Gene-Environment Interactions

What is gene-environment interaction?

“A different effect of an environmental exposure on disease risk in persons with different genotypes," or, alternatively, *"a different effect of a genotype on disease risk in persons with different environmental exposures.”*

Ottman, Prev Med 1996

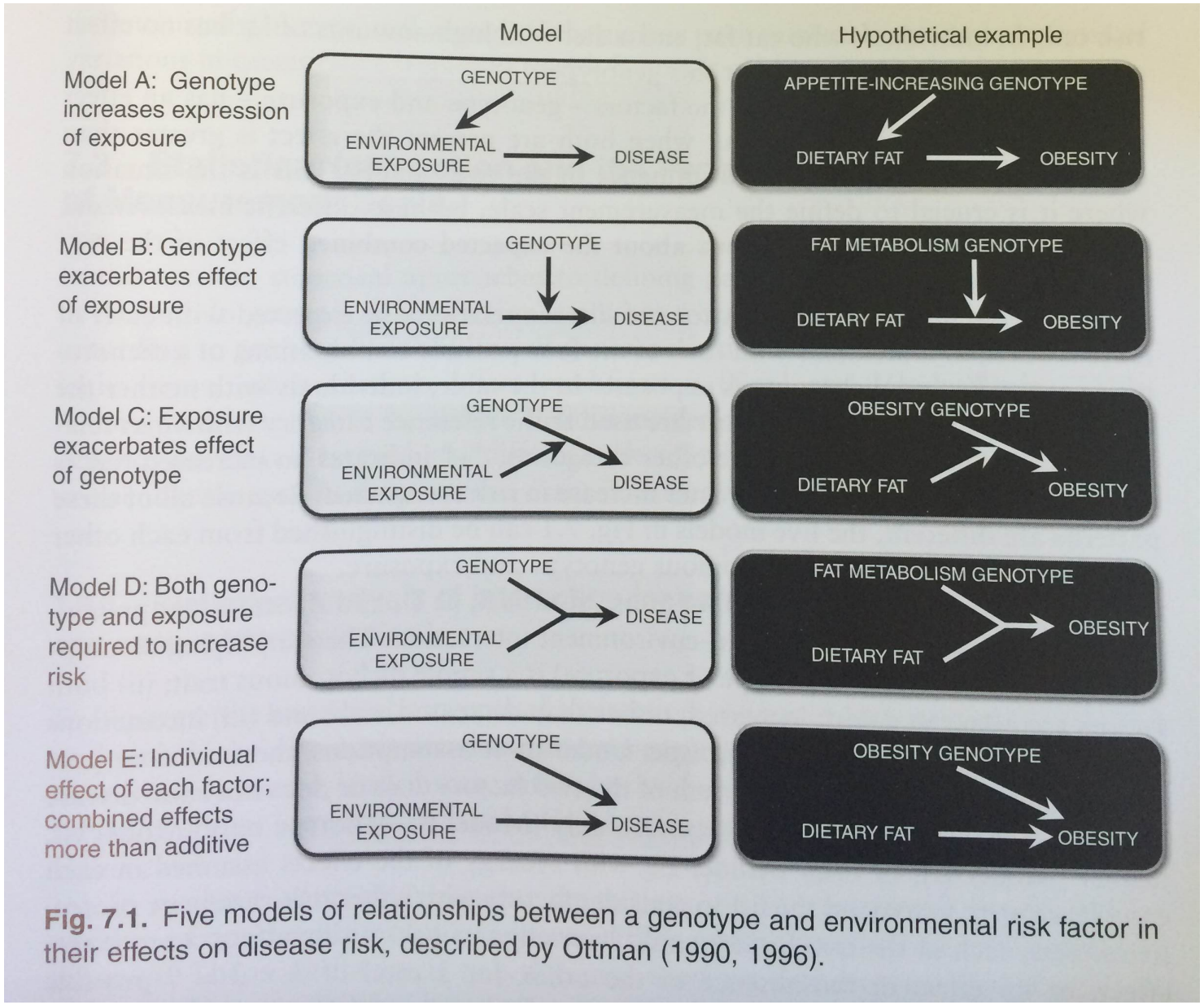


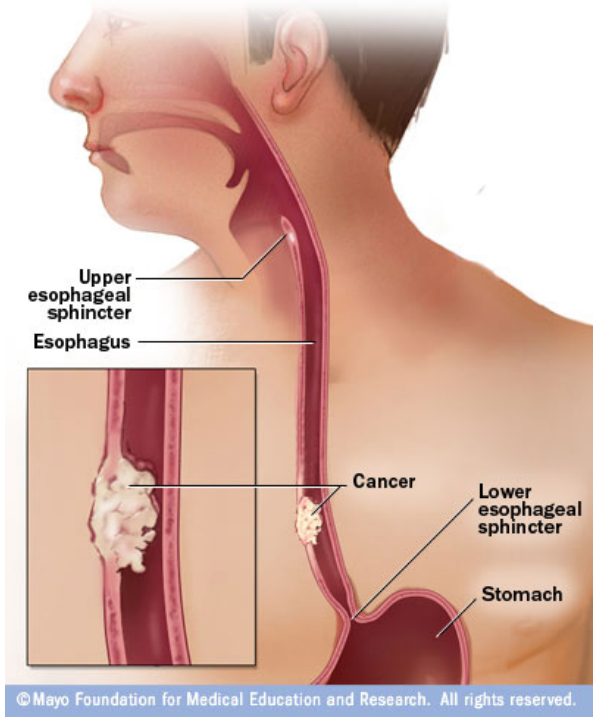
Fig. 7.1. Five models of relationships between a genotype and environmental risk factor in their effects on disease risk, described by Ottman (1990, 1996).

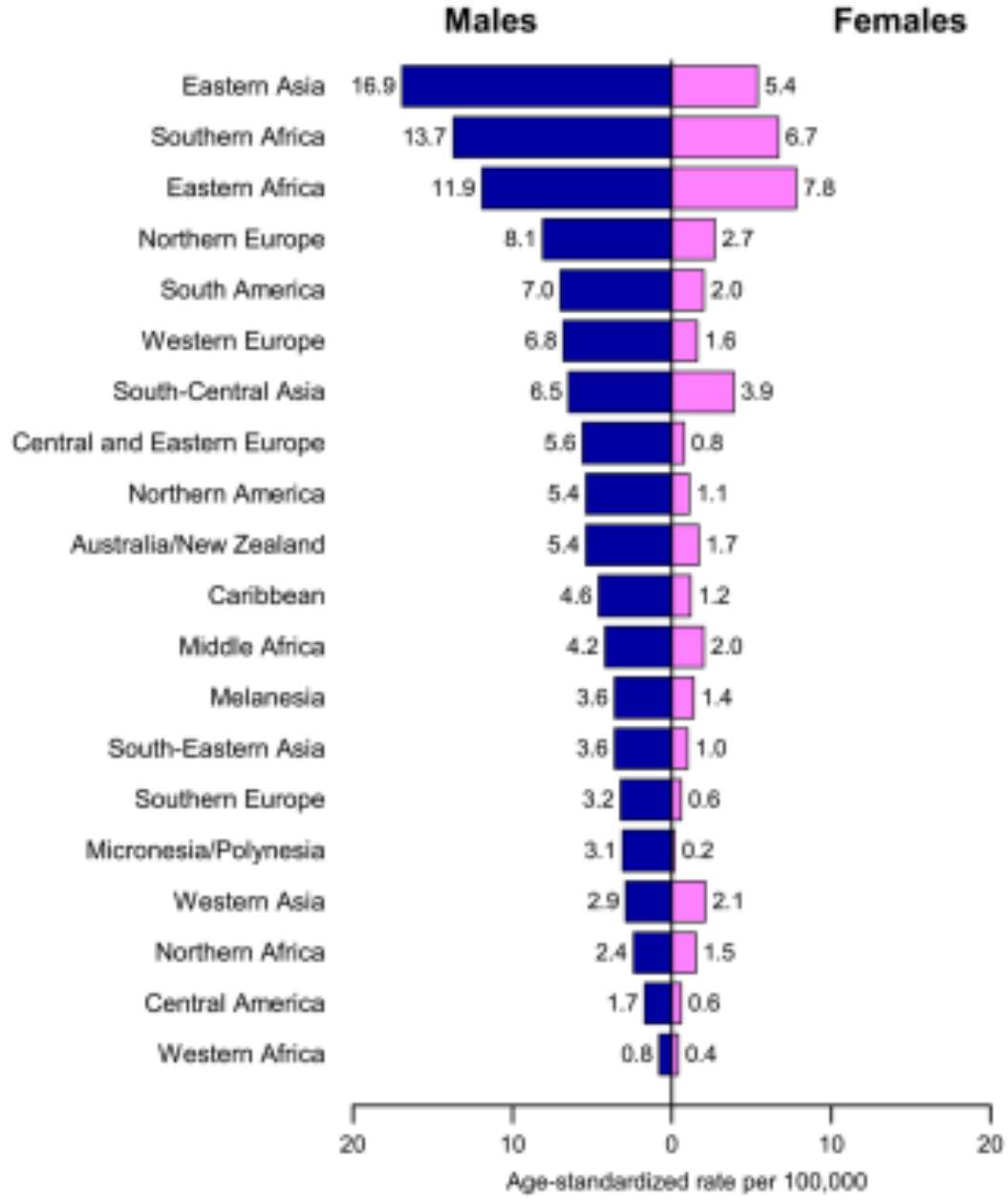
Why study Gene-Environment Interactions?

