Pharmacogenetics of Tobacco Smoking and Lung Cancer

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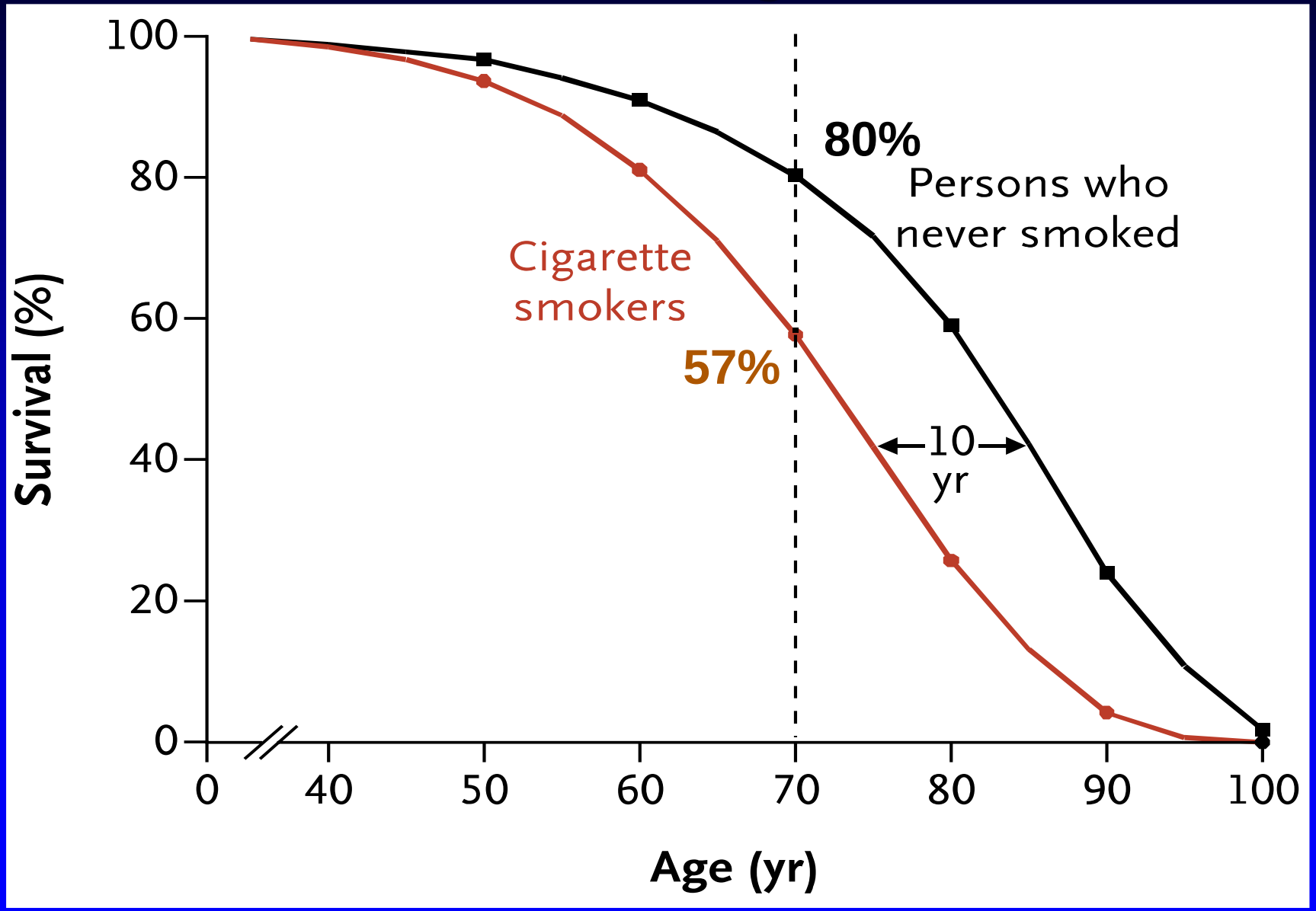
CANCER CENTER

GAME-ON
Consortium

Smokers live 10 years less

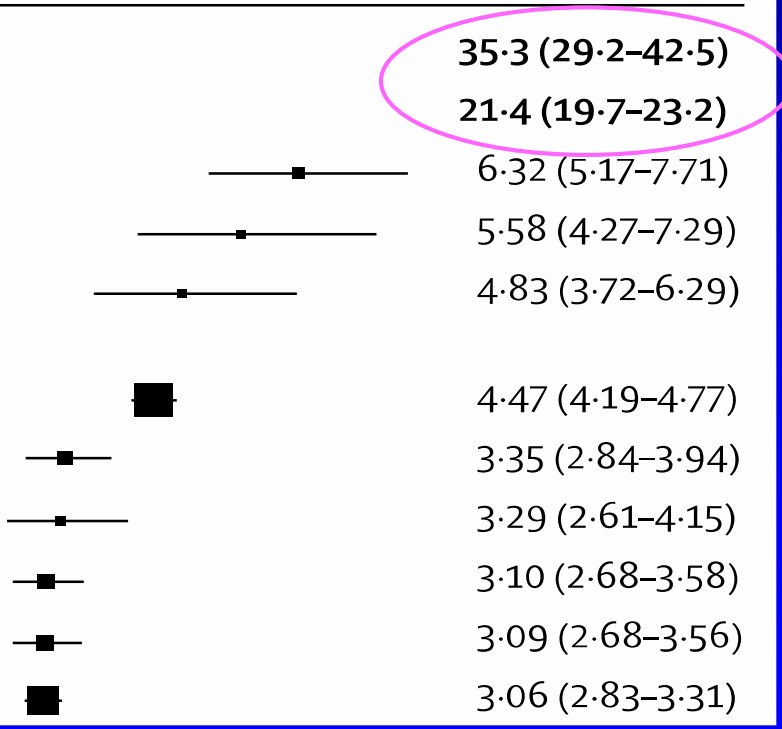


Peto et al., 2010

Smoking: a leading cause of premature death

- Currently:
  - >1 billion smokers globally
  - 6 million death each year
- Projected: 1 billion deaths during 21st century

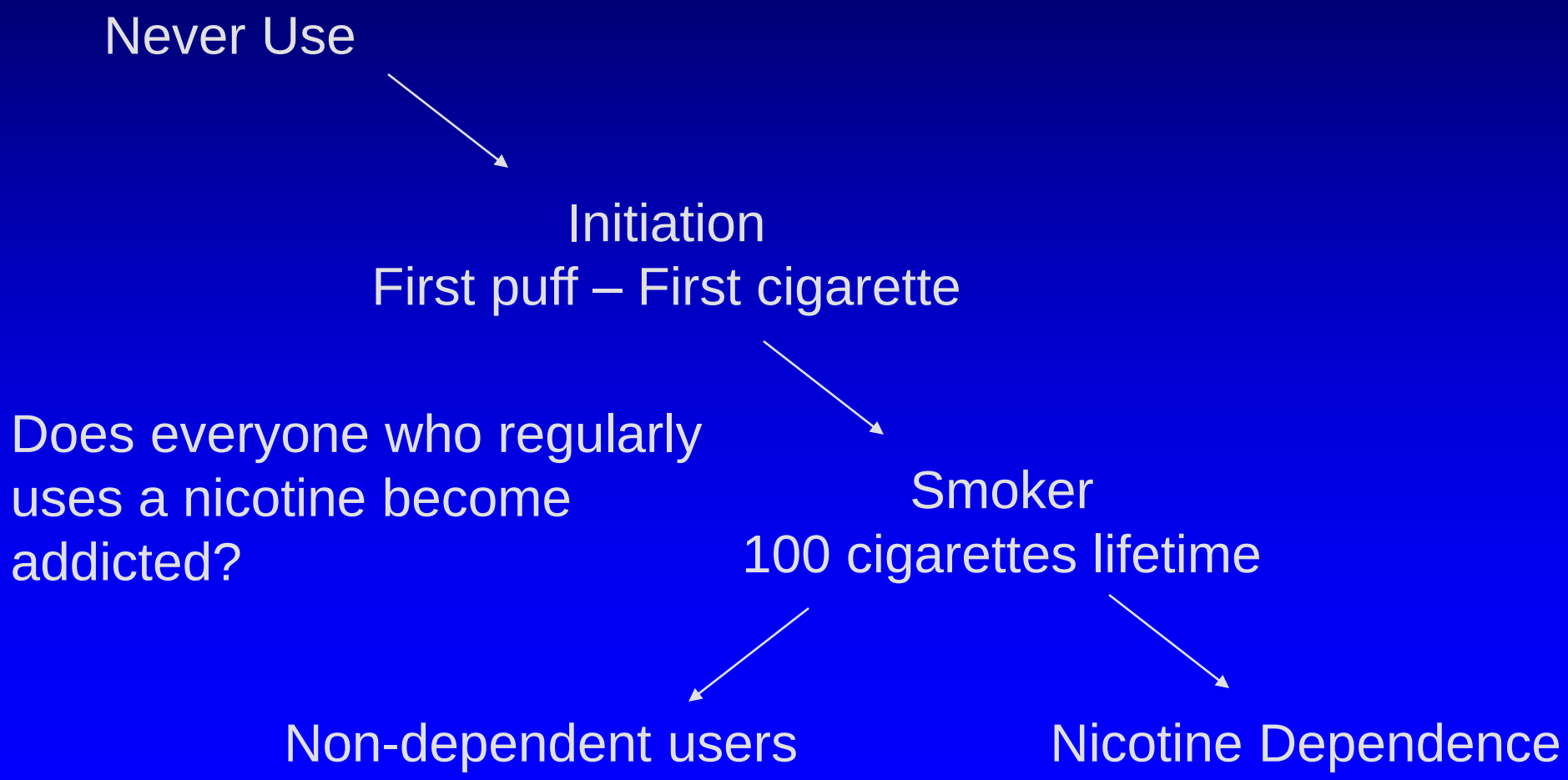
	Number of deaths		RR (95% CI)
	Current smoker	Never smoker	
Chronic lung disease (J40–44)	1789	121	**35·3 (29·2–42·5)**
Cancer of lung (C34)	5633	698	**21·4 (19·7–23·2)**
Aortic aneurysm (I71)	330	164	6·32 (5·17–7·71)
Intestinal ischaemia (K55)	183	91	5·58 (4·27–7·29)
Cancer of mouth, pharynx, larynx, nasal cavity, or sinuses (C00–14,30–32)	204	91	4·83 (3·72–6·29)
Coronary heart disease (I21–25)	2726	1732	4·47 (4·19–4·77)
Cirrhosis or alcoholic liver (K70,74)	478	256	3·35 (2·84–3·94)
Cancer of bladder (C67)	178	156	3·29 (2·61–4·15)
Cancer of oesophagus (C15)	450	397	3·10 (2·68–3·58)
Pneumonia (J12–18)	494	408	3·09 (2·68–3·56)
Cerebrovascular disease (I60–69)	1528	1458	3·06 (2·83–3·31)



Pirie et al., 2010


I miss my lung, Bob.
California Department Of Health Services.
Funded By The Tobacco Tax Initiative.

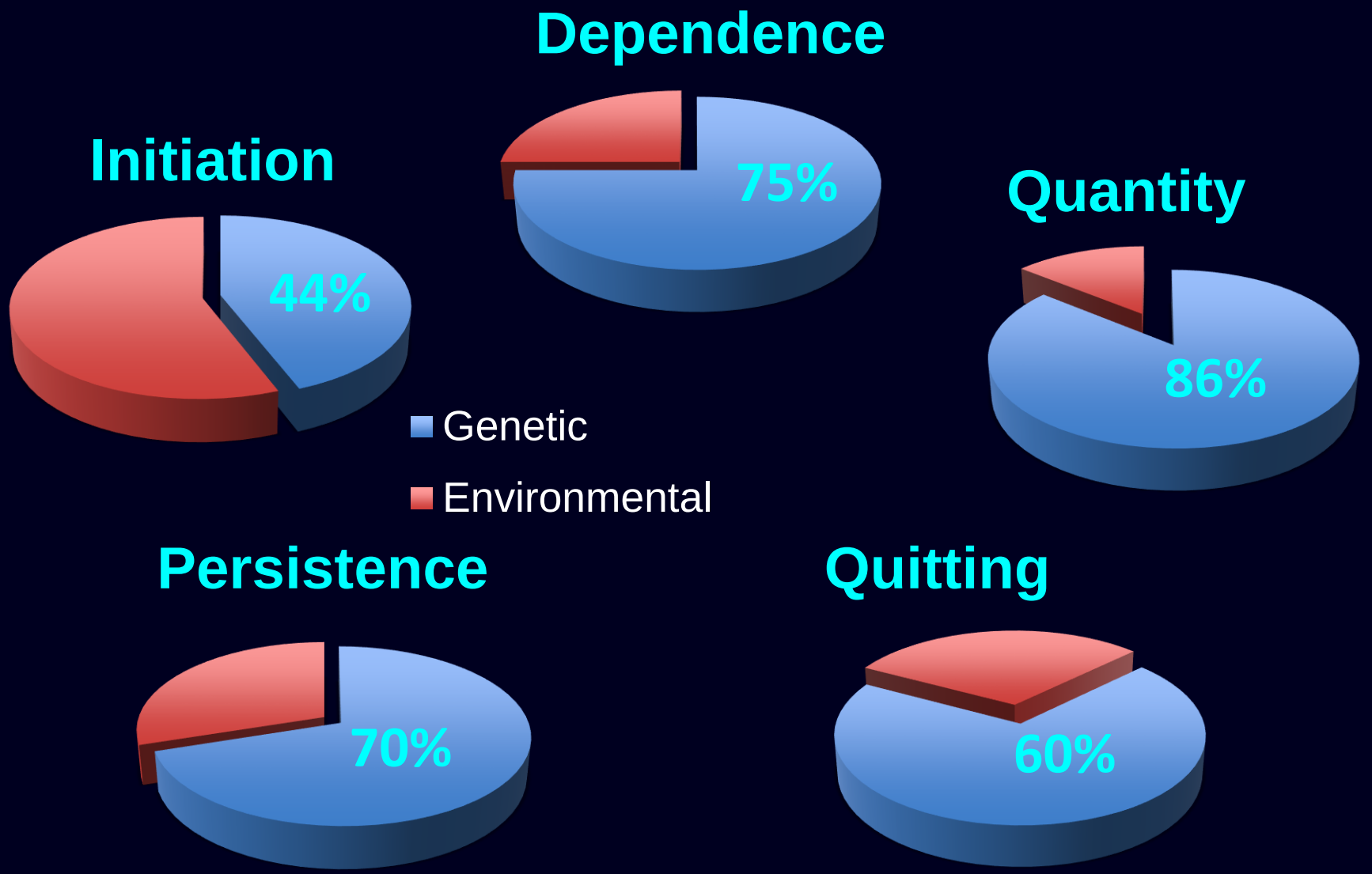
Model of Nicotine Dependence - A many step process



