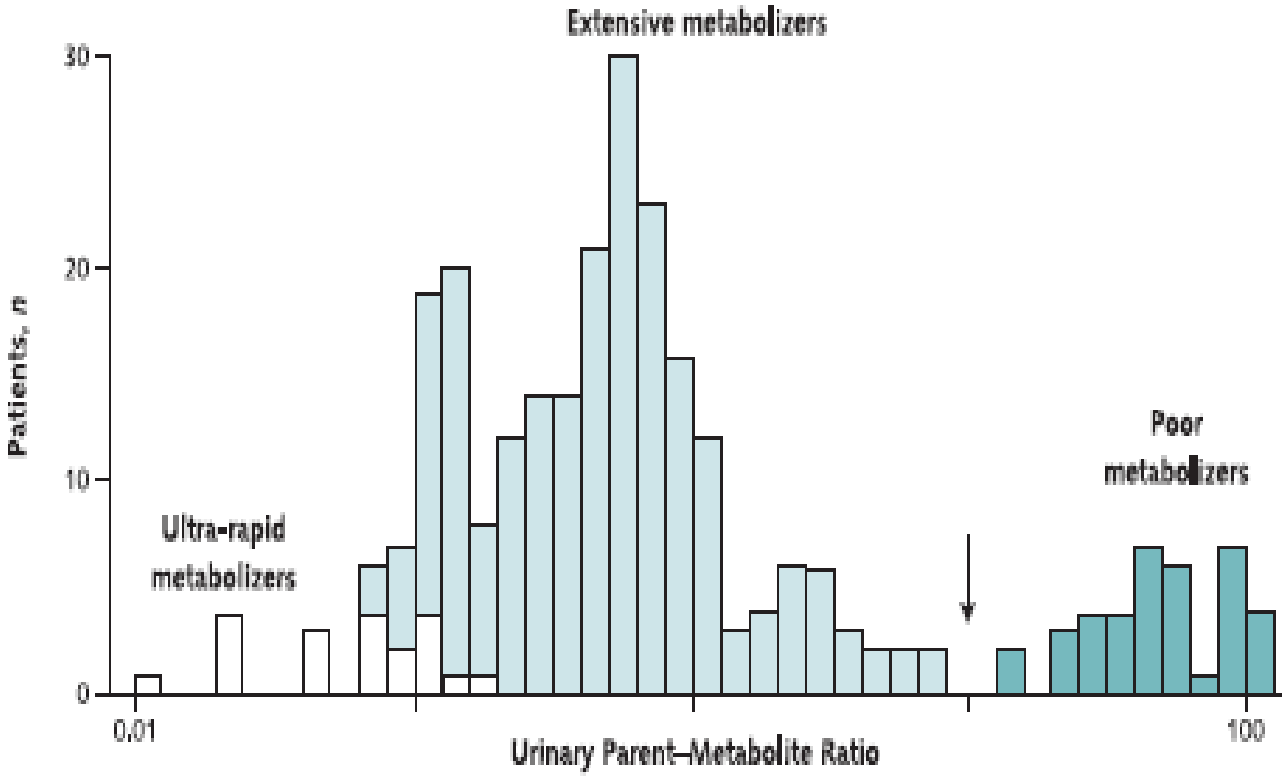
CYP2D6 Polymorphisms

- Over 100 polymorphisms in CYP2D6

Major variant alleles	*Mutation*	*Consequence*	*Allele frequencies (%)*			
			Caucasians	*Asians*	*Black Africans*	*Ethiopians and Saudi Arabians*
*CYP2D6*2xn*	Gene duplication/ multiduplication	Increased enzyme activity	1–5	0–2	2	10–16
*CYP2D6*4*	Defective splicing	Inactive enzyme	12–21	1	2	1–4
*CYP2D6*5*	Gene deletion	No enzyme	2–7	6	4	1–3
*CYP2D6*10*	P34S, S486T	Unstable enzyme	1–2	51	6	3–9
*CYP2D6*17*	T107I, R296C, S486T	Altered affinity for substrates	0	0	20–35	3–9

- Based on these polymorphisms, patients are phenotypically classified as:
  - Ultrarapid metabolizers
  - Extensive metabolizers
  - Poor metabolizers

CYP2D6 Phenotypes



Roden DM et al. Ann Intern Med 2006; 145:749-57

Metabolism Pharmacogenetics

Table 1. Pharmacogenetics of Phase I Drug Metabolism.*

NEJM 2003; 348: 529-537

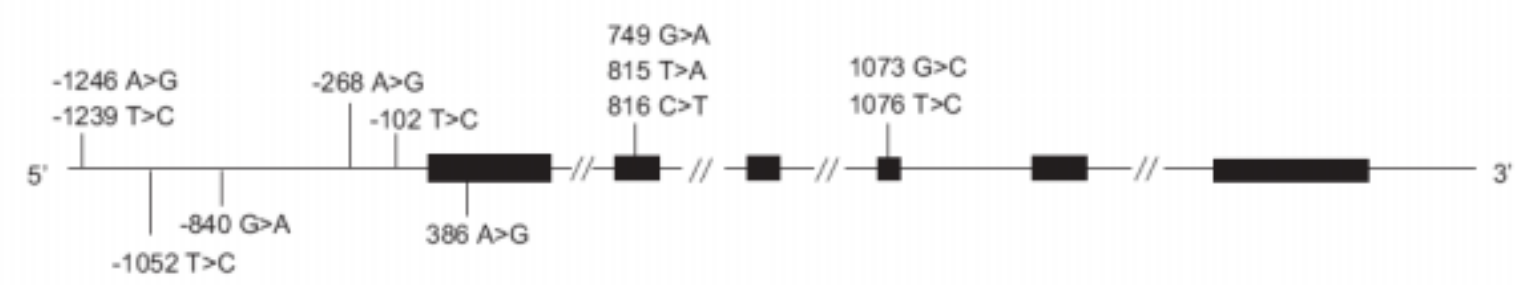
Drug-Metabolizing Enzyme	Frequency of Variant Poor-Metabolism Phenotype	Representative Drugs Metabolized	Effect of Polymorphism
Cytochrome P-450 2D6 (CYP2D6)	6.8% in Sweden 1% in China[17]	Debrisoquin[15] Sparteine[16] Nortriptyline[23] Codeine[27,28]	Enhanced drug effect Enhanced drug effect Enhanced drug effect Decreased drug effect

Poor metabolizers have decreased analgesic effect from tramadol

Ultra-rapid metabolizers, common among Asians, will increase the efficacy of codeine

UGT2B7

12 single SNPs, partly linked (3 haplotypes and 6 genotypes)



Genetic variability in the gene coding for the UGT2B7 enzyme does not seem to be important for differences in morphine pharmacokinetics

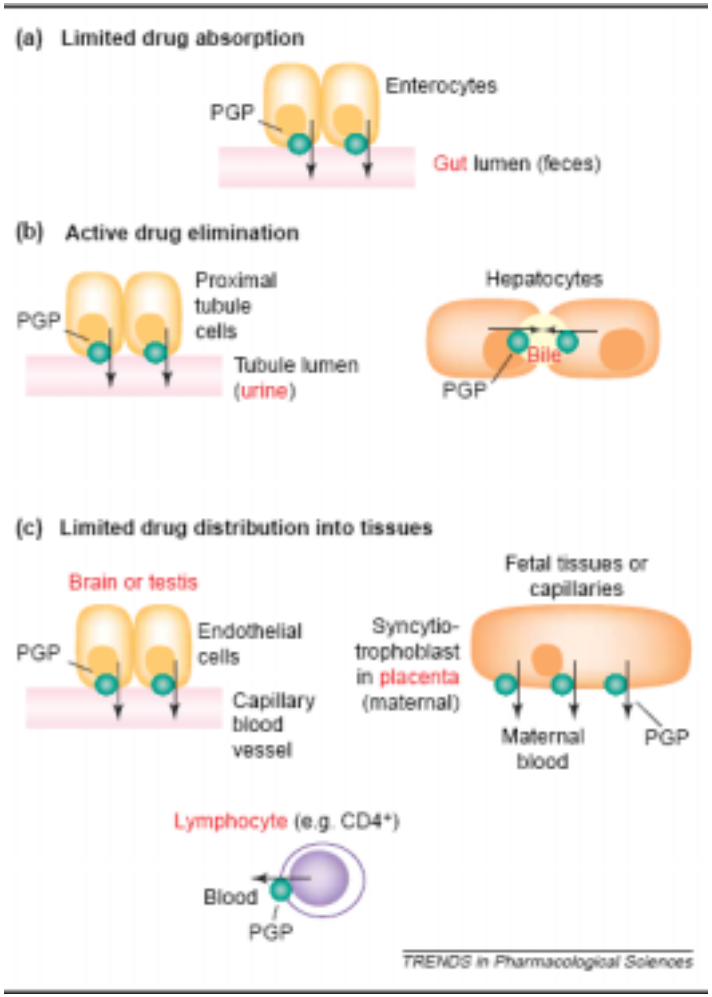
This seems to be in agreement with the notion that serum concetratrions of morphine and M6G are not closely associated with clinical outcomes, i.e., analgesia and adverse effects