U.S. Population Screening and Nicotine Dependence

Collaborative Genetic Study of Nicotine Dependence, Bierut

Genetics of Smoking



Vink, *et al.* 2005; True, *et al.* 1997, Koopmans, *et al.* 1999

Table 1 Heritability estimates for different drugs of abuse

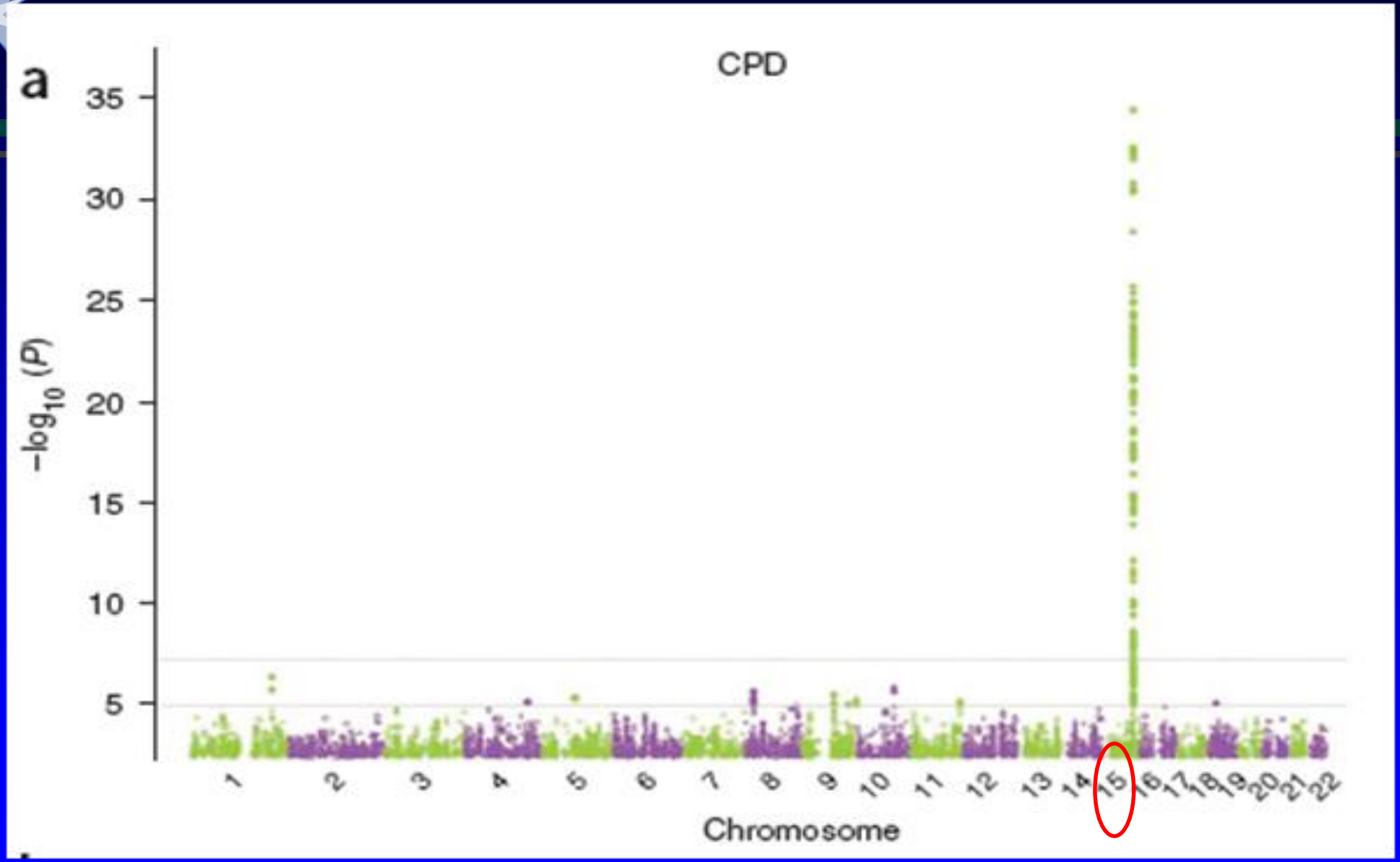
Phenotype	Heritability estimates
Smoking	
Persistence	28–84%
Cigarette consumption	45–86%
Nicotine dependence	31–75%
Nicotine withdrawal symptoms	26–48%
Smoking cessation	50–58%
Alcoholism	
Alcohol abuse/dependence	50–70%
Consumption levels	45–58%
Problem drinking	8–50%
Opiates/heroin	
Abuse and/or dependence	43–60%
Sedatives	
Abuse and/or dependence	29–58%
Psychostimulants	
Abuse and/or dependence	42–74%

These studies have been reviewed in greater detail elsewhere.[3,4,6,7,50] A list of the primary references can be found in **Supplementary Table S2** online.

Twin, family and adoption studies in humans, together with animal studies, have provided the foundation for genetic effects on substance use, abuse and dependence.

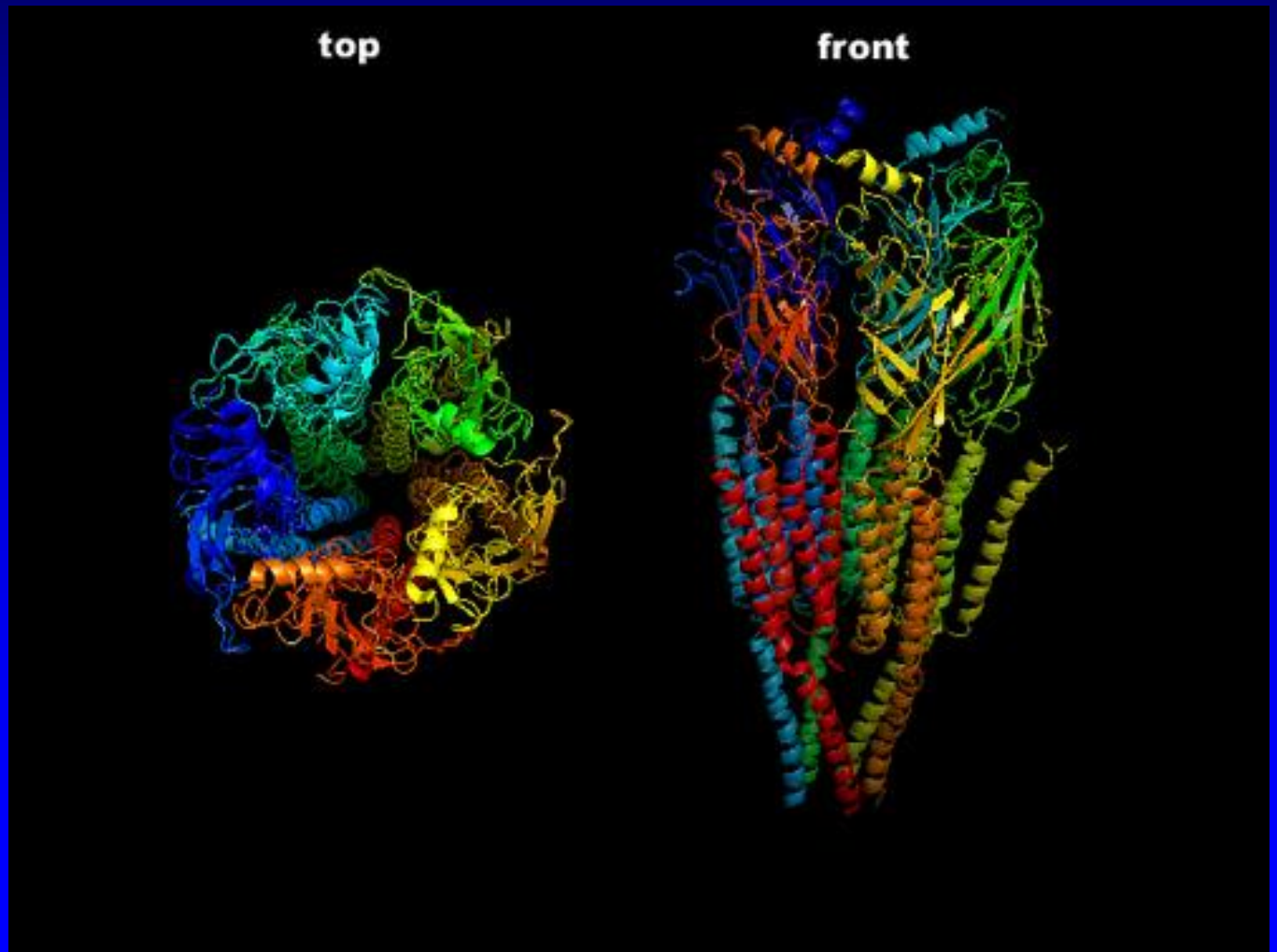
Ho, Goldman, Heinz, Kaprio, Kreek, Li, Munafò, Tyndale.
Breaking barriers in the genomics and pharmacogenetics of drug addiction.
Clin Pharmacol Ther. 2010 Dec;88(6):779-91.

Chromosome 15q25 Is Important for Smoking



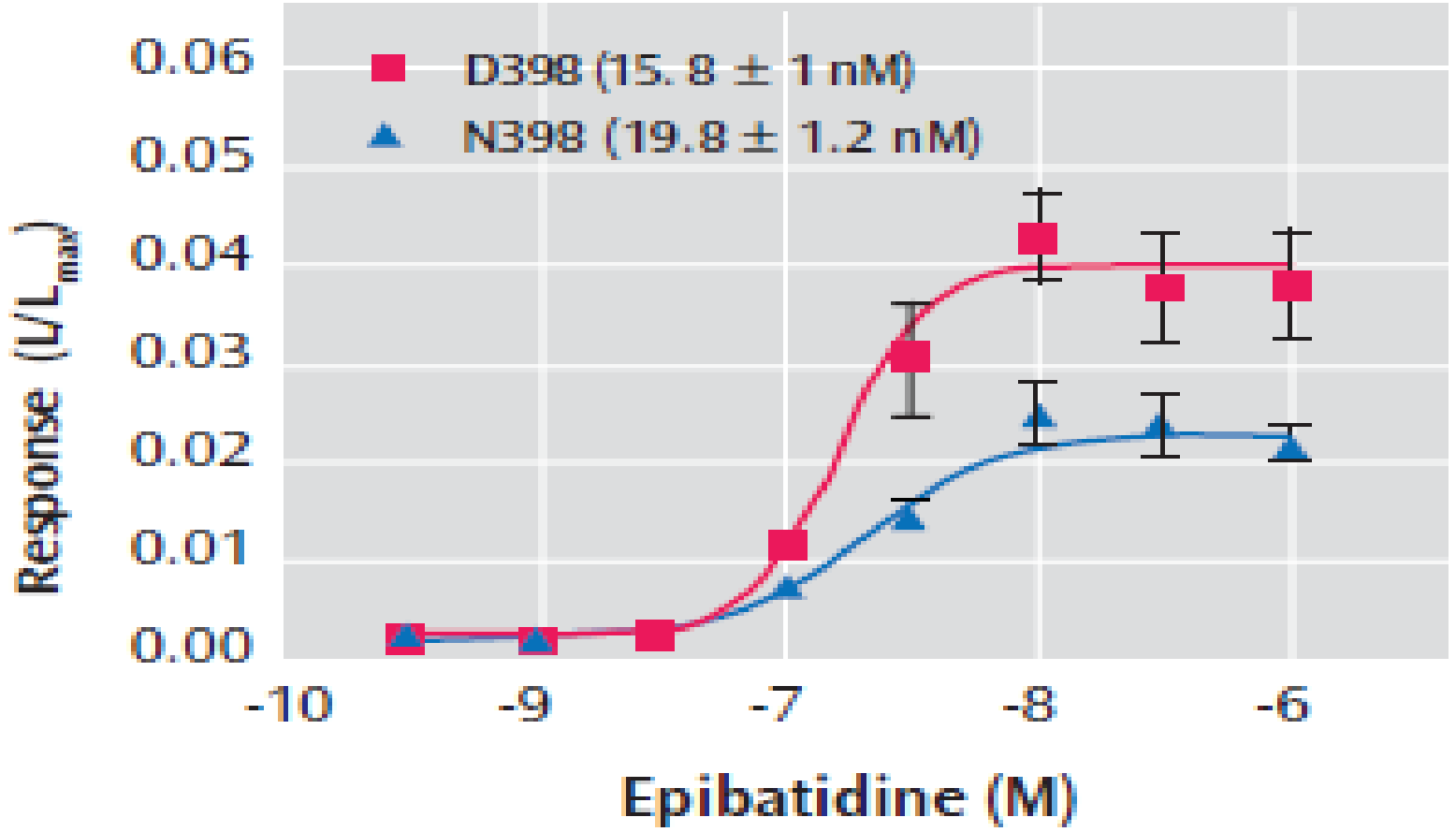
The Tobacco and Genetics Consortium (2010) *Nature Genetics*

Nicotinic Receptors are Homo- or Heteropentamers



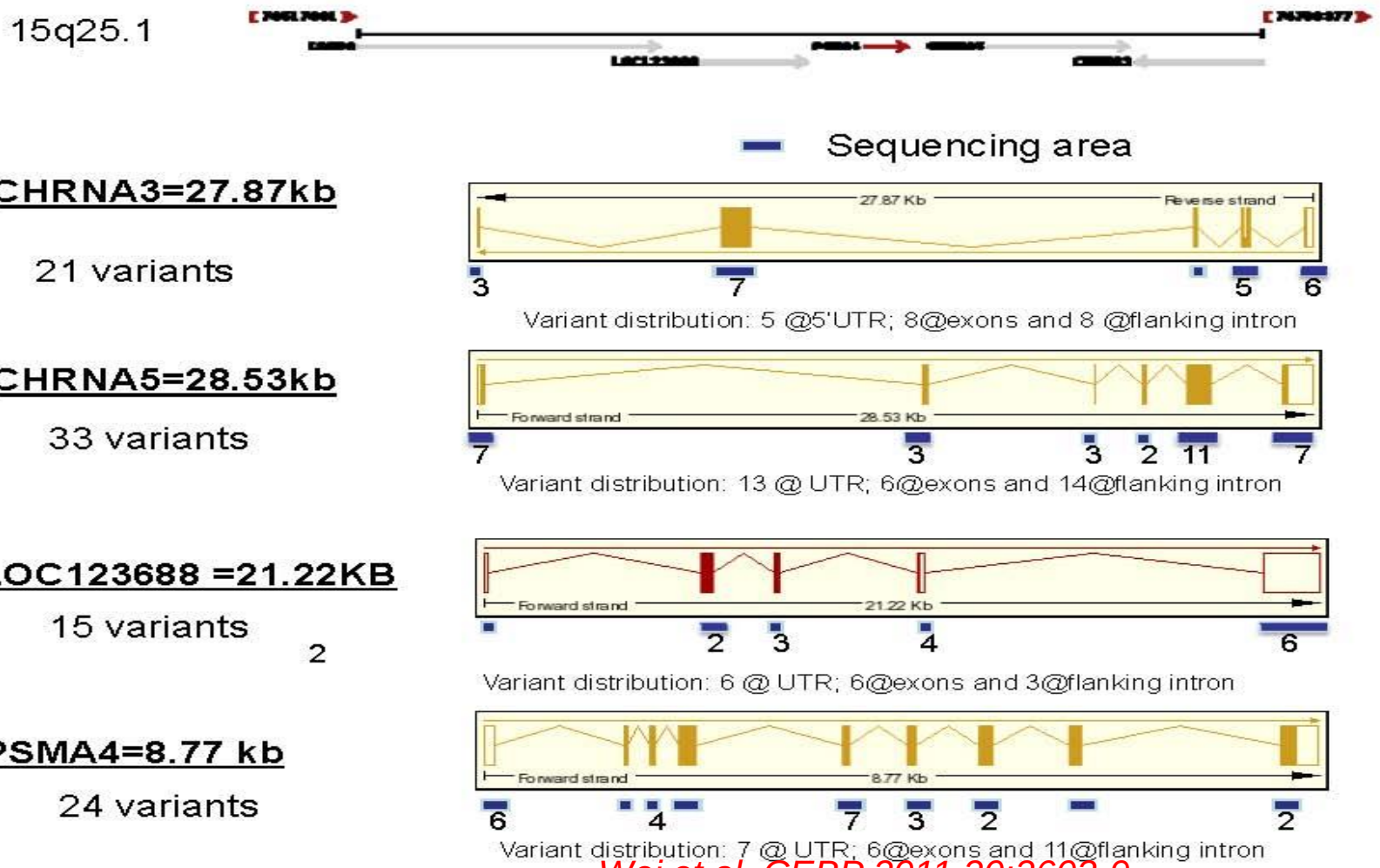
α_3
β_4
α_5
low level expression in brain

SNP rs16969968 affects maximal response to agonist



Bierut, Am J Psych 165:1163, 2008

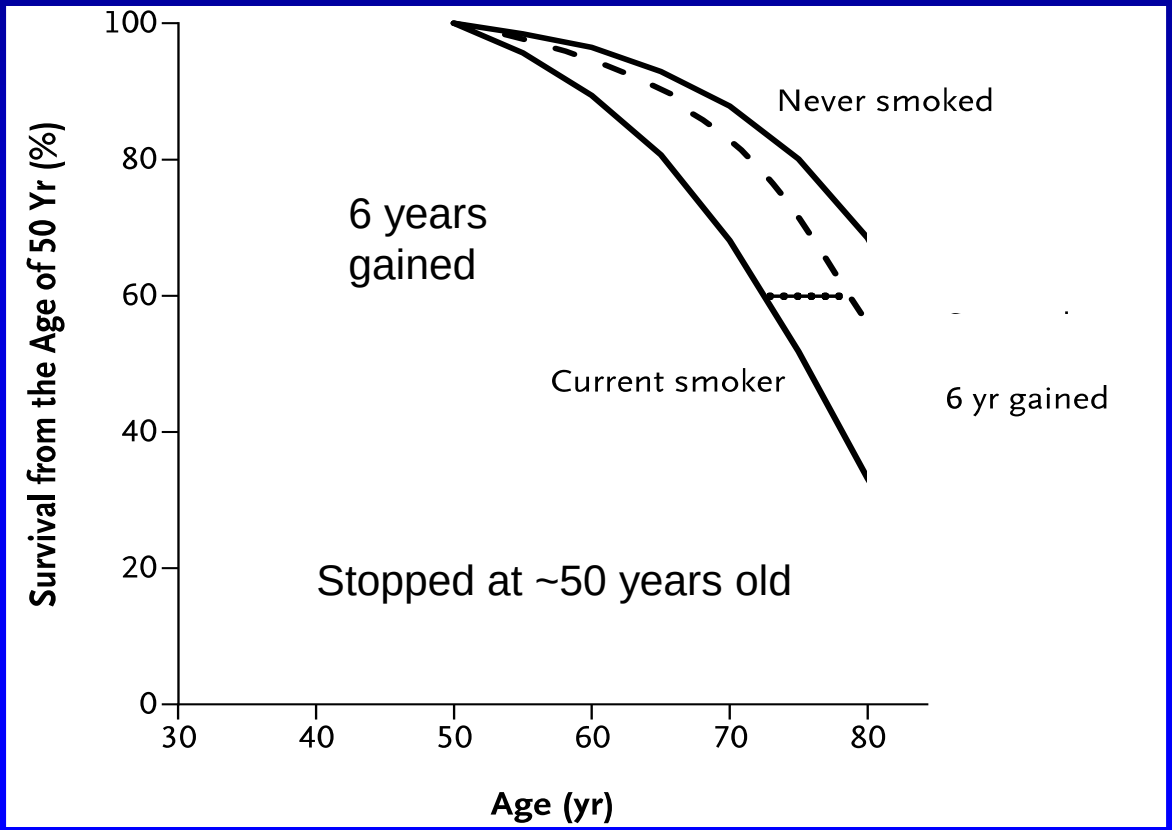
Chromosome $15_q25.1$ region contains specialized nicotinic receptor variants



Wei et al. CEBP 2011 20:2603-9

Smoking Cessation

- 70% of smokers say they would like to quit
- 40% quit for at least 1 day each year, but 80% of them relapse within a month
- Only 3% of smokers quit successfully each year



Benowitz et al., 2010;
Jha et al., 2013

Is *CHRNA5-A3-B4* involved in cessation?