3. Pharmacogenetics of opioids

Blood brain barrier

P-glycoprotein

- Morphine is transported out from the brain through the BBB by P-glycoprotein efflux transporter



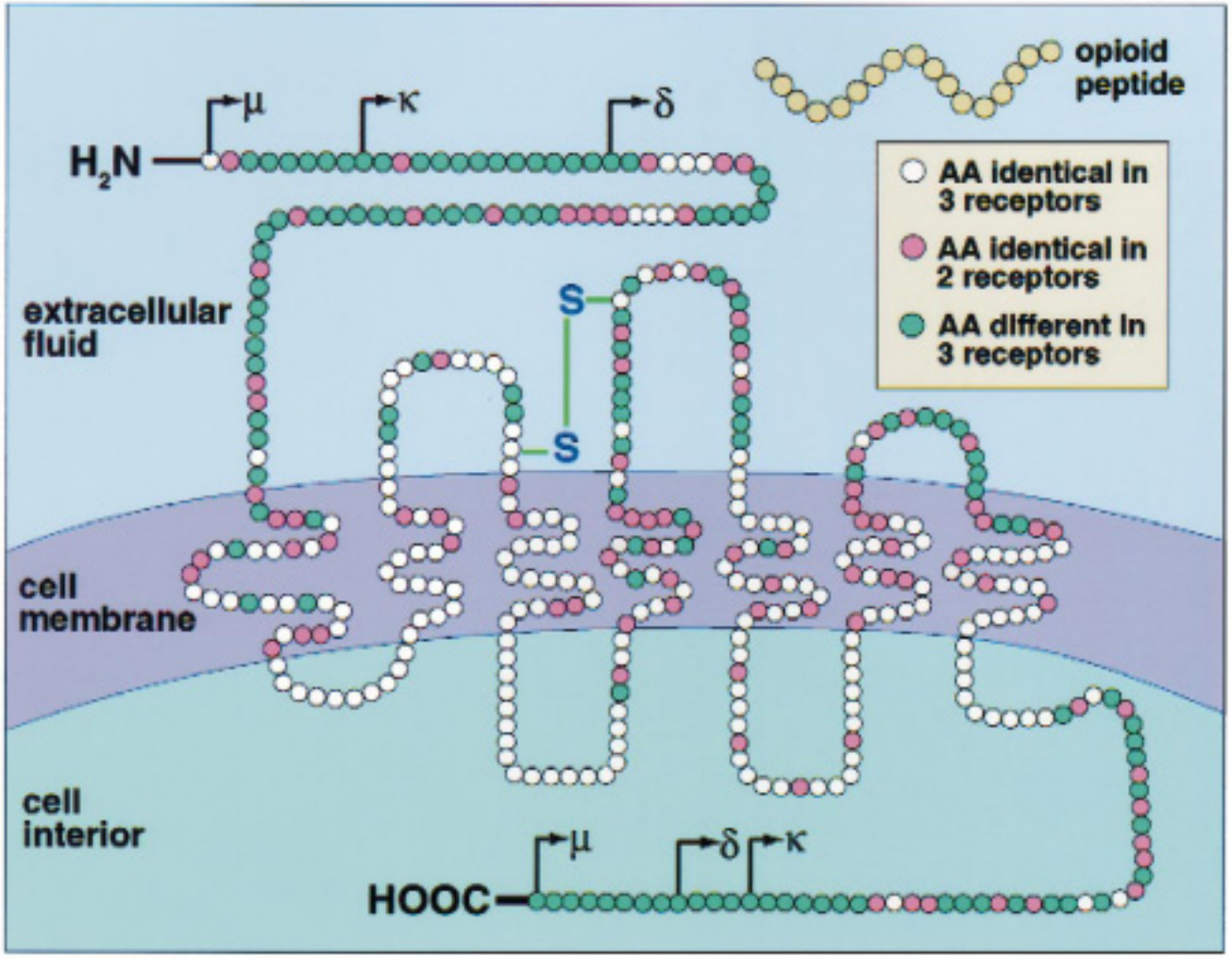
P-glycoprotein

- P-glycoprotein is coded by ABCB1 gene
- The most investigated ABCB1 polymorphism is the exon 26 SNP, C3435T
- It is observed with a frequency of 50-60% in Caucasians, 40-50% in Asians, 10-30% in Africans
- In animal studies a decreased function of the transporters increase the CNS concentration and analgesic efficacy of morphine