Study Design

U Wisconsin - TTURC

- N=1073, European Ancestry
- Pharmacotherapy arms (Bupropion, NRT, combo) and one placebo arm

- Cessation

Abstinence at 60 days

Time to relapse over 60 days

***CHRNA5-A3-B4* Haplotypes**

- rs16969968

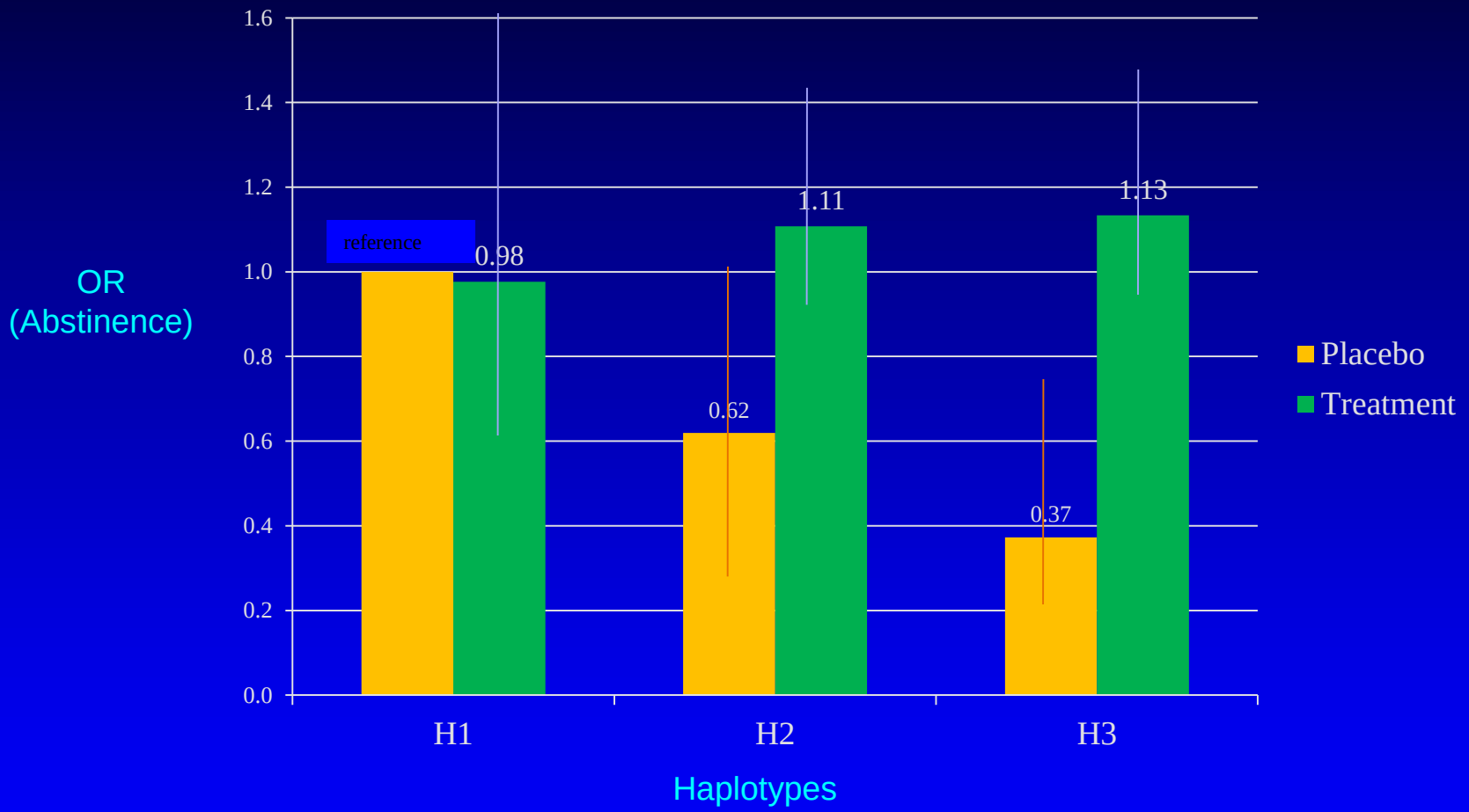
  Non-synonymous coding, Amino acid change in *CHRNA5*

- rs680244

  CHRNA5 mRNA levels in brain and lung

- Combination of 2 variants
  - H1 (G_C, 20.8%)
  - H2 (G_T, 43.7%)
  - H3 (A_C, 35.5%)

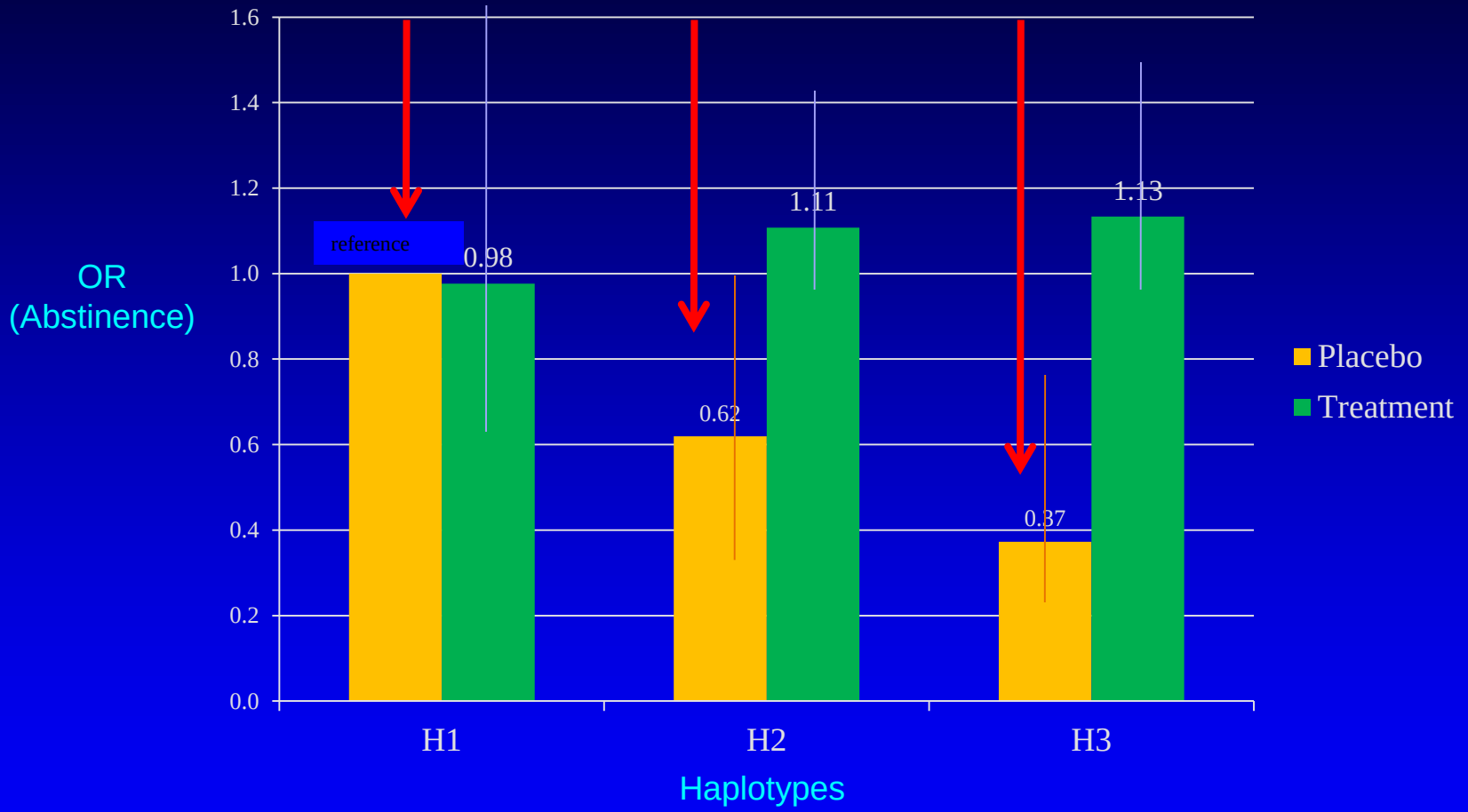
Haplotypes predict cessation and response to medication



Haplotypes (rs16969968, rs680244):
H1=G_C(20.8%)
H2=G_T(43.7%)
H3=A_C(35.5%)

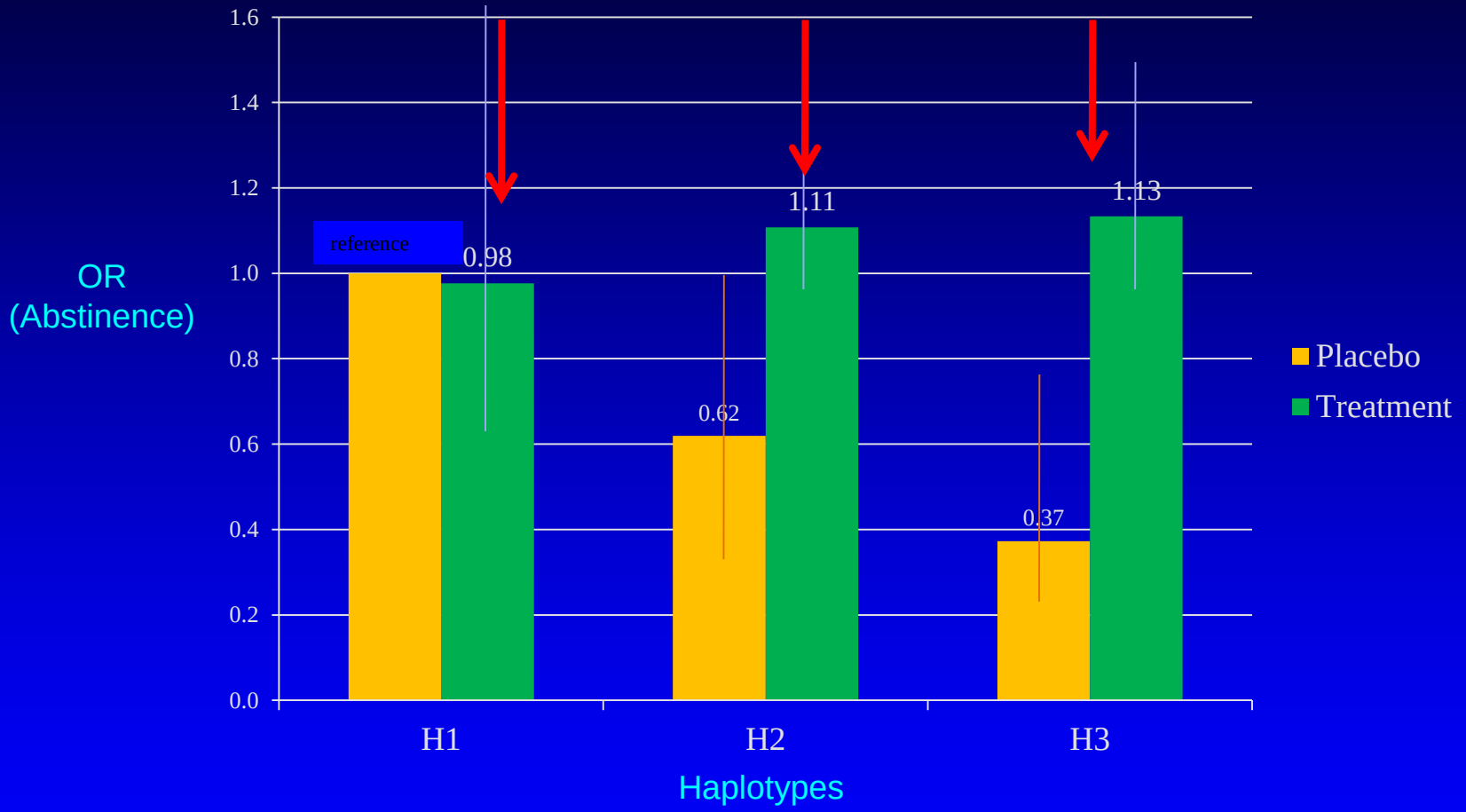
Chen et al, 2012, Am J Psychiatry

Haplotypes predict abstinence in individuals receiving placebo medication



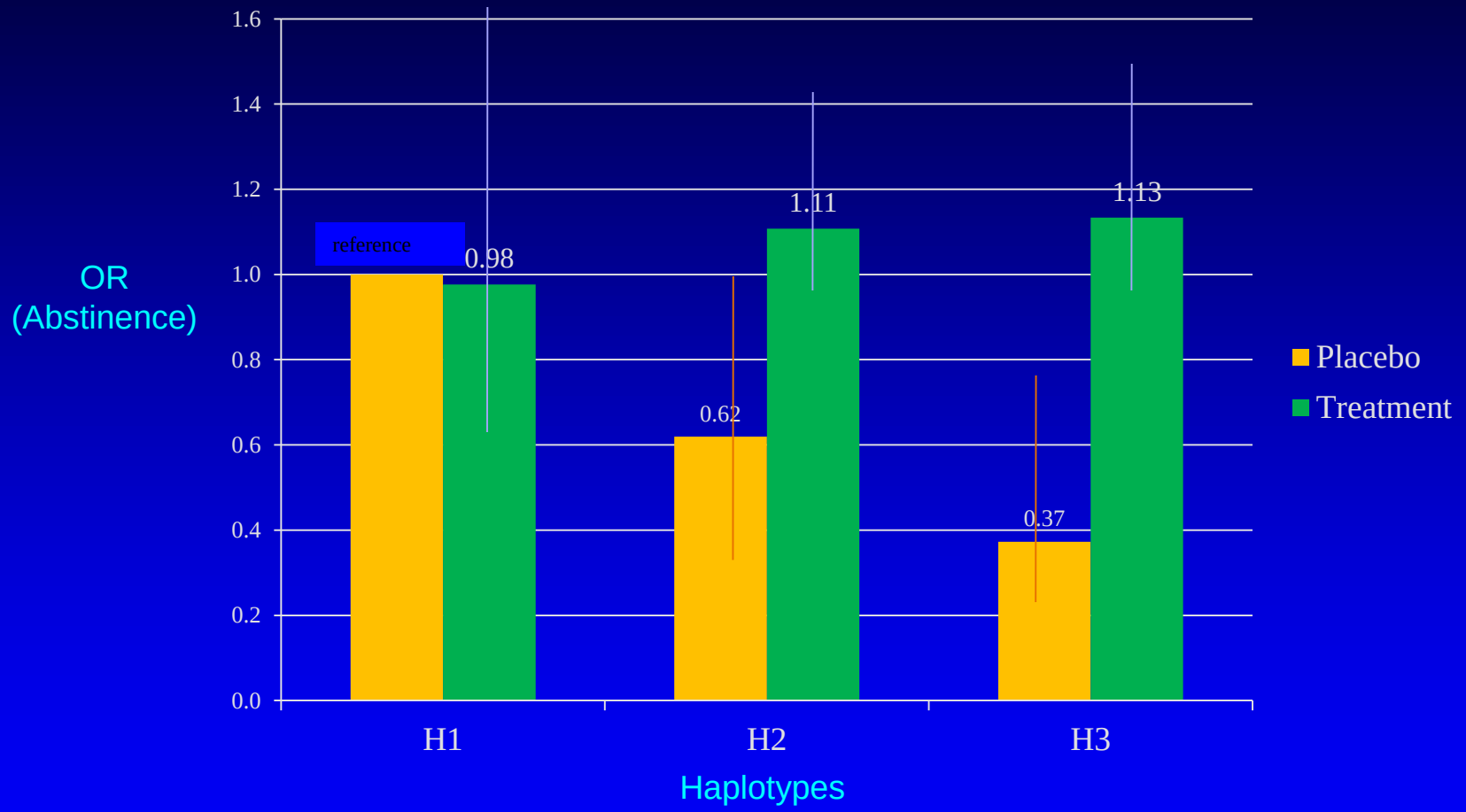
Chen et al, 2012, Am J Psychiatry

Haplotypes do not predict abstinence in individuals receiving active medication



Chen et al, 2012, Am J Psychiatry

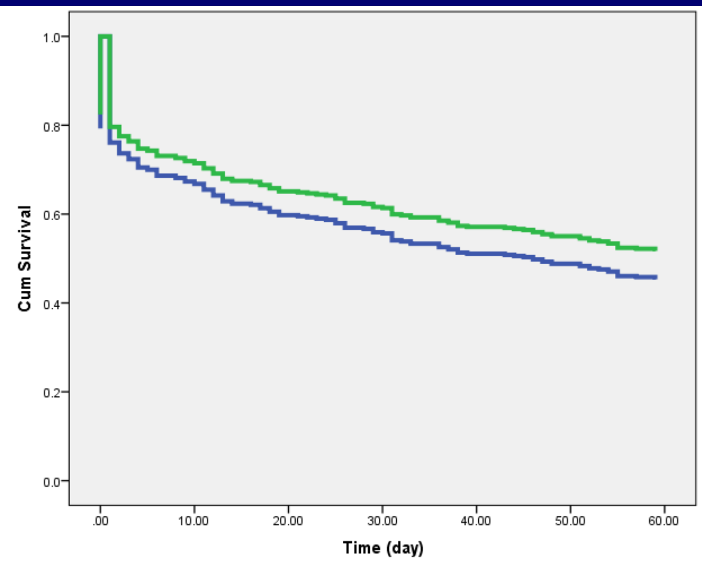
A Significant Genotype by Treatment Interaction



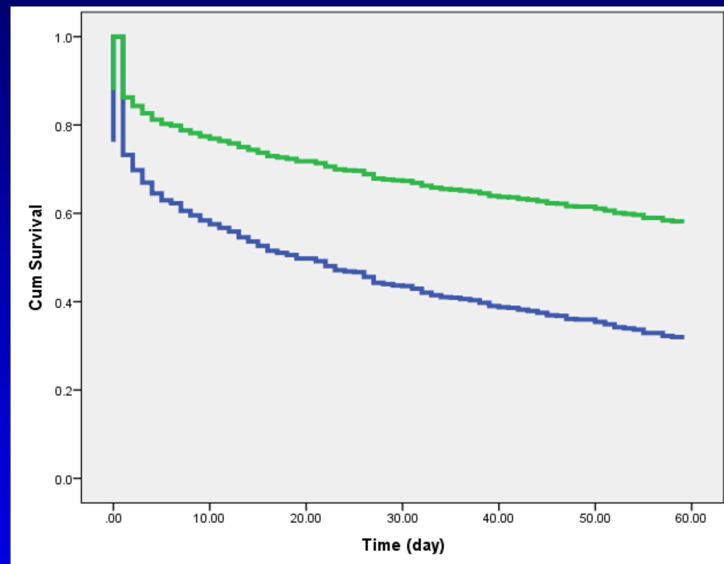
The interaction of haplotypes and treatment is significant (X^2=8.97, df=2, p=0.011)

Response to Treatment Differs by Haplotype

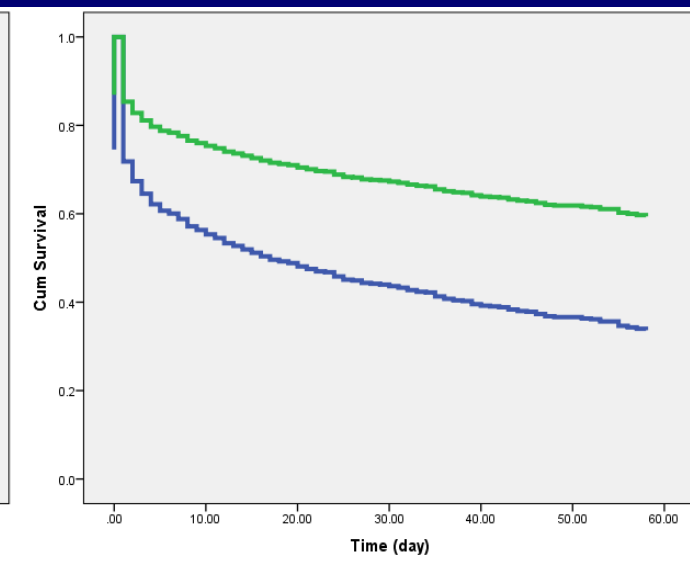
a. Haplotype H1 (G_C)
RH=0.83, p=0.36



b. Haplotype H2 (G_T)
RH=0.48, $p=2.7*10^{-8}$



c. Haplotype H3 (A_C)
RH=0.48, $p=9.7*10^{-7}$



Placebo
Active Treatment

Chen et al, 2012, Am J Psychiatry

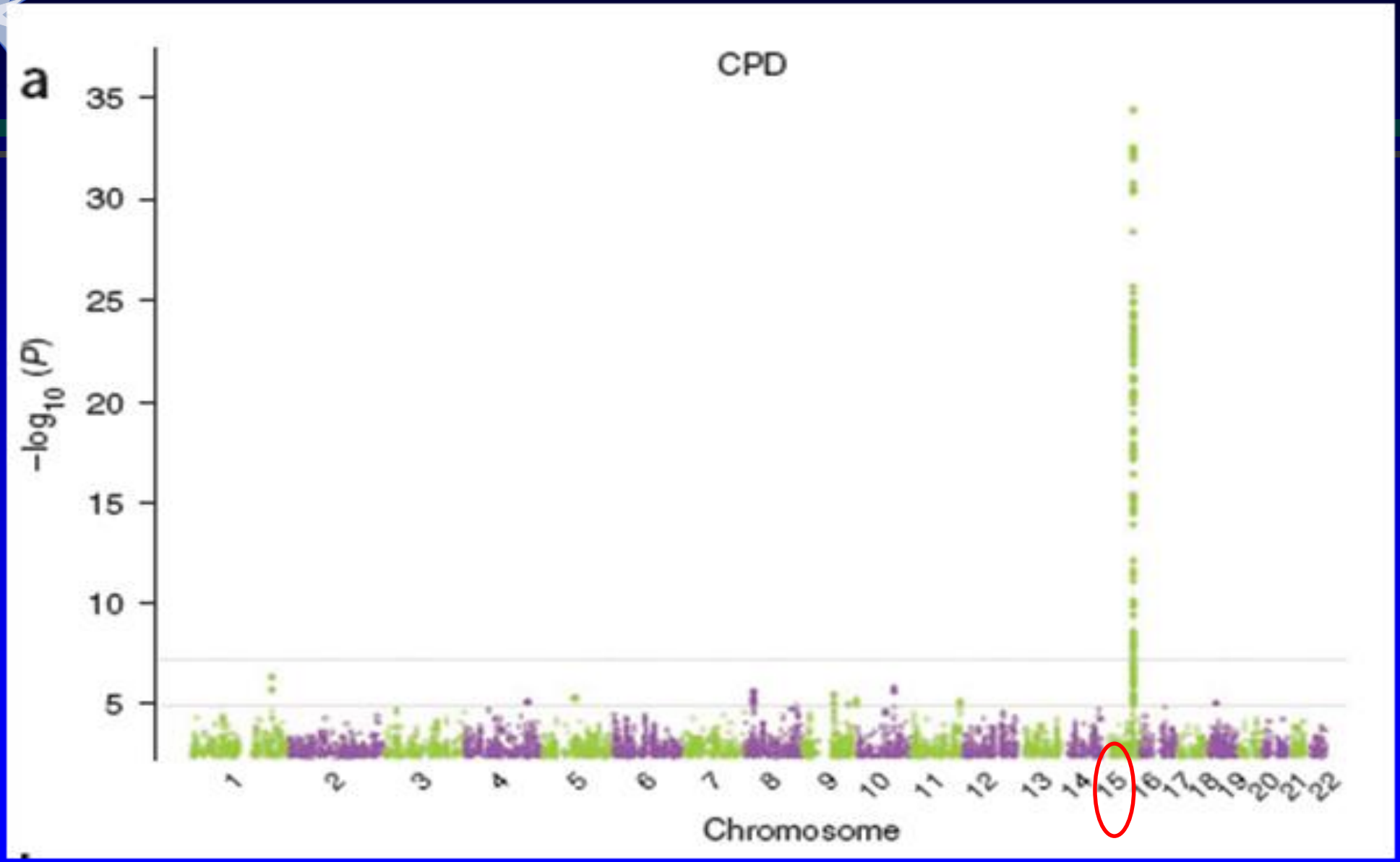
Genetics can predict prognosis & inform treatment

- Smokers with the low risk haplotype (H1/G_C)
  - can quit more successfully without medication
  - do not benefit from medication

- Smokers with the high risk haplotype (H3/A_C)
  - have more difficulty quitting without medication
  - can benefit from medication with a 3-fold increase in cessation success

  - is associated with a 2-year delay in age of quitting

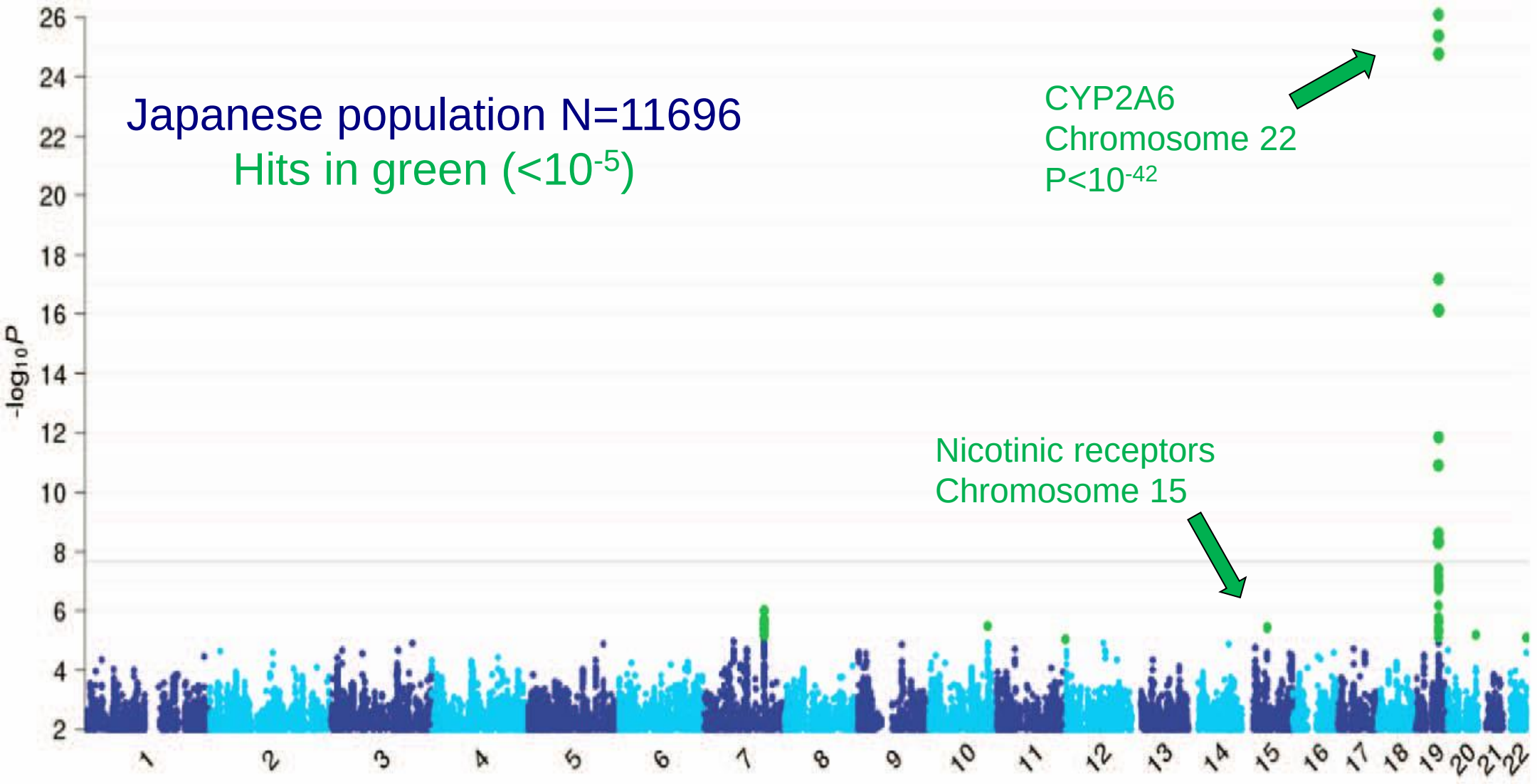
Chromosome 15q25 Is Important for Smoking



The Tobacco and Genetics Consortium (2010) *Nature Genetics*

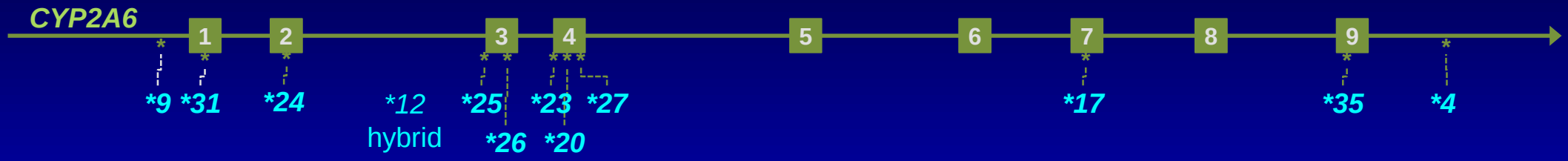
Manhattan plot for all CNPs and SNPs in the genome-wide analysis of Cigarettes per day

Kumasaka, Aoki, Okada, Takahashi, Ozaki, Mushiroda, Hirota, Tamari, Tanaka, Nakamura,Kamatani, Kubo, PLOS one 2012



CYP2A6 Genotyping and Statistical Methods

CYP2A6 genotyping:



- Reduced or null activity alleles *(CYP2A6*4, *9, *17, *20, *23-*27, *31, *35)*
- Grouped by predicted metabolic activity

CYP2A6 normal (54%)

vs.