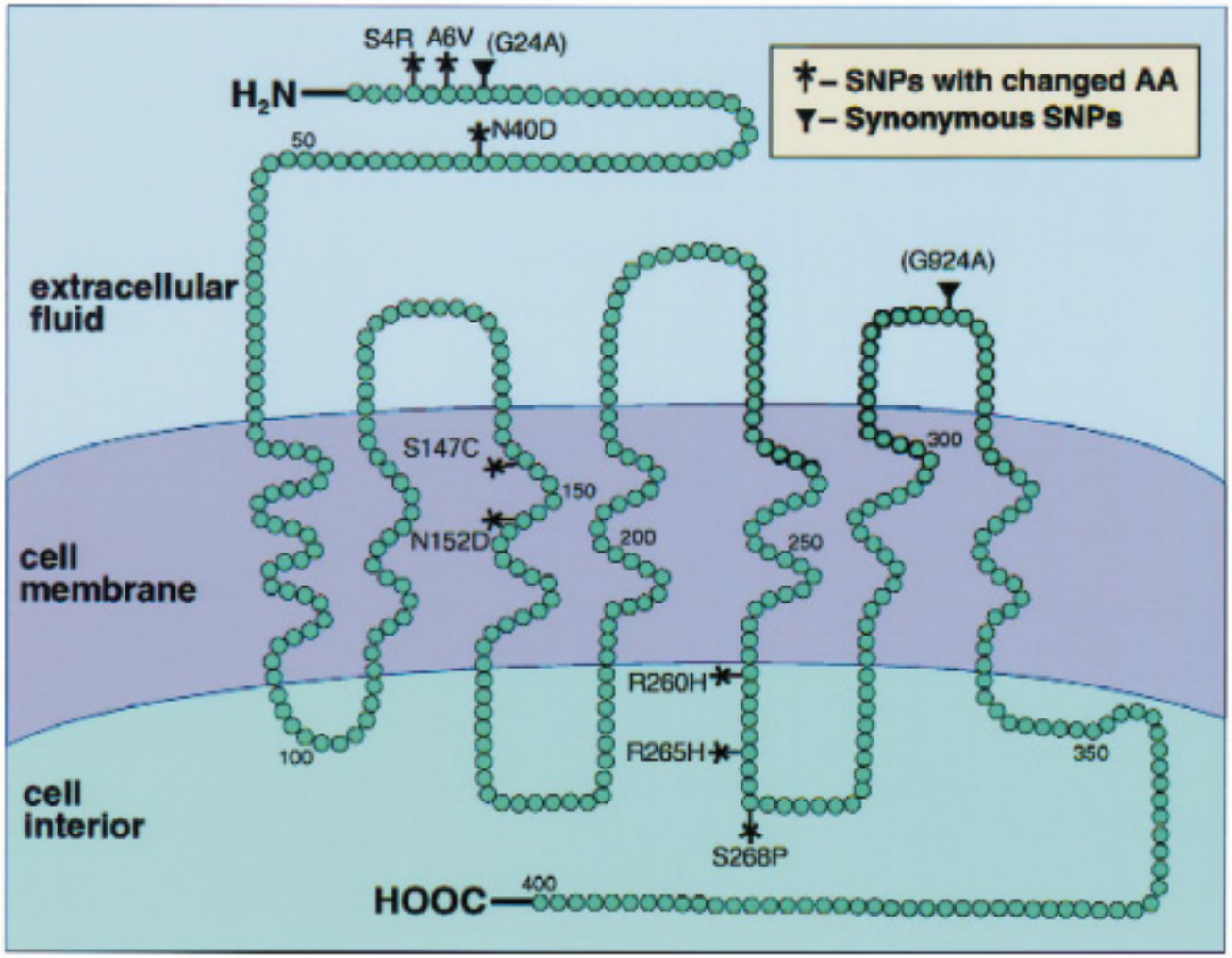
4. Pharmacogenetics of opioids

Receptor

Human Opioid Receptors Mu, Delta, and Kappa



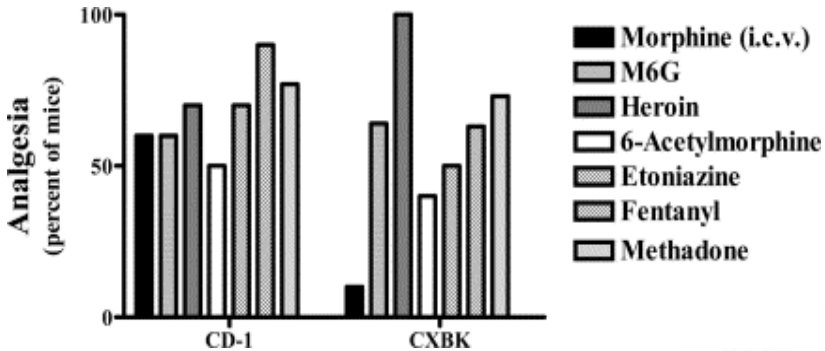
Human Mu Opioid Receptor

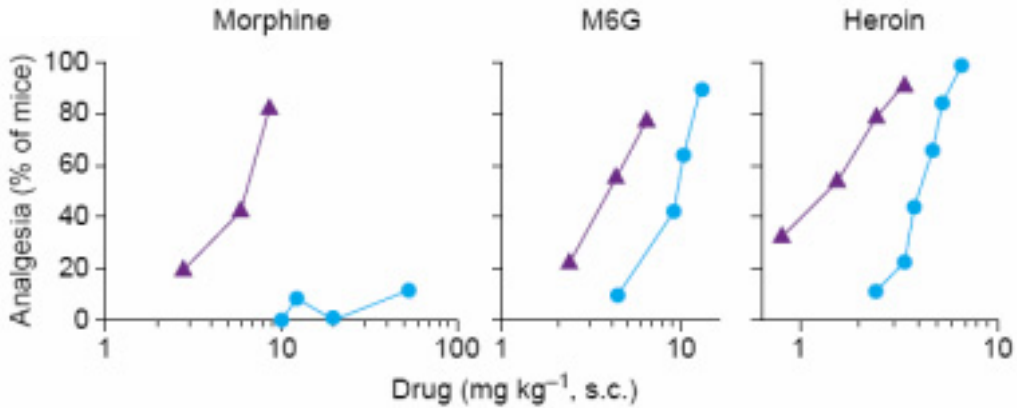


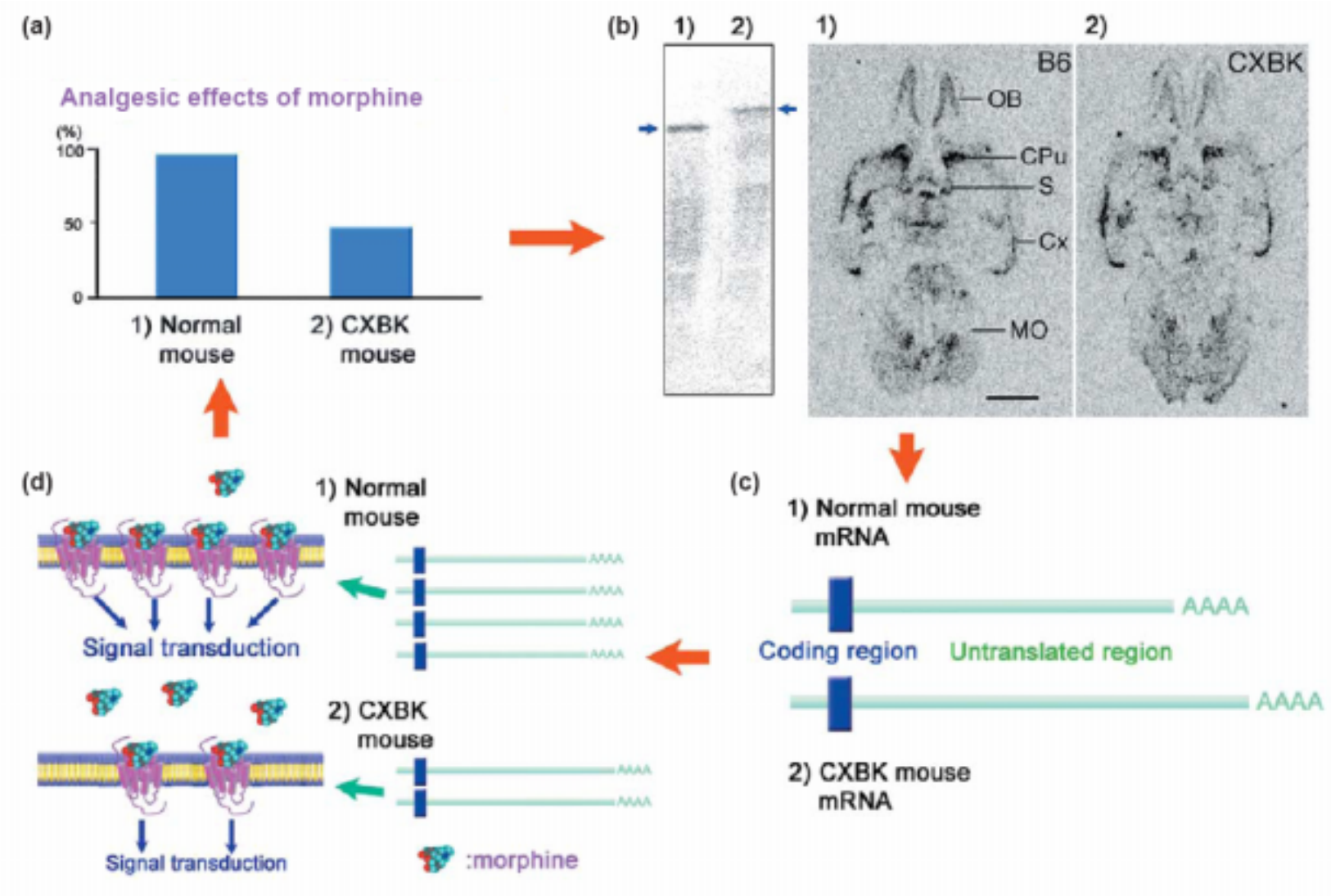
European Journal of Pharmacology 410 (2000) 249–268

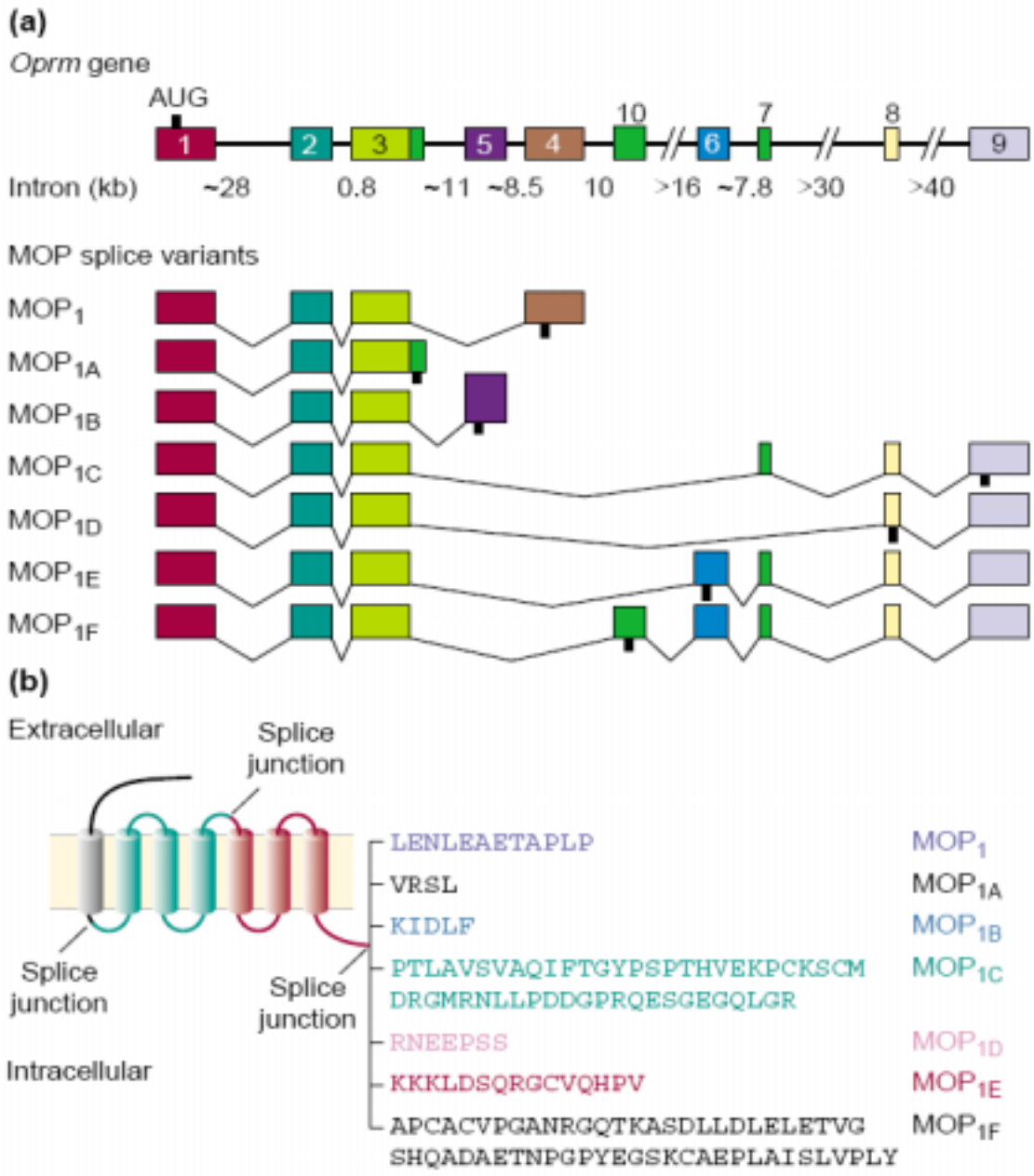
Incomplete cross tolerance and multiple mu opioid peptide receptors