CYP2A6 reduced (46%)

High

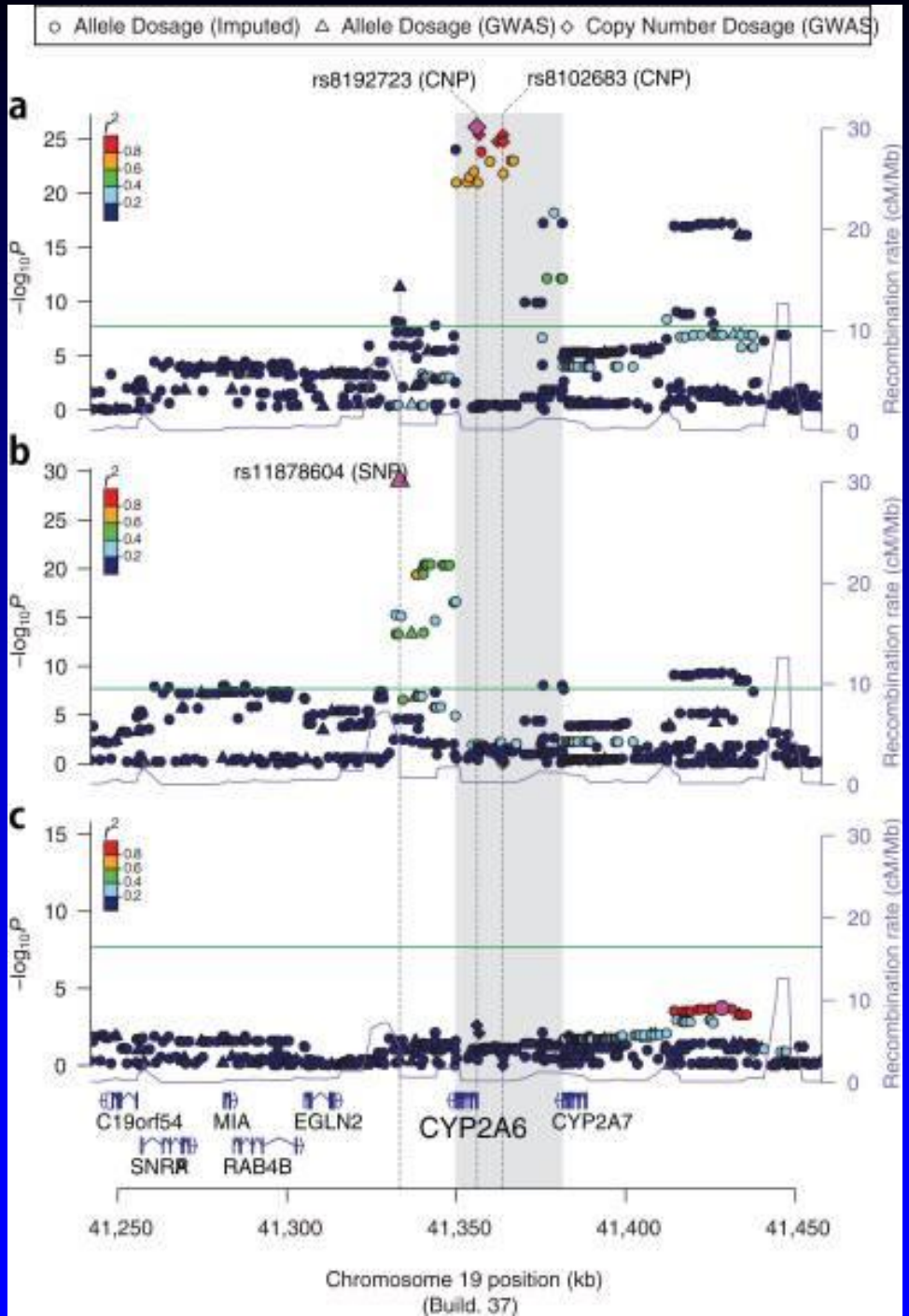
Risk

Low

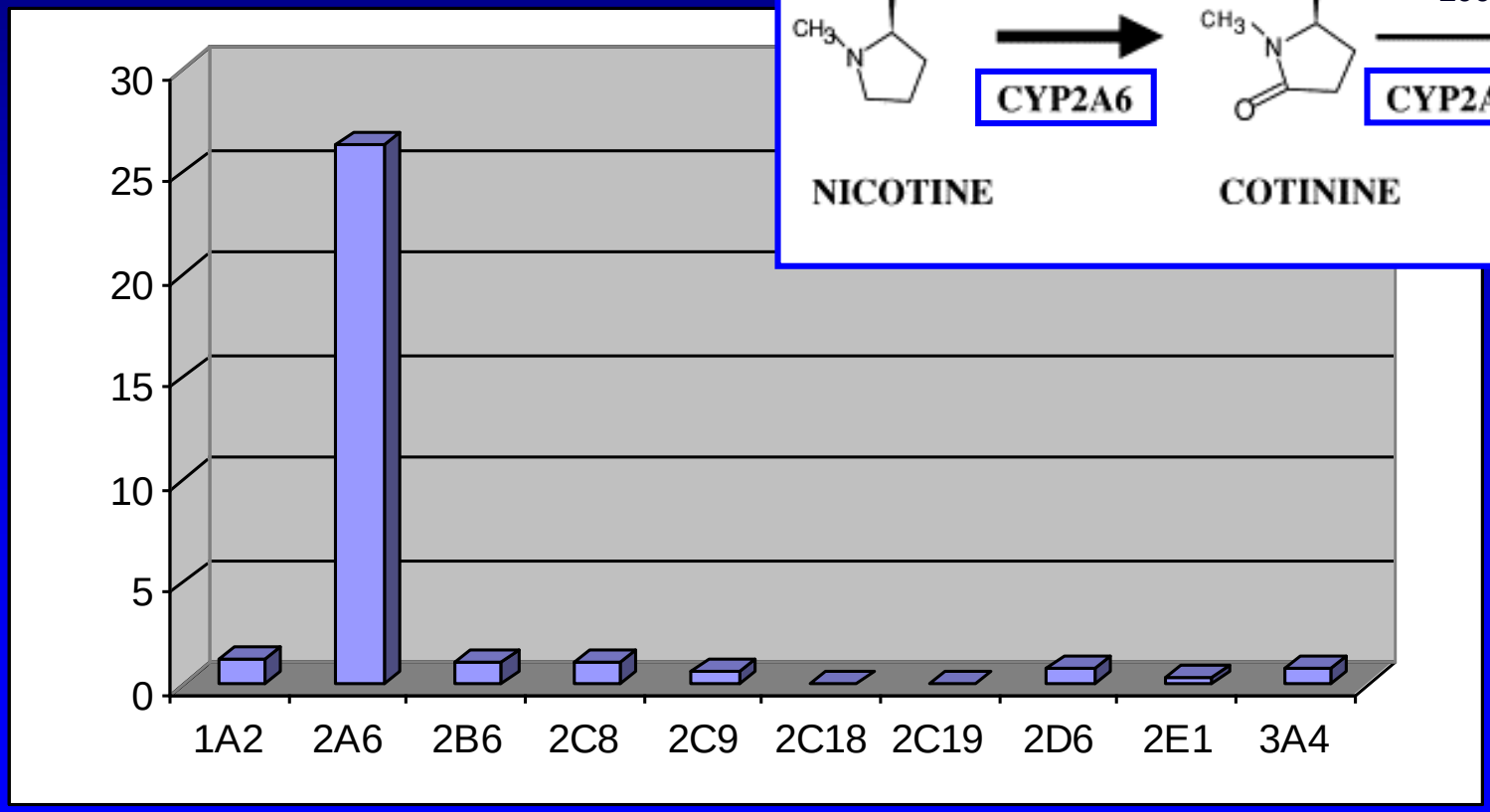
a) Before conditioning

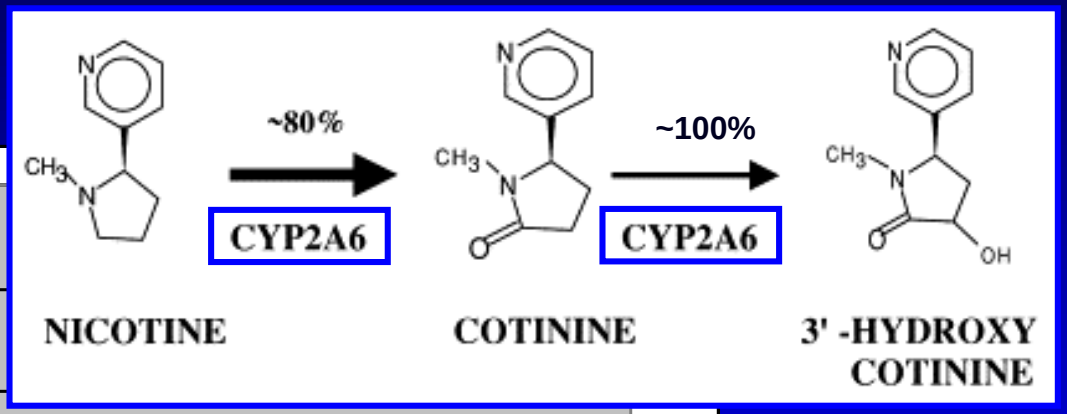
b) After conditioning on rs8102683 (CNV)

c) After conditioning on rs8102683 and rs11878604



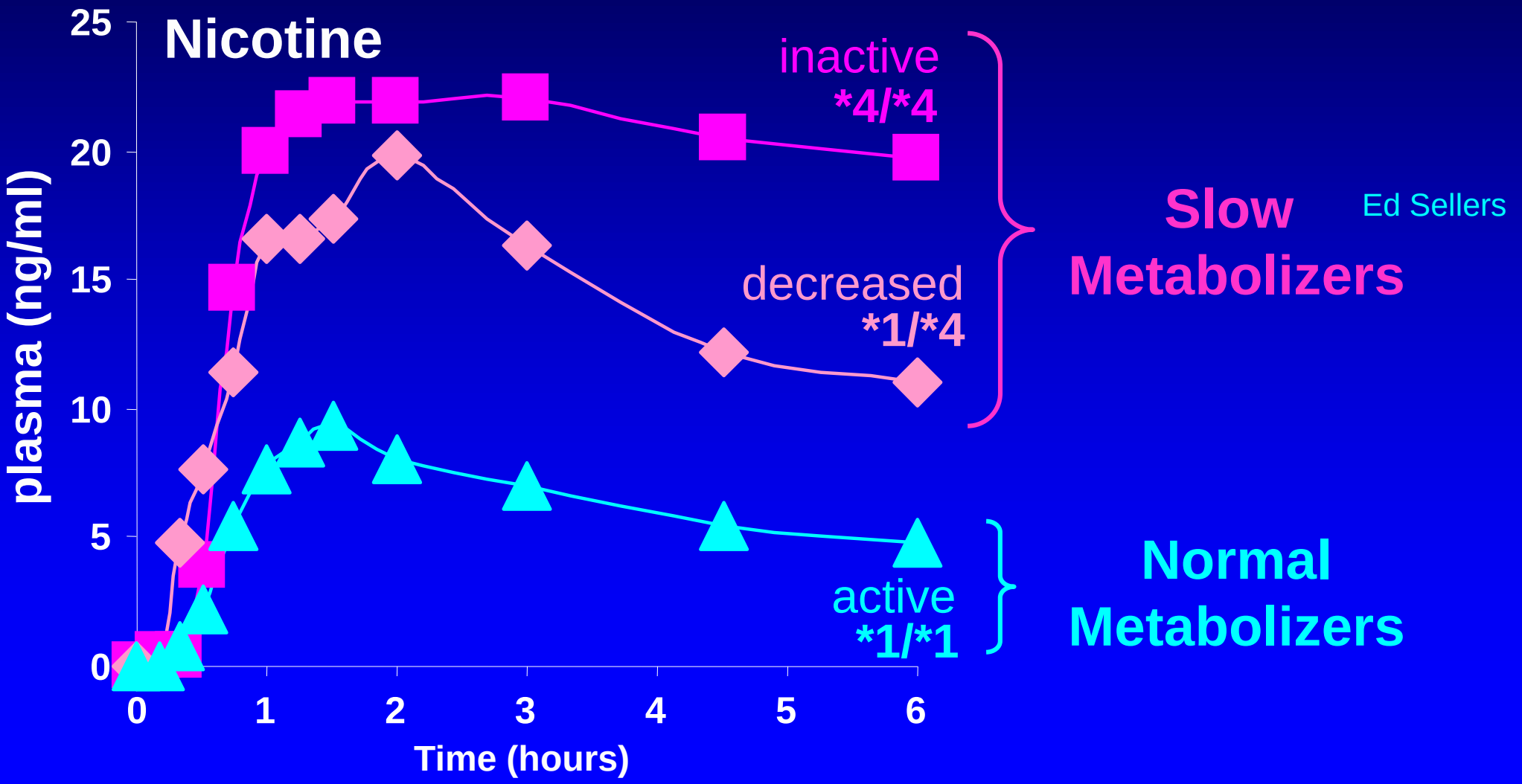
Relative Nicotine (61 μM) metabolism by Expressed CYPs (Baculovirus): CYP2A6





Genetically Reduced CYP2A6 increases nicotine plasma levels

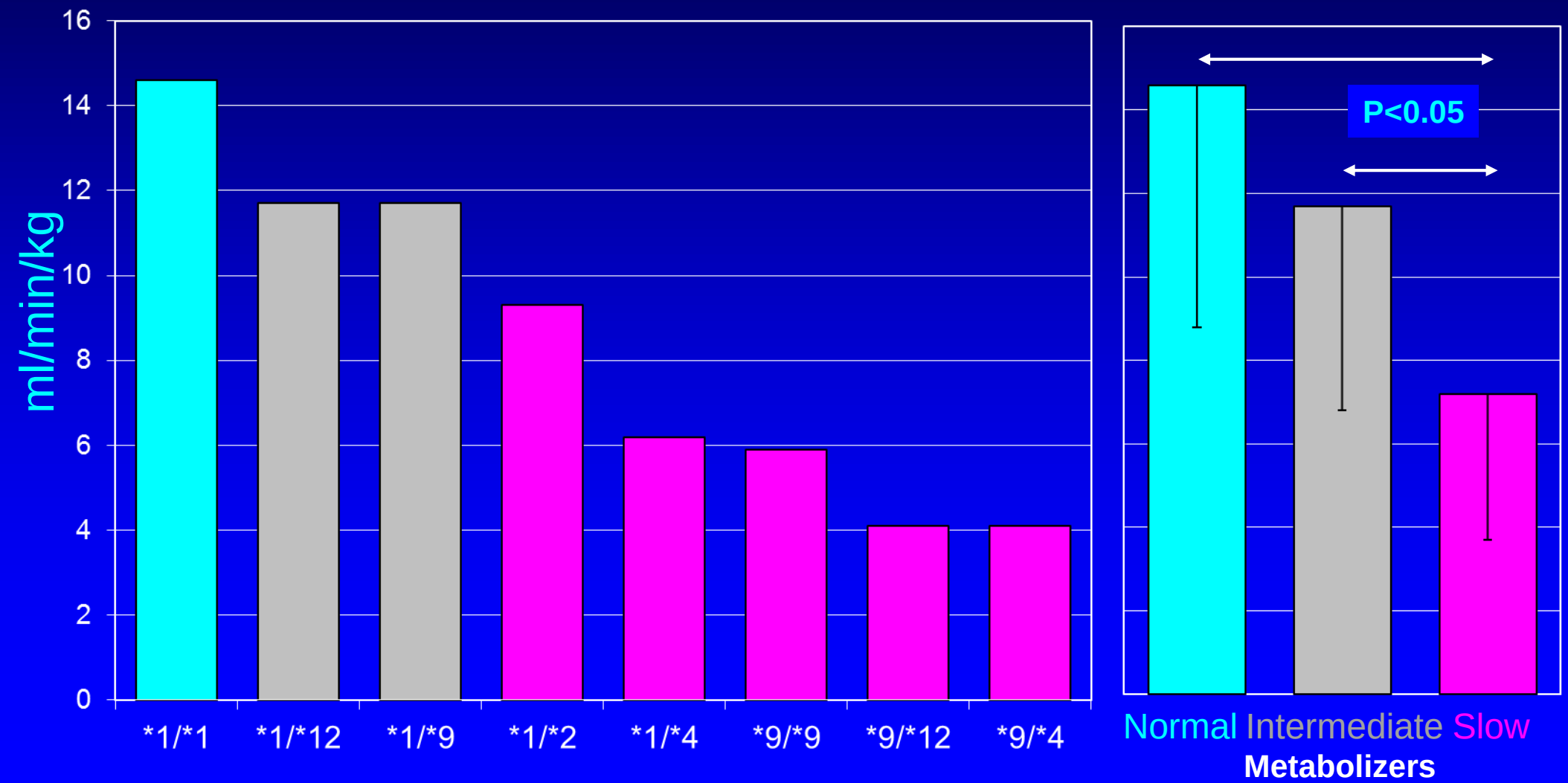
Nicotine 4 mg base, oral Japanese subjects



Ed Sellers

CYP2A6 Genotype:
Fractional Clearance of Plasma NIC to COT
(Twin NIC infusion cohort)

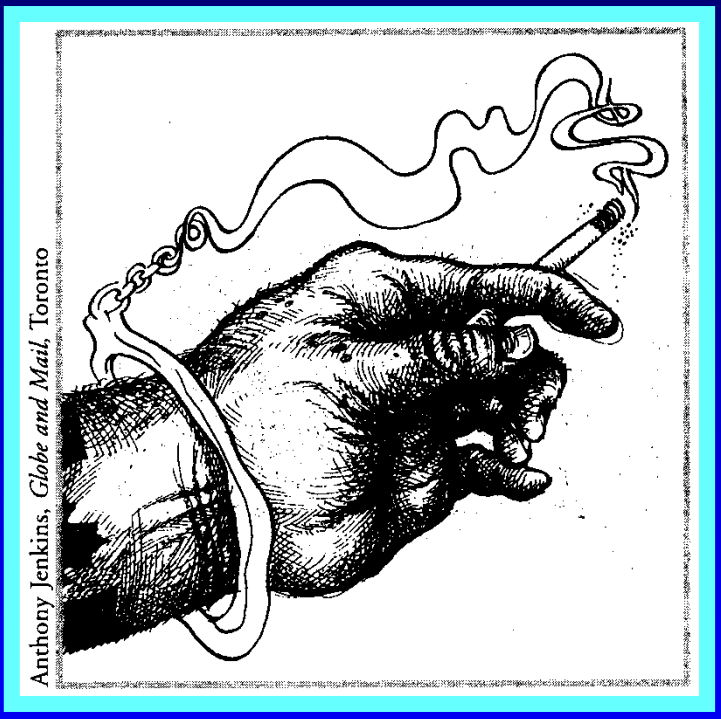
Benowitz CPT 2006



Does slow nicotine inactivation alter the amount smoked?

Rationale:

- Dependent smokers adjust their smoking behavior to maintain nicotine levels
- Amount smoked ≈ 85% genetic
  - **Koopmans JR et al, *Behav Genet* 29(6): 383-93., 1999.**
  - **Kaprio J et al, *Int J Epidemiol* 11(4): 378-86., 1982.**



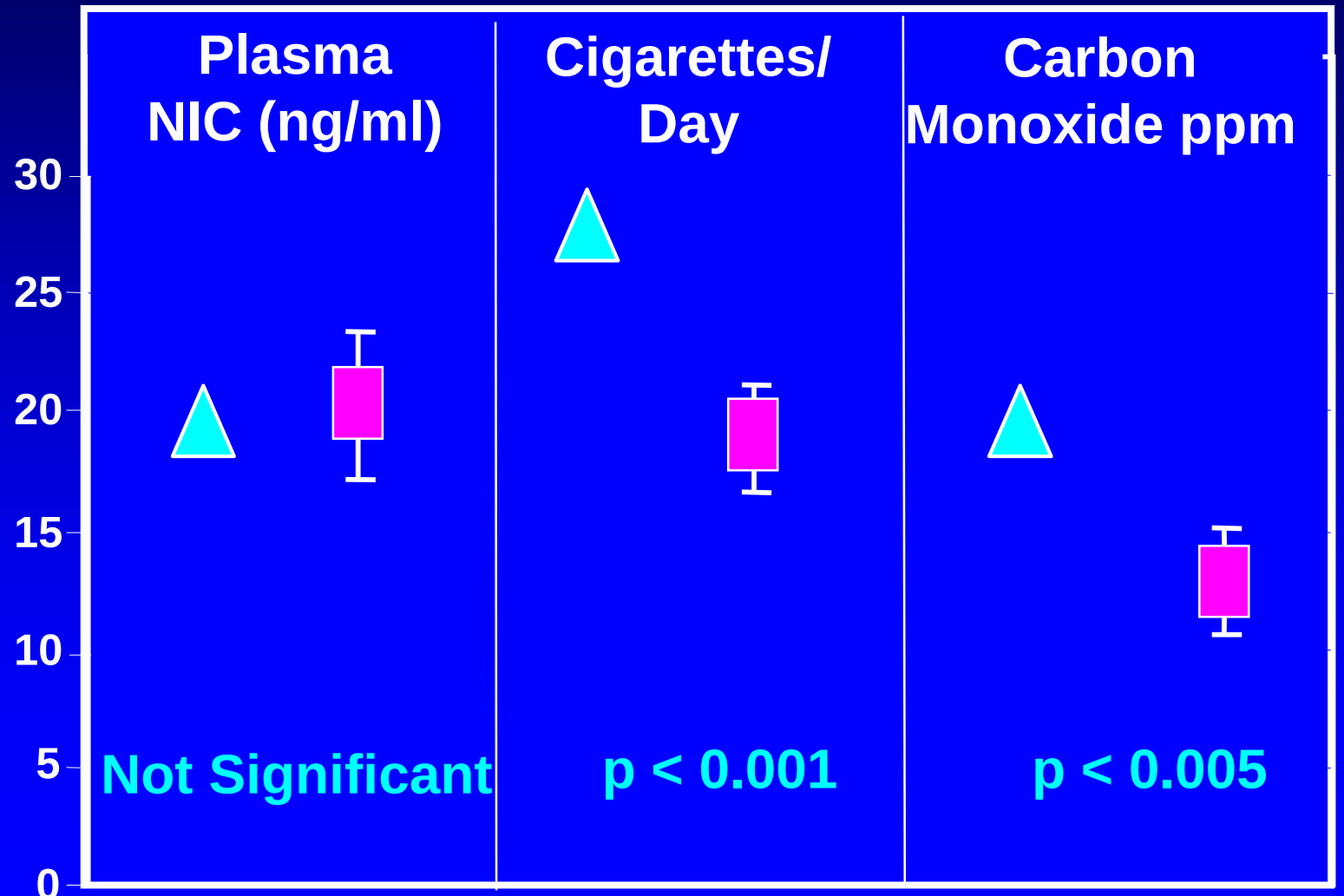
Hypothesis:

- Genetically slow nicotine metabolizers who are dependent smokers will smoke fewer cigarettes per day

Slow metabolism decreases smoking

(# of cigarettes smoked and breath CO)

Rao et al., Mol Pharm 2002

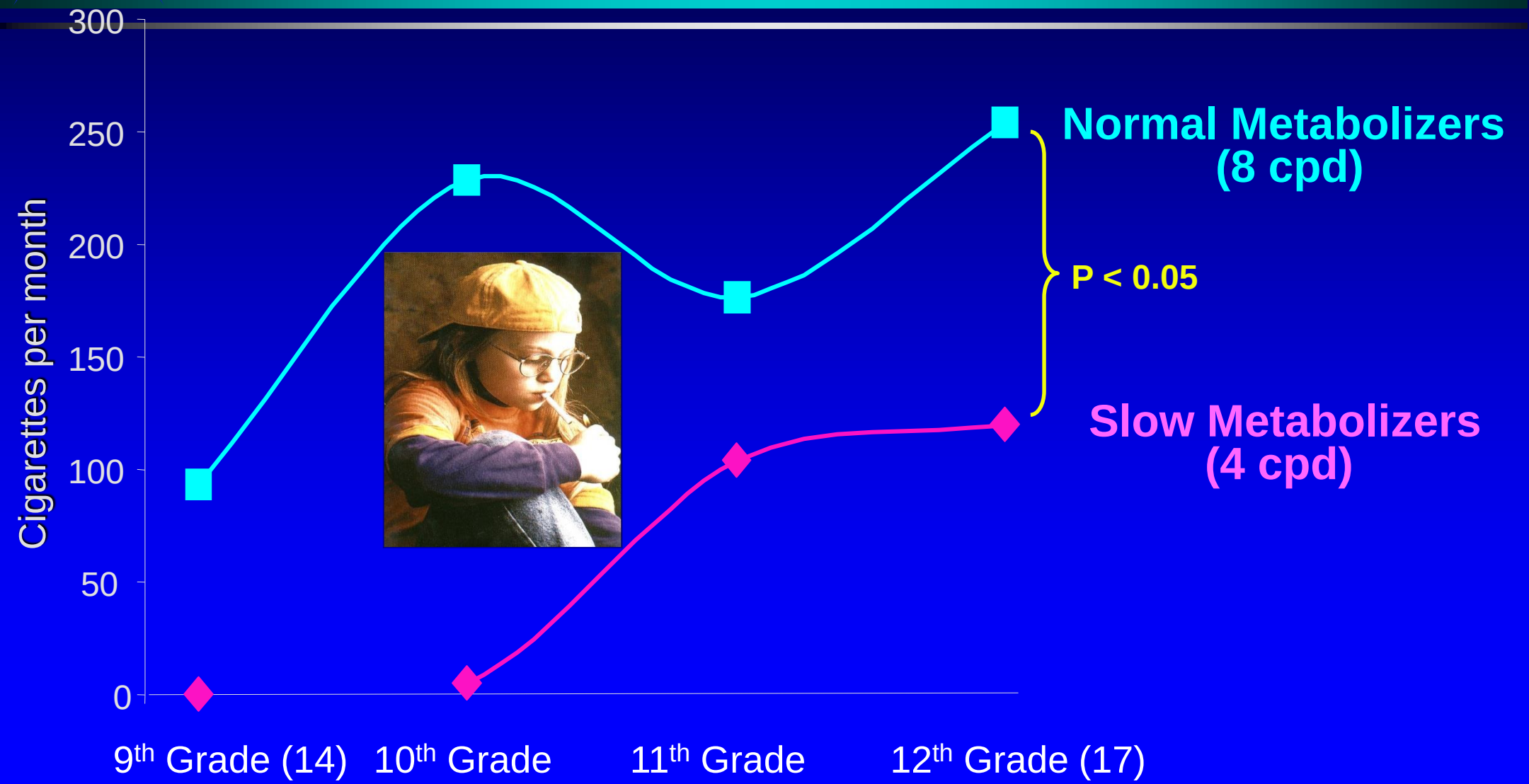


Normal Metabolizers *1/*1 n=277

Slow Metabolizers *1/*2 &*1/*4 n=14

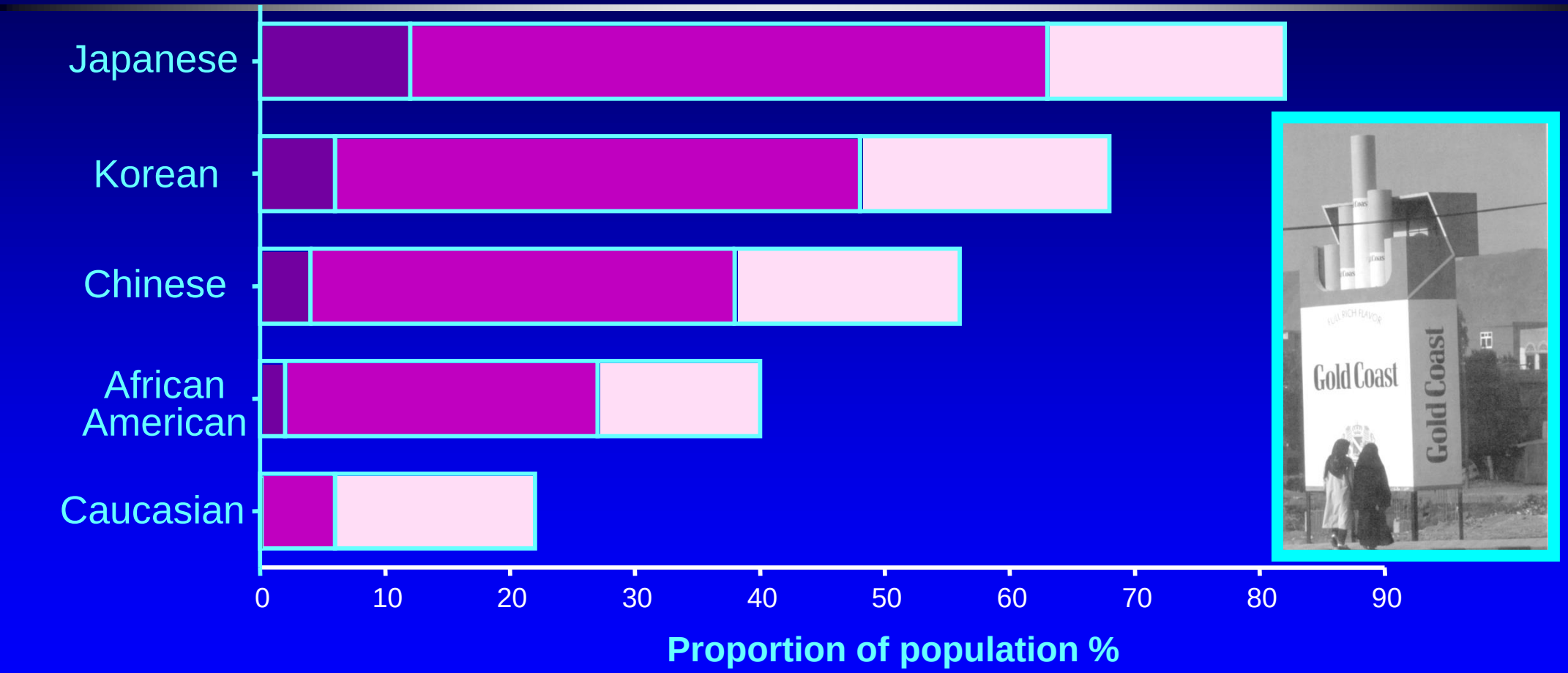
Slow metabolizers smoke fewer cigarettes, Even at very early stages of smoking

Audrain-McGovern, Pediatrics 2007



*Frequencies of CYP2A6 activity groups varies among ethnic groups (*2, *4-*10,*12, *17) N=2000*