TRENDS in Pharmacological Sciences Vol.22 No.2 February 2001






(a)
Analgesic effects of morphine
(%)
100
50
0
1) Normal mouse
2) CXBK mouse
(b)
1)
2)
1)
2)
B6
CXBK
OB
CPu
S
Cx
MO
(c)
1) Normal mouse mRNA
AAAA
Coding region
Untranslated region
AAAA
2) CXBK mouse mRNA
(d)
1) Normal mouse
Signal transduction
2) CXBK mouse
Signal transduction
:morphine


(a)
Oprm gene
AUG
1
2
3
5
4
10
6
7
8
9
Intron (kb)
~28
0.8
~11
~8.5
10
>16
~7.8
>30
>40
MOP splice variants
MOP1
MOP1A
MOP1B
MOP1C
MOP1D
MOP1E
MOP1F
(b)
Extracellular
Splice junction
Splice junction
Splice junction
Intracellular
LENLEAETAPLP
MOP1
VRSL
MOP1A
KIDLF
MOP1B
PTLAVSVAQIFTGYPSPTHVEKPCKSCM
DRGMRNLLPDDGPRQESGEGQLGR
MOP1C
RNEEPSS
MOP1D
KKKLDSQRGCVQHPV
MOP1E
APCACVPGANRGQTKASDLLDLELETVG
SHQADAETNPGPYEGSKCAEPLAISLVPLY
MOP1F

SNPs and Other Polymorphisms in Coding Region of Opioid Receptor Genes

	# Polymorphisms Identified by our Laboratory*	# of Additional Polymorphisms	
Mu opioid receptor	11	6	17
Kappa opioid receptor	8	1	9
Delta opioid receptor	0	2	2

**Without or with prior identification or later verification by other groups*

LaForge, Yuferov and Kreek, 2000; LaForge and Kreek, 2002

Single Nucleotide Polymorphisms in Human Mu Opioid Receptor Gene

Variant (nucleotide position)	Exon location	Protein domain	Corresponding amino acid change	Allele frequency
A118G	1	N-terminus	Asn 4 Asp (N40D)	10.5% (26 heterozygous; 3 homozygous)
C17T	1	N-terminus	Ala 6 Val (A6V)	6.6% (14 heterozygous; 3 homozygous)
G24A	1	N-terminus	Synonymous mutation	2% (6 heterozygous)
G779A	3	CL3	Arg 260 His (R260H)	<1% (1 heterozygous)
G942A	3	EL3	Synonymous mutation	<1% (1 heterozygous)

** Nucleotide position 1 is first base of the start codon.*

Bond et al., 1998

Allelic Frequencies of the Variant Allele of the A118G Single Nucleotide Polymorphism of the Human μ-Opioid Receptor Gene in Diverse Populations

Ethnicity or population	Bergen et al. (1997)	Bond et al. (1998)	Gelernter et al. (1999)	Szeto et al (2001)	Tan et al (2003)	Bart et al (2003)
Asian						
Japanese			0.485 (34)			
Han Chinese				0.362 (297)		
Chinese					0.351 (208)	
Thai					0.438 (56)	
Malay					0.446 (156)	
Indian					0.442 (137)	
Southwest Native American			0.163 (367)			
Caucasian						
European American	0.105 (100)	0.115 (52)	0.141 (543)			
Finnish Caucasian	0.122 (324)					
Swedish Caucasian						0.107 (187)
Hispanic		0.142 (67)	0.117 (47)			
African American		0.016 (31)	0.028 (144)			
Other (populations in Israel)						
Ethiopian			0.170 (49)			
Bedouin			0.080 (43)			
Ashkenazi			0.210 (93)			

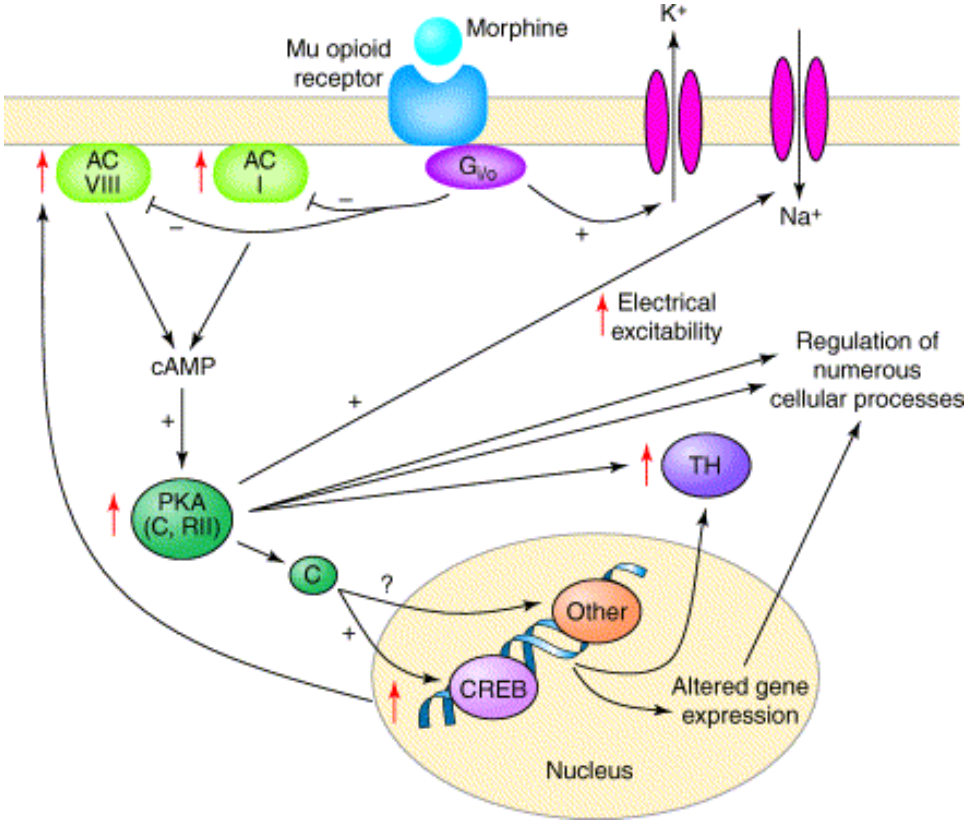
LaForge, Yuferov and Kreek, 2003

MOR A118G SNP

- Patients need significant more morphine and achieve less pain relief
- Causes a decrease in opioid potency by a factor of 2-3
- Studies on the molecular consequence have produced inconsistent results.
- Binding and signalling seem unaffected; may be decreased receptor transcription

5. Pharmacogenetics of opioids

Intracellular signalling


Morphine
Mu opioid receptor
K+
Na+
AC VIII
AC I
Gi/o
cAMP
PKA (C, RII)
C
?
Other
CREB
Altered gene expression
Nucleus
TH
Electrical excitability
Regulation of numerous cellular processes