Malaiyandi et al, CPT 2005; Mwenifumbo et al., PG &G 2005, DAA 2007

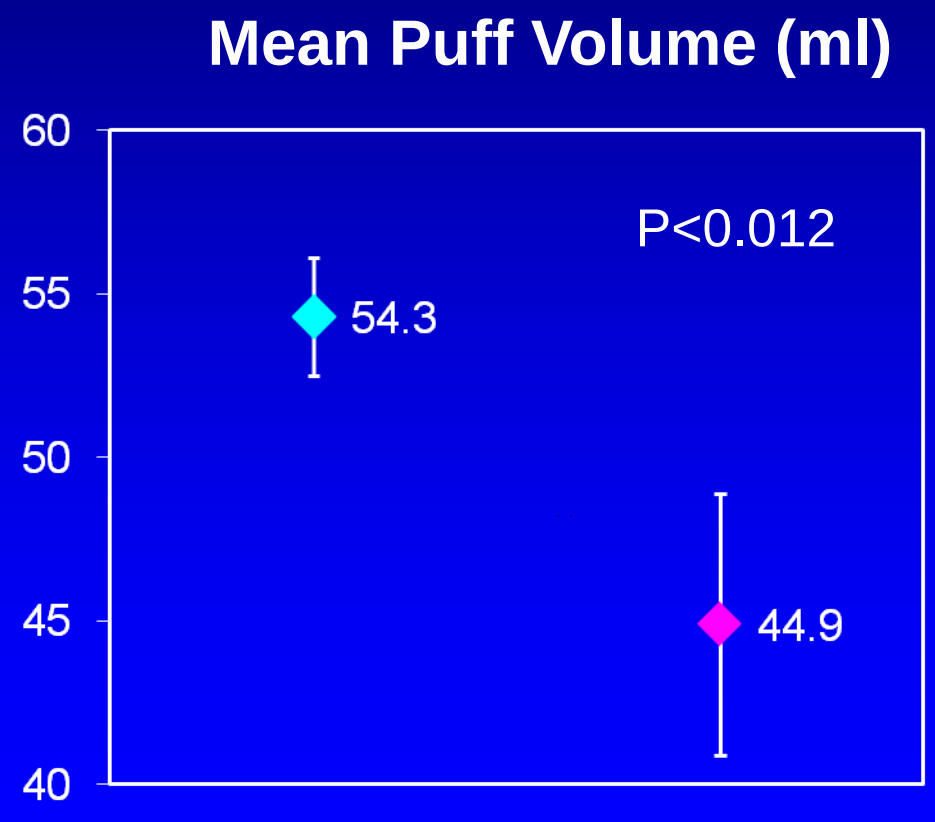


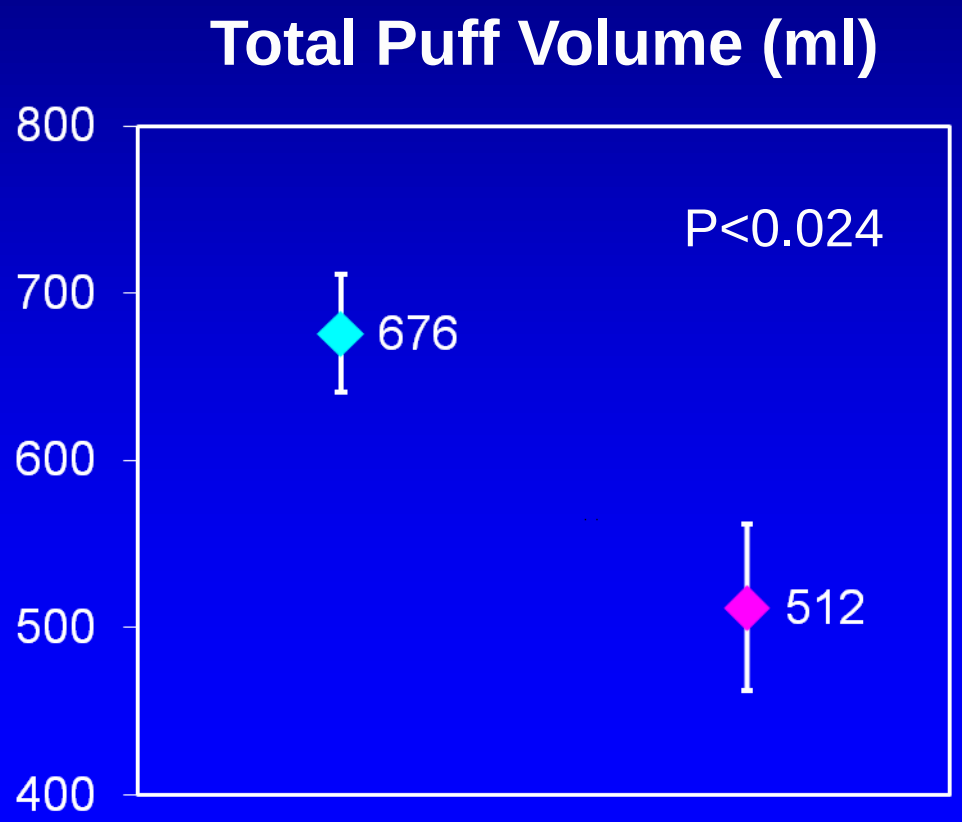
Topography: CYP2A6 Slow metabolizers take smaller puffs

Andrew Strasser
NTR 2007

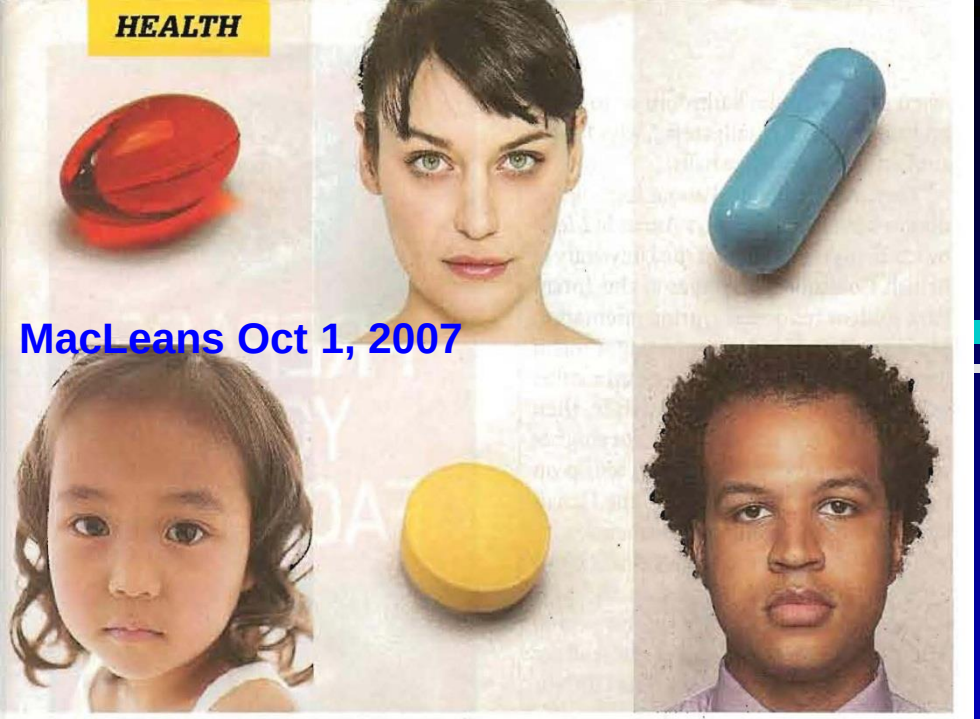
◆ Normal Metabolizers

◆ Slow Metabolizers



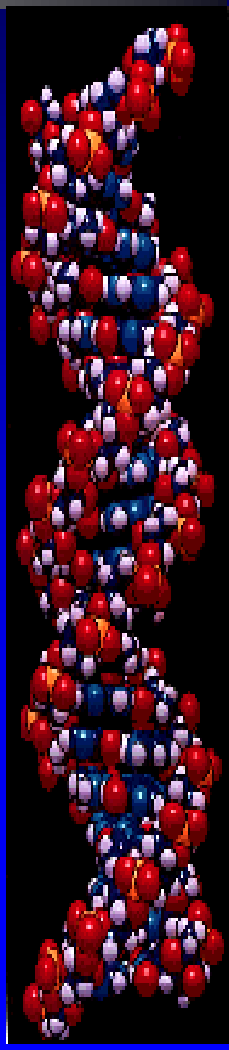


Pharmacogenetics of Treatment



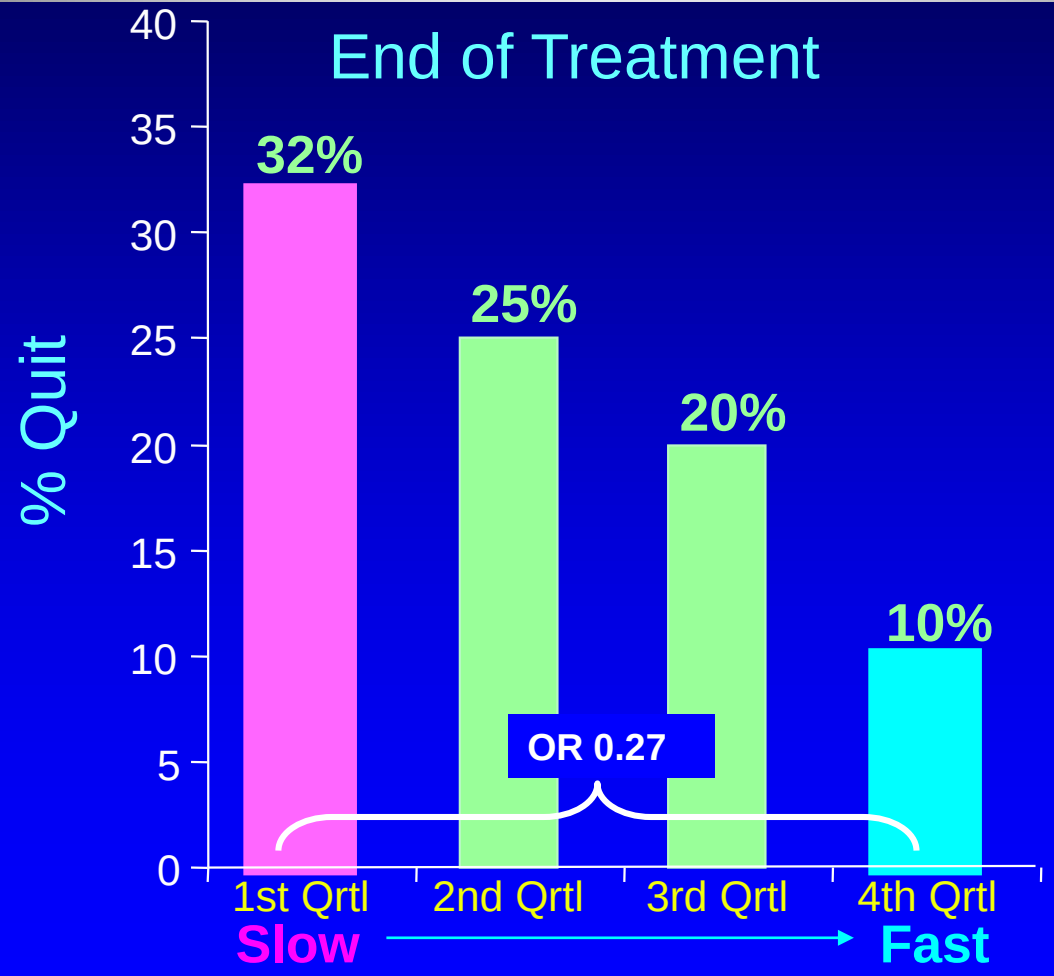
Current treatment: low efficacy

- Genetic variation in response
- Tailor medication to genetic make-up
- Discover novel targets for drug development



Slow Metabolizers have better quit rates on placebo

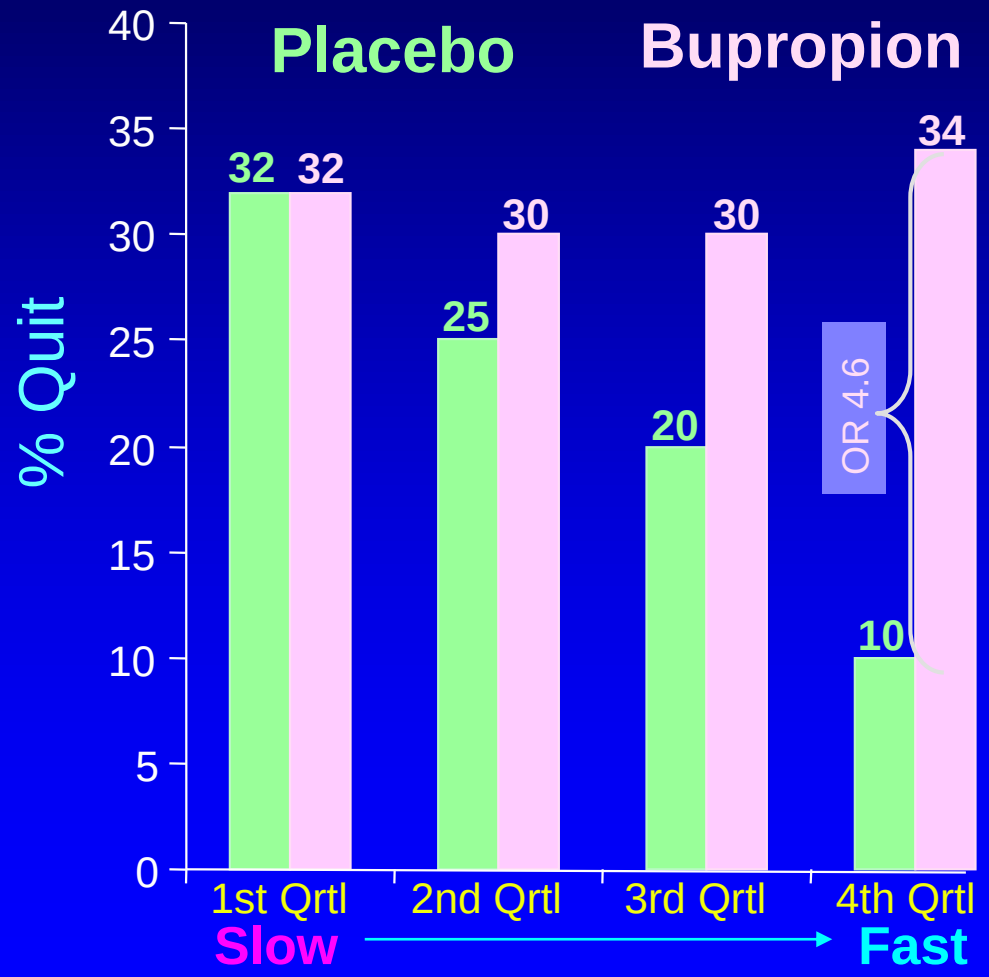
Patterson et al 2008



Placebo Arm

3-HC: Cotinine Ratio in Quartiles

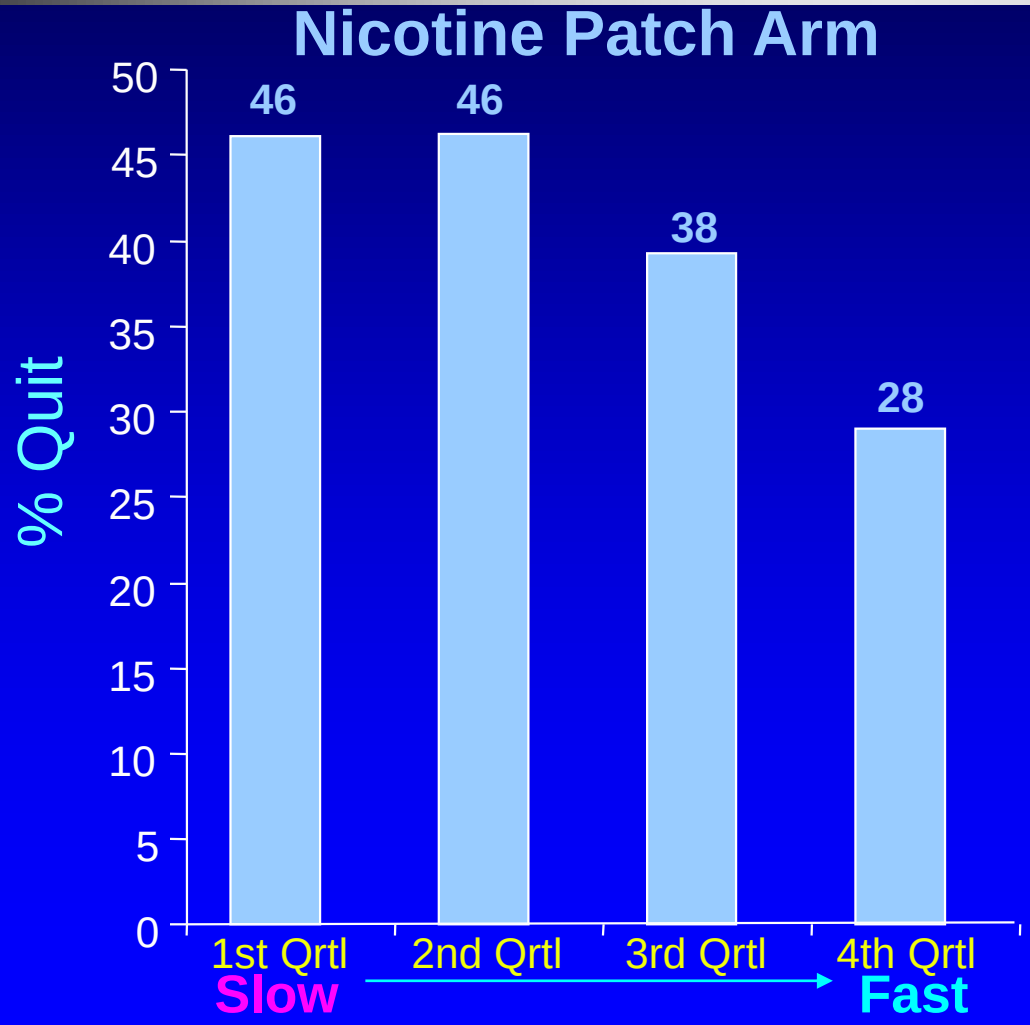
Fast Metabolizers quit poorly on placebo, but respond well to Bupropion



End of Treatment

Slow metabolizers respond well to Nicotine Patch

Lerman et al., Clin. Pharm. Ther. 2006, 2010

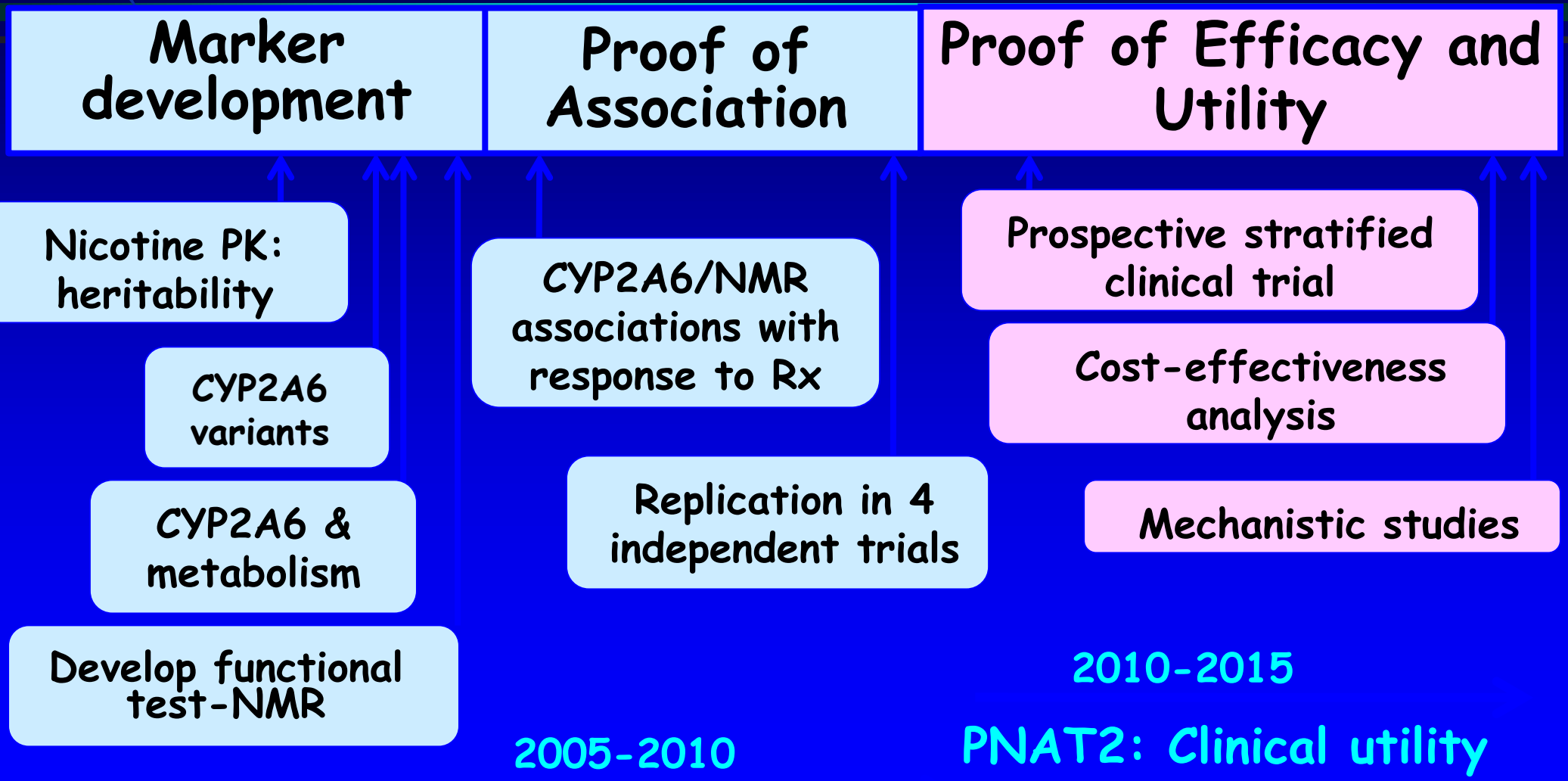


Replicated in multiple retrospective studies

Also looked at extended treatment
- even better for slow metabolizers
- no gain for fast metabolizers

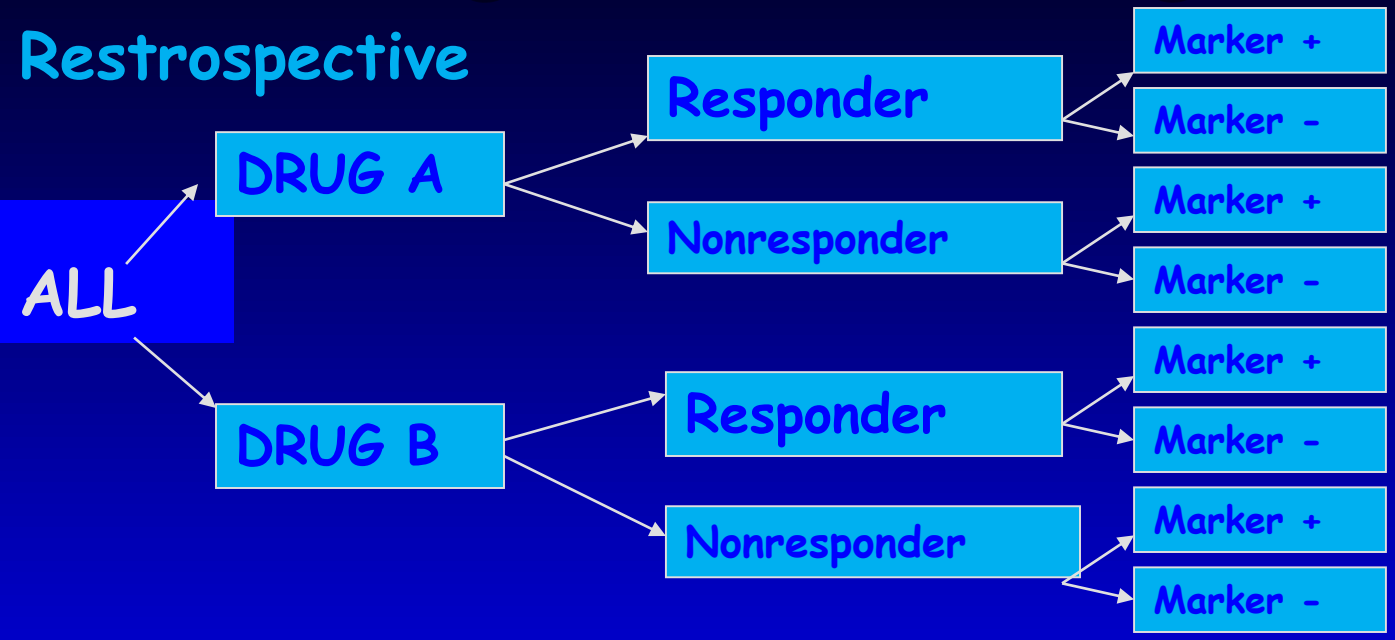
Higher dose nicotine patch (pilot)
- may help faster metabolizers

Identification of a reliable marker for nicotine addiction treatment



Pharmacogenomic Trial Designs

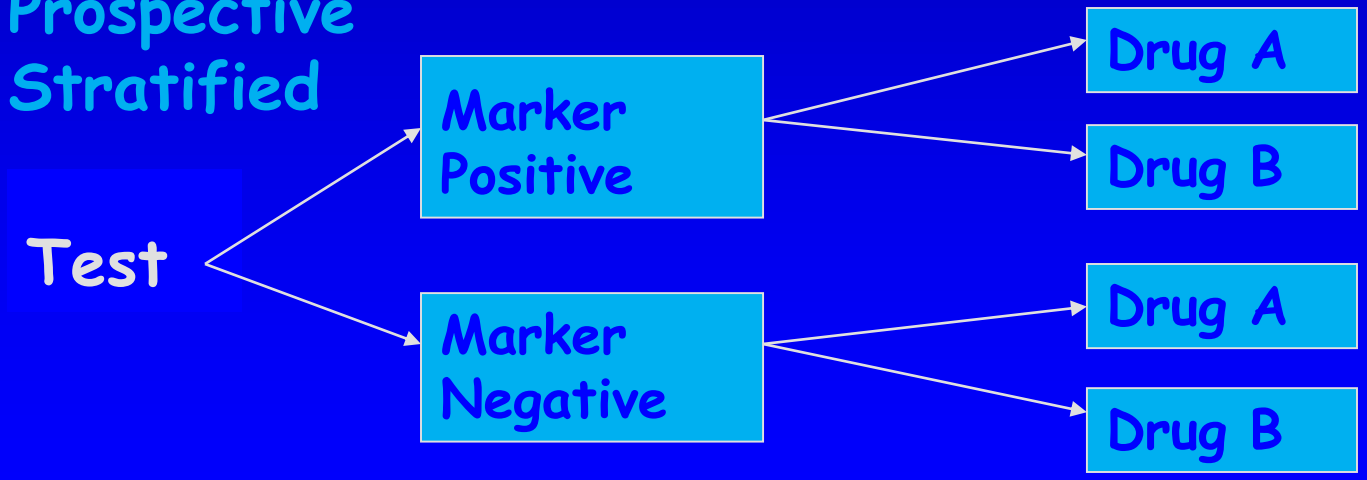
Restrospective



Best Uses:
- Marker unknown at trial initiation
- Hypothesis generation
- Independent validation

Limitations:
- Unbalanced groups
- Reduced power
- Missing data

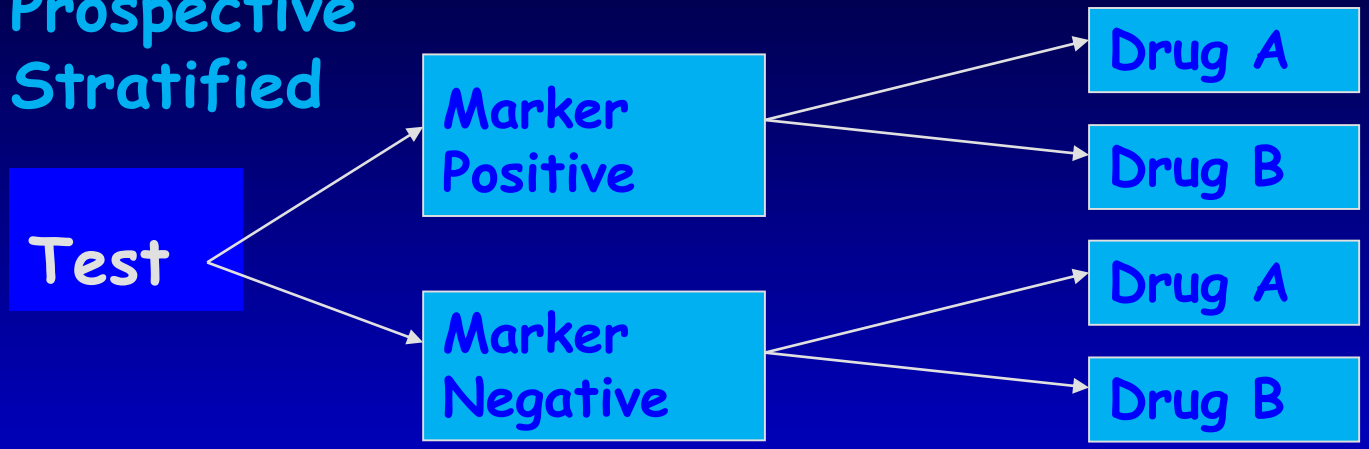
Prospective Stratified



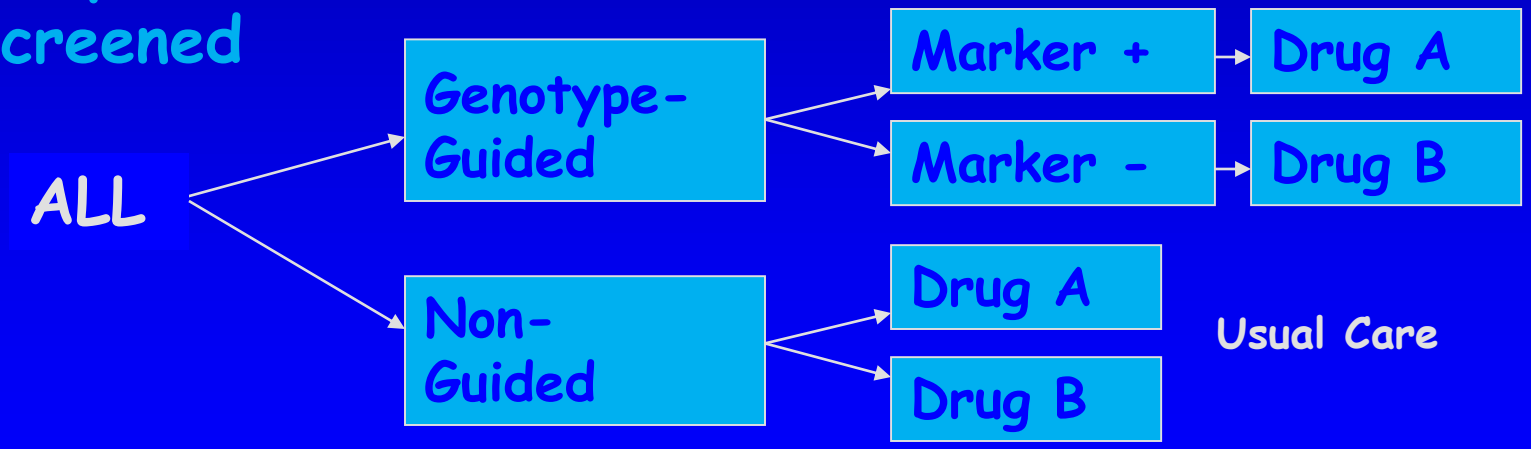
Advantages:
- Trial based on a piori hypothesis
- Allows for "enrichment"
- Balances Tx assignment

Pharmacogenomic Trial Designs

Prospective Stratified



Prospective Screened



Usual Care

High ecological ('real world') validity

True Test of Whether Personalized Medicine is Effective?

Lost in Translation?

- Increase generalizability to clinical practice settings
- Demonstrate improvement of health outcomes and cost-effectiveness
- Establish evidence-based guidelines
- Enhance adoption in clinical practice

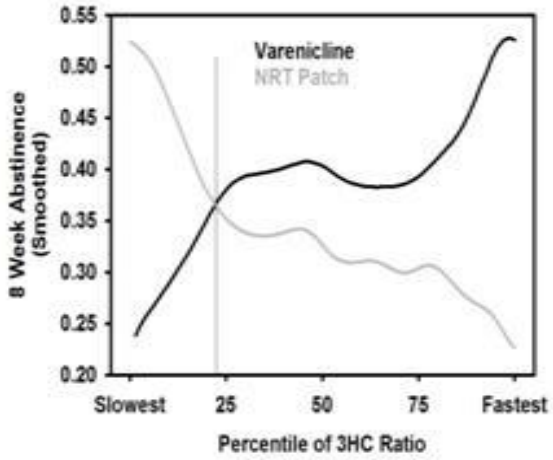
Khoury, Genomic Medicine, 2009

Prospective Randomized Trial

Slow Metabolizers
(oversampled)

N=675

Test



N=675

Normal Metabolizers

A Placebo

B Transdermal Nicotine

C Varenicline

D Placebo

E Transdermal Nicotine

F Varenicline

Smokescreen® Genotyping Array

A platform for genetic research on smoking, addiction, and treatment approaches

Summary

- Genotyping array with 646,247 markers designed for studies of addiction, smoking, downstream consequences and treatment
- Developed as part of a SBIR contract with the National Institute on Drug Abuse (NIDA)
- High coverage in multiple populations (African, Asian, European)
- Available companion services by BioRealm
  - Unified quality control and analysis
  - Software interface to results
  - Genotyping at partner labs

GAME-ON OncoArray

Common Content – 40K

Fine-mapping of common cancer susceptibility loci (*TERT*, 8q24 (proximal and distal to *MYC*), *HNF1B*, *TET2*, *RAD51B*, 11q13, *MERIT40*, *MDM4)*

Ancestry Informative Markers

Cross-Site meta analysis

Pharmacogenetic components

eQTL (Height, Weight, BMI, WHR, Menarche, Menopause etc)

Other cancers published GWAS variants

Chromosome X and mitochondrial DNA variants

GWAS Backbone

260K

Illumina Core

OncoChip

600K

beadtypes

Cancer Specific Variants

Lung

Colon

Breast

Prostate

Ovarian

(proportional allocation)

Challenges in application of pharmacogenetics for smoking cessation

- Two different loci, each having multiple variants, influence smoking cessation. Genetic markers are effective for CHRNAs but serum markers may be better for characterizing CYP2A6 variation.
- Will smokers seeking to quit wait for test results before starting therapy?