Stat6

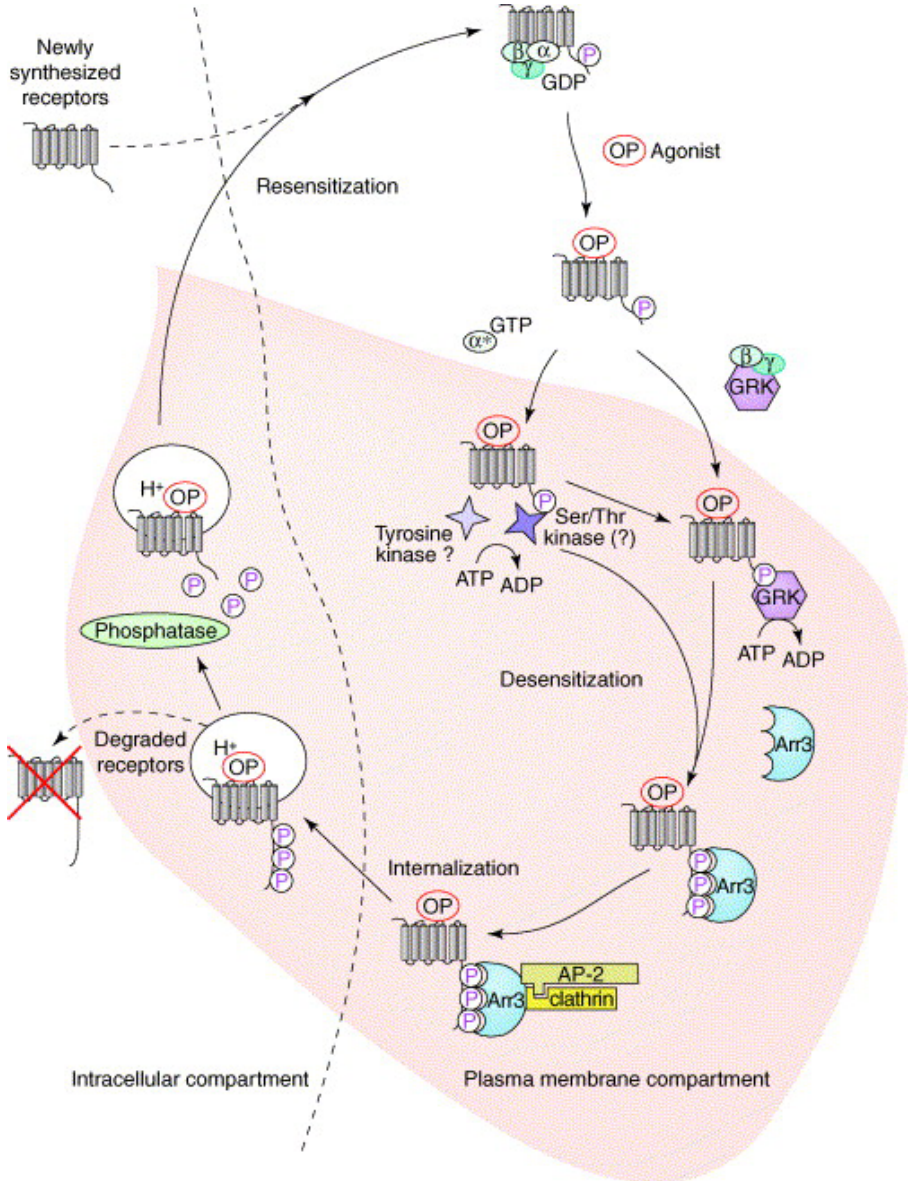
- transcription factor that can alter MOR gene expression

SNP	Genotype	Genotype frequency		Allele	Allele frequency		Allele carriage	
		Control ($n=117$)	Switcher ($n=39$)		Control ($n=117$)	Switcher ($n=39$)	Control ($n=117$)	Switcher ($n=39$)
(c) Stat6 gene								
−1714 C/T intron 1	CC	0.51	0.31	C	0.7	0.59	0.89	0.87
	CT	0.38	0.56	T	0.3	0.41	0.49*	0.69*
	TT	0.11	0.13					

Switchers had increased carriage of the T allele (−1714 C/T) and a significant difference in the allelic frequency at 9065 C/T ($\chi^2=3.86$, $P=0.049$) in the *stat6* gene. No

The Pharmacogenomics Journal (2005) 5, 324–336

www.nature.com/tpj



Current Opinion in Pharmacology

β-arrestin

- Intracellular protein important in desensitization of MOR and trafficking of internalized receptors

SNP	Genotype	Genotype frequency		Allele	Allele frequency		Allele carriage	
		Control (n=117)	Switcher (n=39)		Control (n=117)	Switcher (n=39)	Control (n=117)	Switcher (n=39)
1082 G/A intron 1	GG	0.38	0.44	G	0.61	0.71	0.83*	0.97*
	GA	0.44	0.54	A	0.39	0.29	0.62	0.56
	AA	0.17	0.03					
7757 A/G intron 9	TT	0.61	0.56	T	0.79	0.76	0.98	0.95
	TC	0.38	0.38	C	0.21	0.24	0.39	0.44
	CC	0.02	0.05					
8622 T/C exon 11	CC	0.19*	0.03*	C	0.4*	0.27*	0.63	0.51
	CT	0.43*	0.49*	T	0.6*	0.73*	0.81*	0.97*
	TT	0.38*	0.49*					
8864 A/G intron 11	GG	0.38	0.46	G	0.62	0.72	0.85*	0.97*
	GA	0.48	0.52	A	0.38	0.28	0.62	0.54
	AA	0.15	0.03					

The Pharmacogenomics Journal (2005) 5, 324–336

www.nature.com/tpj

6. Pharmacogenetics of opioids

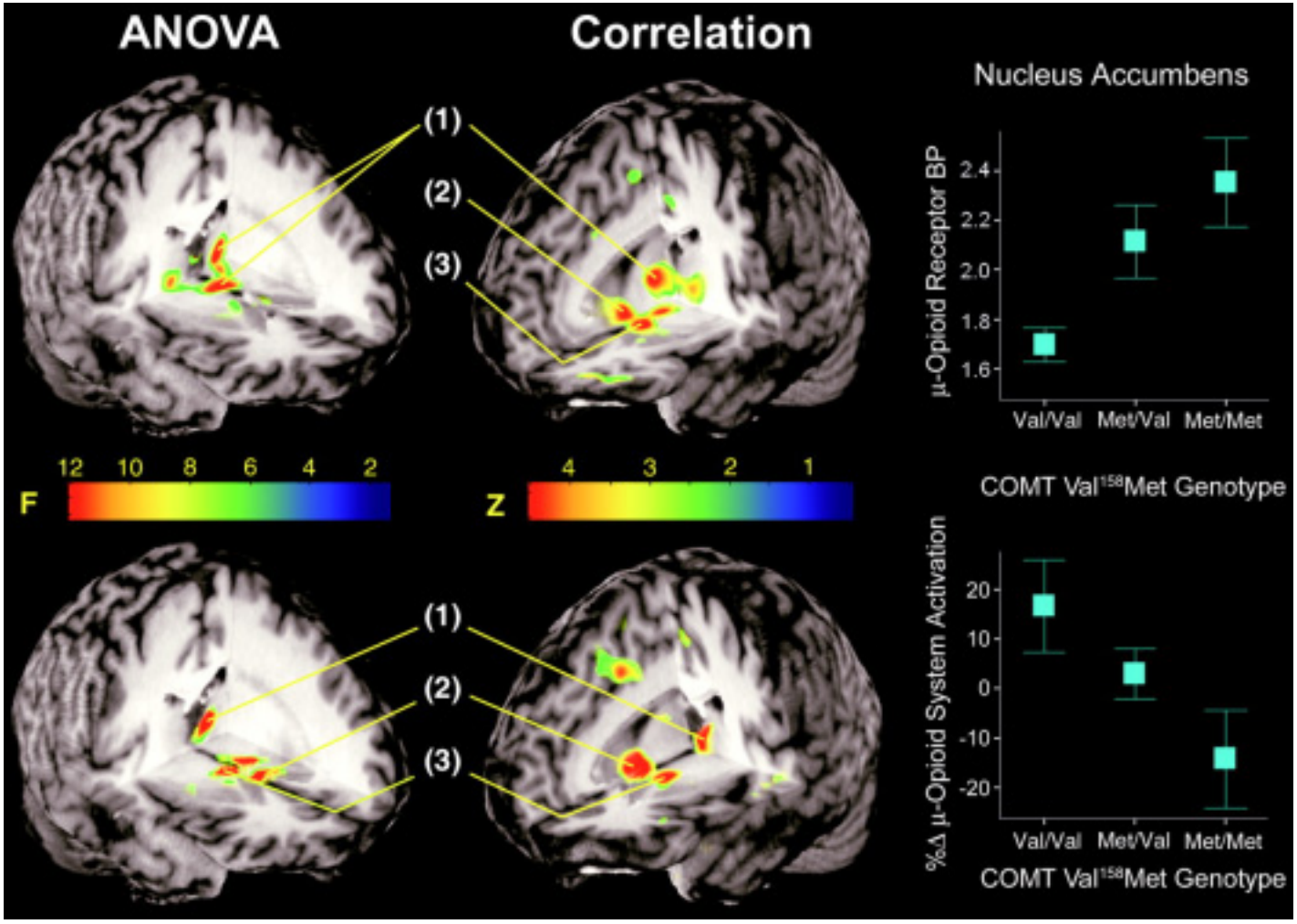
Biological modulators

COMT

- Cathecol-O-methyltransferase metabolizes cathecolamines
- Frequent polymorphism results in a valine (Val) to methionine (Met) substitution
- Variant leads to a low-function enzyme which fails to degrade dopamine, which may cause a depletion of enkephalin and compensatory upregulated expression of MOR

COMT *val158met* Genotype Affects -Opioid Neurotransmitter Responses to a Pain Stressor

Science **299**, 1240 (2003);



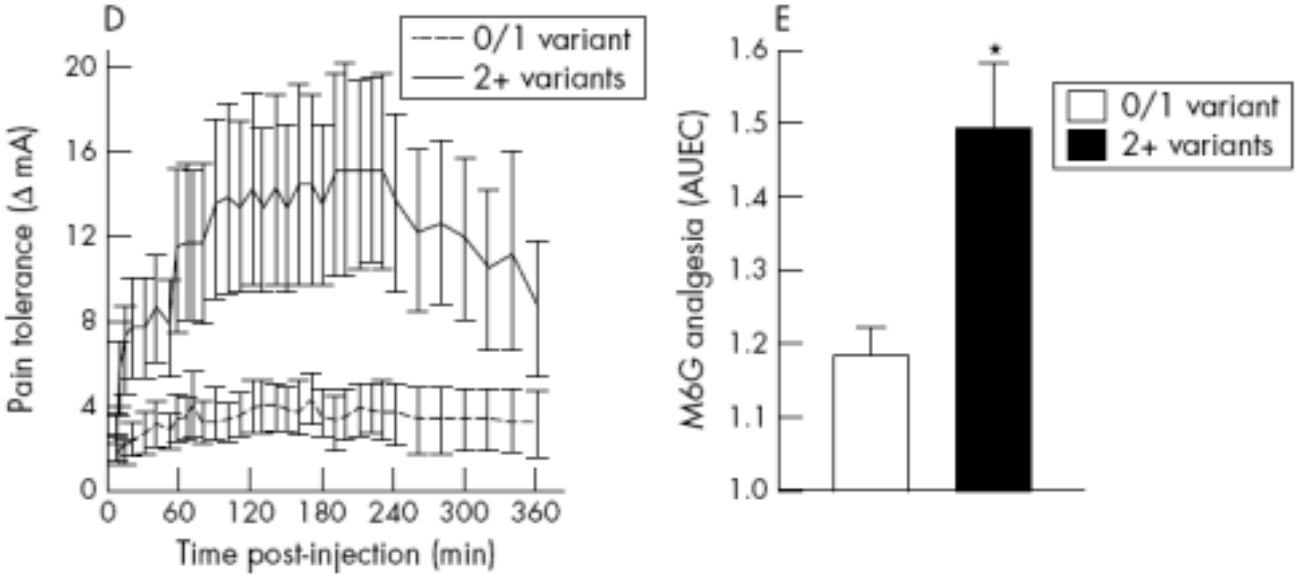
Melanocortin-1 receptor

- Analgesic effects of morphine and M6G are increased in humans with non-functional alleles due to less anti-opiod action
- Seems specific for women

Melanocortin-1 receptor gene variants affect pain and μ-opioid analgesia in mice and humans

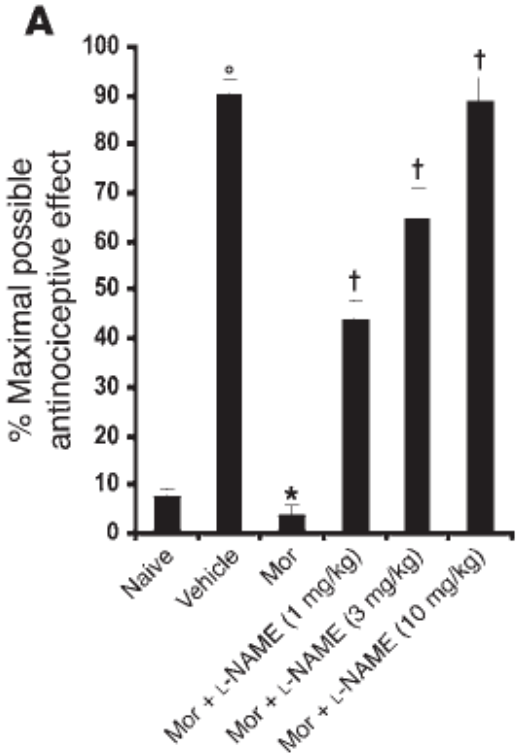
J S Mogil, J Ritchie, S B Smith, K Strasburg, L Kaplan, M R Wallace, R R Romberg, H Bijl, E Y Sarton, R B Fillingim and A Dahan

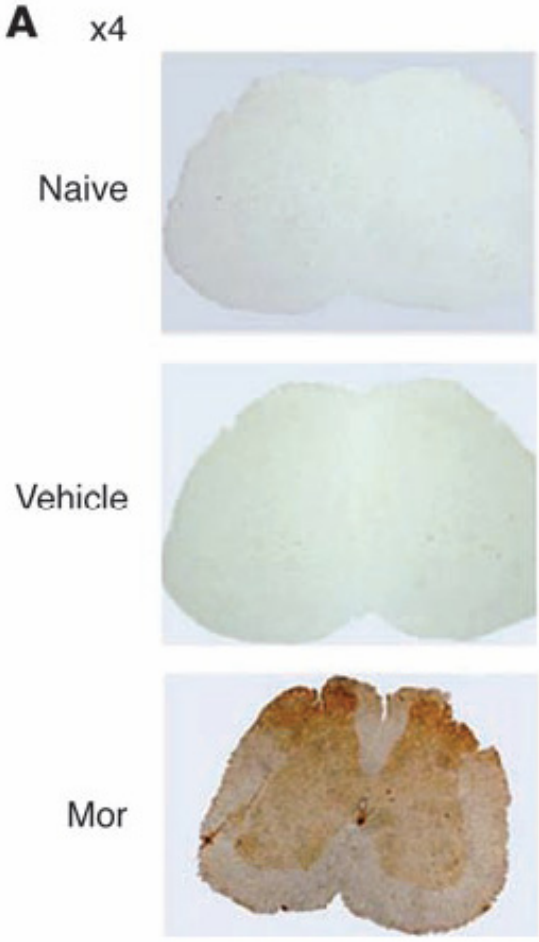
J. Med. Genet. 2005;42;583-587
doi:10.1136/jmg.2004.027698

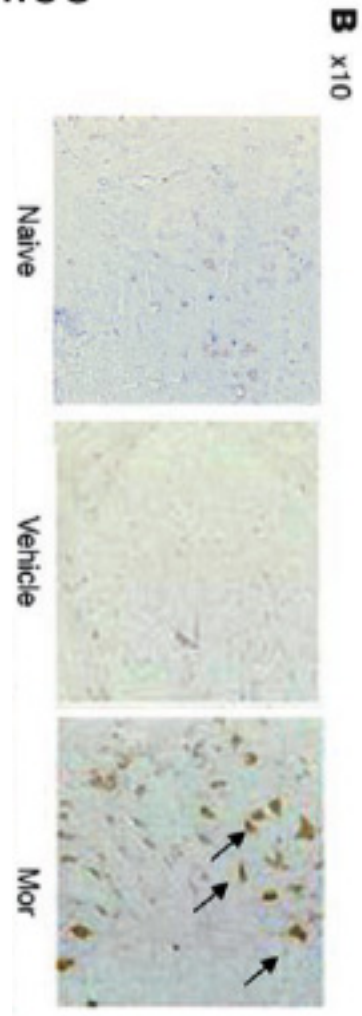


Non functional MC1R is associated with increased pain tolerance and analgesic response to M6G

Therapeutic manipulation of peroxynitrite attenuates the development of opiate-induced antinociceptive tolerance in mice







7. Pharmacogenetics of opioids

Summary

Gene	Variant[a]	SNP reference ID
OPRM1 (μ-opioid receptor)	118A > G	rs17181017
COMT (catechol-*O*-methyl transferase)	472G > A	rs4680
MC1R (melanocortin-1 receptor)	29insA	–
	451C > T	rs1805007
	478C > T	rs1805008
	880G > C	–
CYP2D6 (cytochrome P450 2D6)	2549A > del	–
	1846G > A	rs28371711, rs3892097
	Gene deleted	–
	1707T > del	rs28371709
	2935A > C	–
	1758G > T	–
	Gene duplication/ amplification	–
ABCB1 (P-glycoprotein)	3435C > T	rs1045642

Current evidence for a genetic modulation of the response to analgesics

For carriers of one variant or a combination of variants

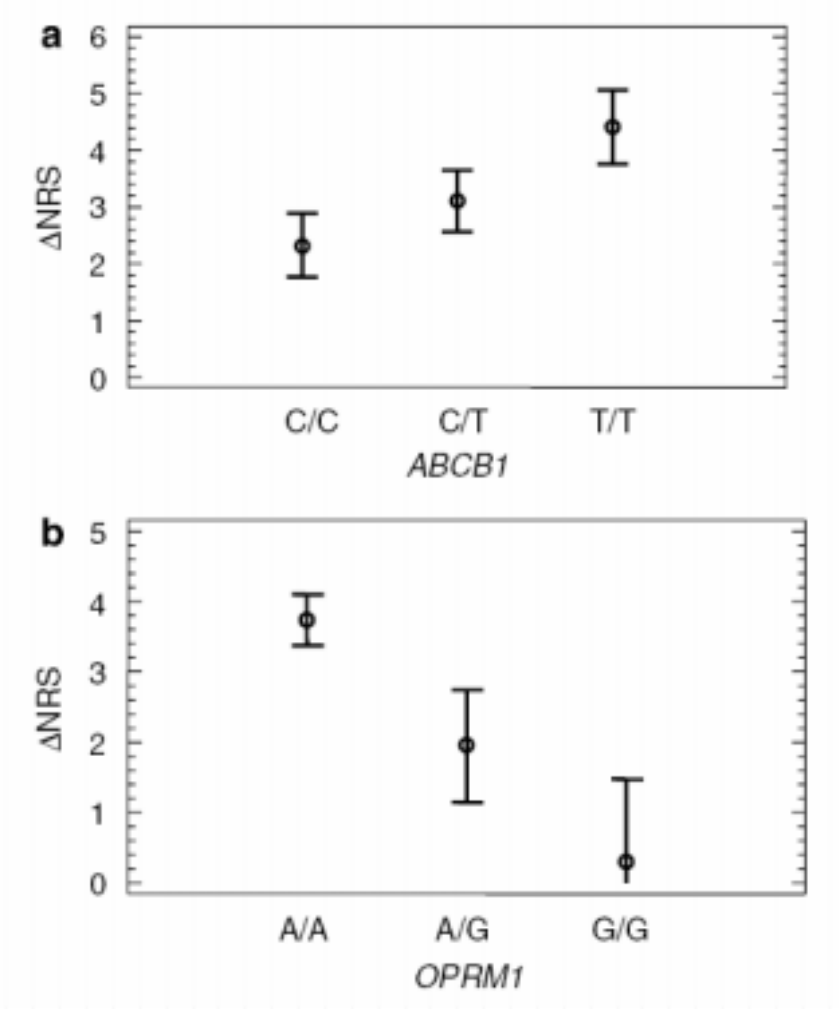
Pharmacogenetic variant with dose adaptation factor			Resulting factor, by which the individual dose may be adapted
OPRM1 118G	*COMT* 472A	2 × *MC1R* nonfunctional SNP	
2	–	–	2
–	0.67	–	0.67
–	–	0.67	0.67
2	0.67	–	1.33
2	–	0.67	1.33
–	0.67	0.67	0.44
2	0.67	0.67	0.89

PAIN

Pain 121 (2006) 1–5

Association of *ABCB1/MDR1* and *OPRM1* Gene Polymorphisms With Morphine Pain Relief

(in press)



145 pts after 1 week of morphine

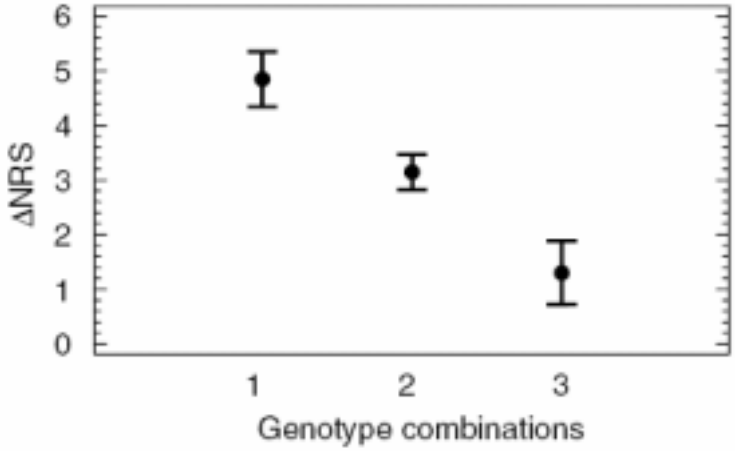


Figure 4 Pain intensity decrease experienced by patients according to their combined genotypes for the two genes: 1 = *OPRM1*: A/A and *ABCB1*: T/T; 2 = *OPRM1*: A/A and *ABCB1*: C/C or C/T; 3 = all other allele combinations. ΔNRS (±CI 95%) as computed by means of multifactor analysis of variance.

Additional players

- Opioids receptors are each coupled with several effector systems including adenylyl cyclases, voltage-dependent Ca channels and G-protein-activated K (GIRK) channels through their interaction with Gi/o protein
- Several known anti-opioid systems, such as nociceptin/orphanin FQ and glutamate-mediated systems
- A variety of neuropeptides act as excitatory neurotransmitters on pain neurons, like subtance P, calcitonin gene-related peptide, somatostatin, cholecystokinin
- Newly discovered

Opioid, cheating on its receptors, exacerbates pain

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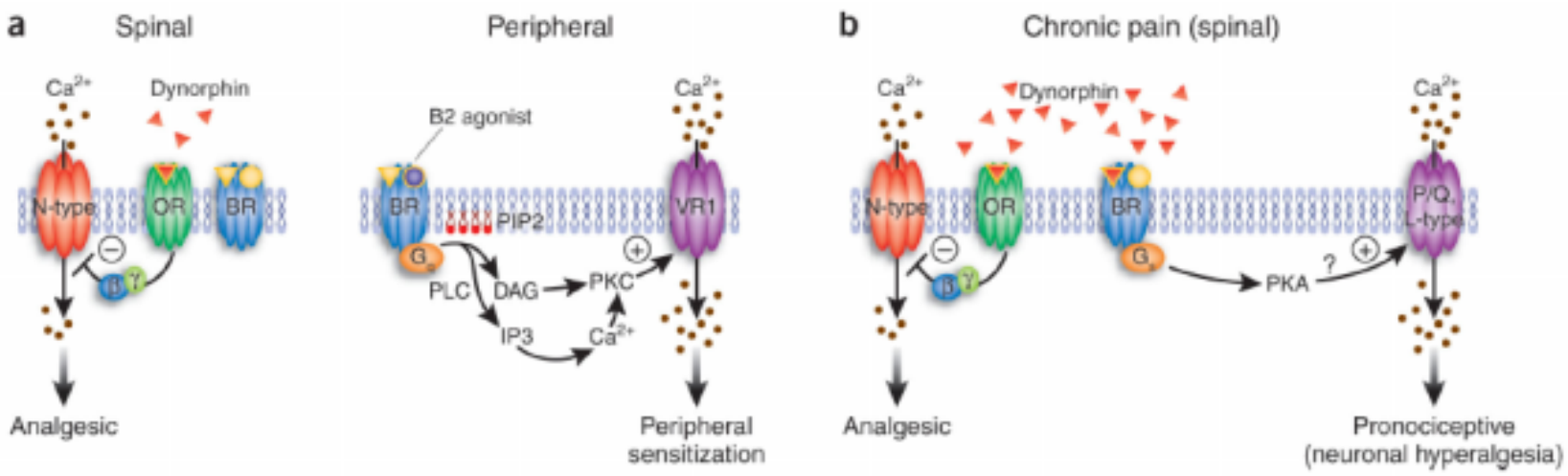
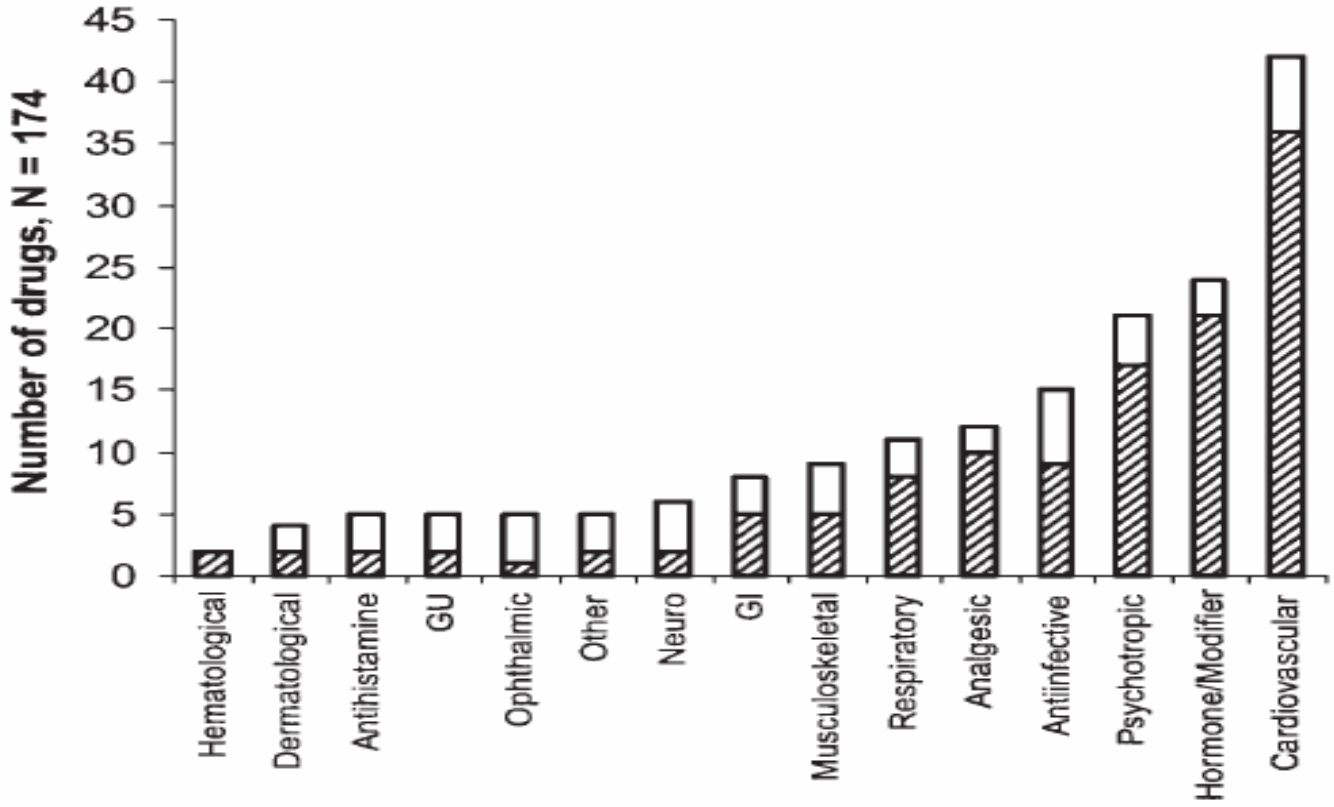


8. Pharmacogenetics of opioids

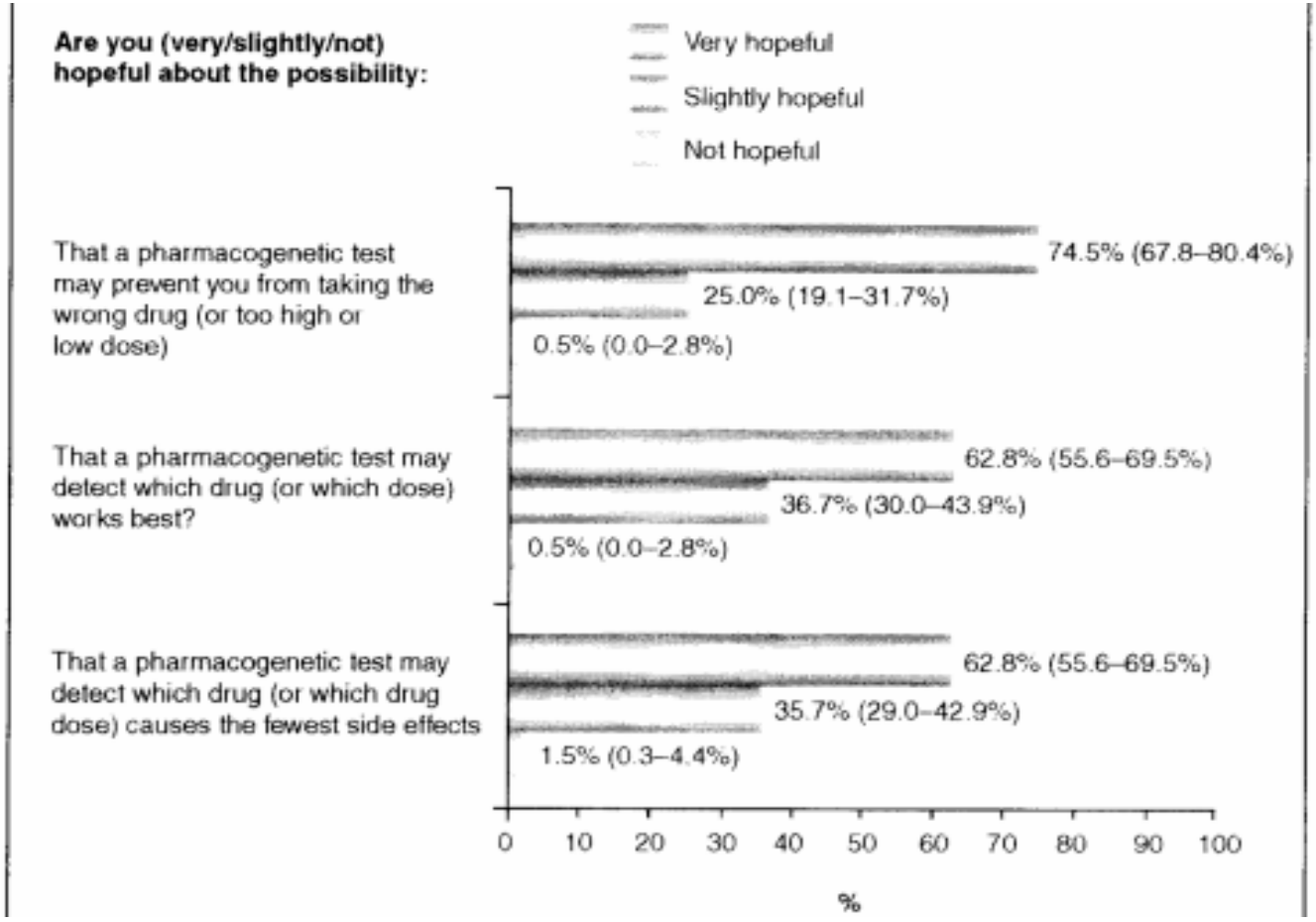
Conclusions

Pharmacogenomics Information in the Published Literature

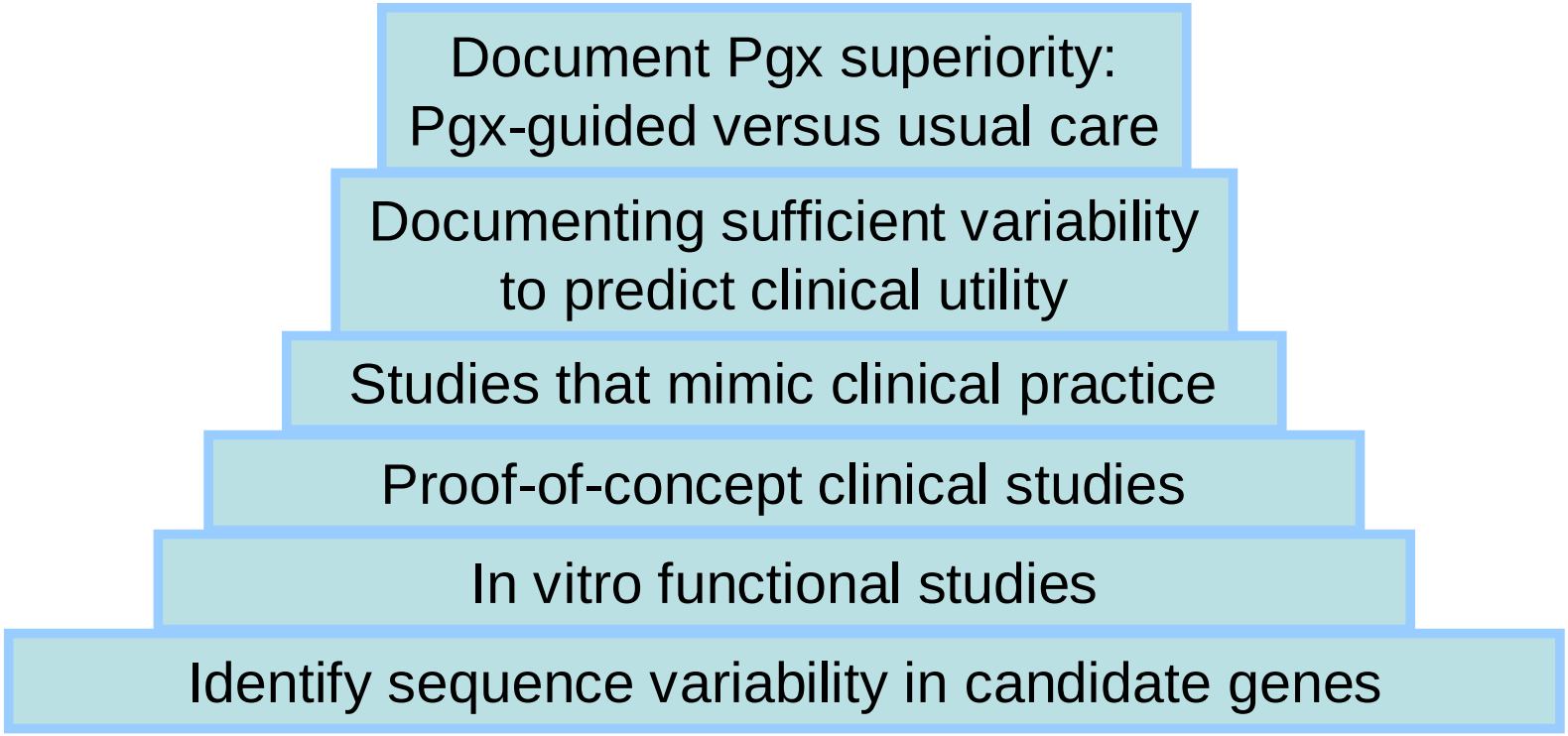


Zineh I et al. Ann Pharmacother. 2006; 40: 639-44

Rogausch et al., *Pharmacogenomics* **7**, 49-59, 2006



Moving Pharmacogenomics to Clinical Practice



Johnson JA. Trends in Genetics 2003; 19: 660-66

Coming future

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

OXY-1: The Pharmacogenetics of Oxycodone Analgesia in Postoperative Pain

This study is currently recruiting participants.
Completion date: december 2007

Sponsored by:	**Odense University Hospital**
Information provided by:	Odense University Hospital
ClinicalTrials.gov Identifier:	NCT00260260

1. Responder (satisfaction with pain treatment in questionnaire and no escape medication) Responder status compared to CYP2D6 genotype

2. Registration of pain, side effects and total amount of oxycodone given compared to CYP2D6 genotype and SNPs

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