- Gain insights about already known genes
  - Information about effect in different strata might give insights in pathways and biology
- Clinical Importance
  - Disease prediction, pharmacogenetics
- A tool in gene discovery
  - Gene only affective in exposed individuals - Environment only affective in gene carriers
  - Incorporating GxE interactions may boost power in association analysis

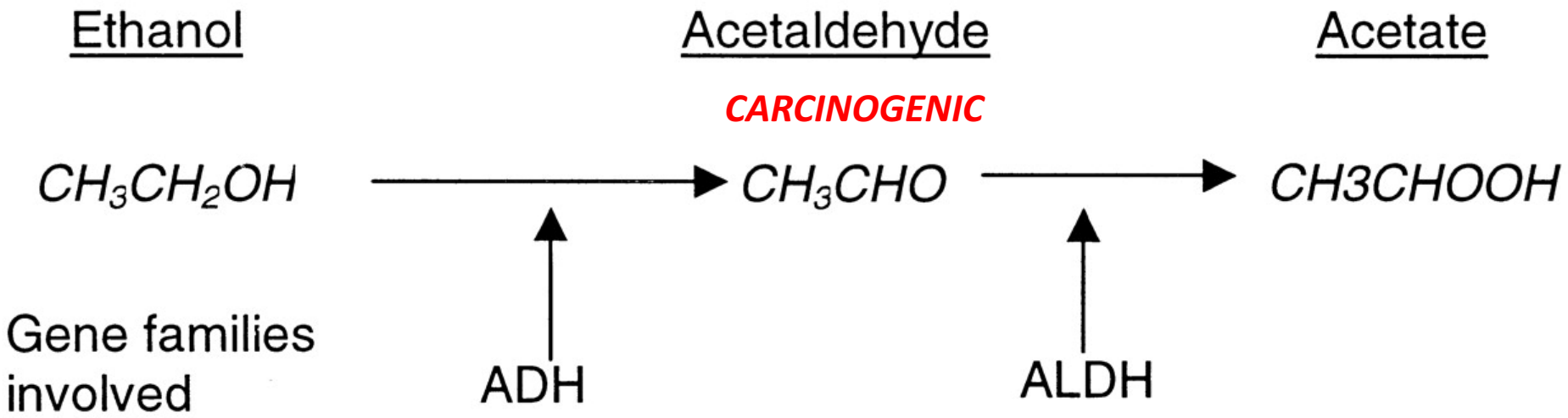
Example: Esophageal cance

- Risk factors: alcohol intake, tobacco use, being a man, Barrett Syndrome, obesity

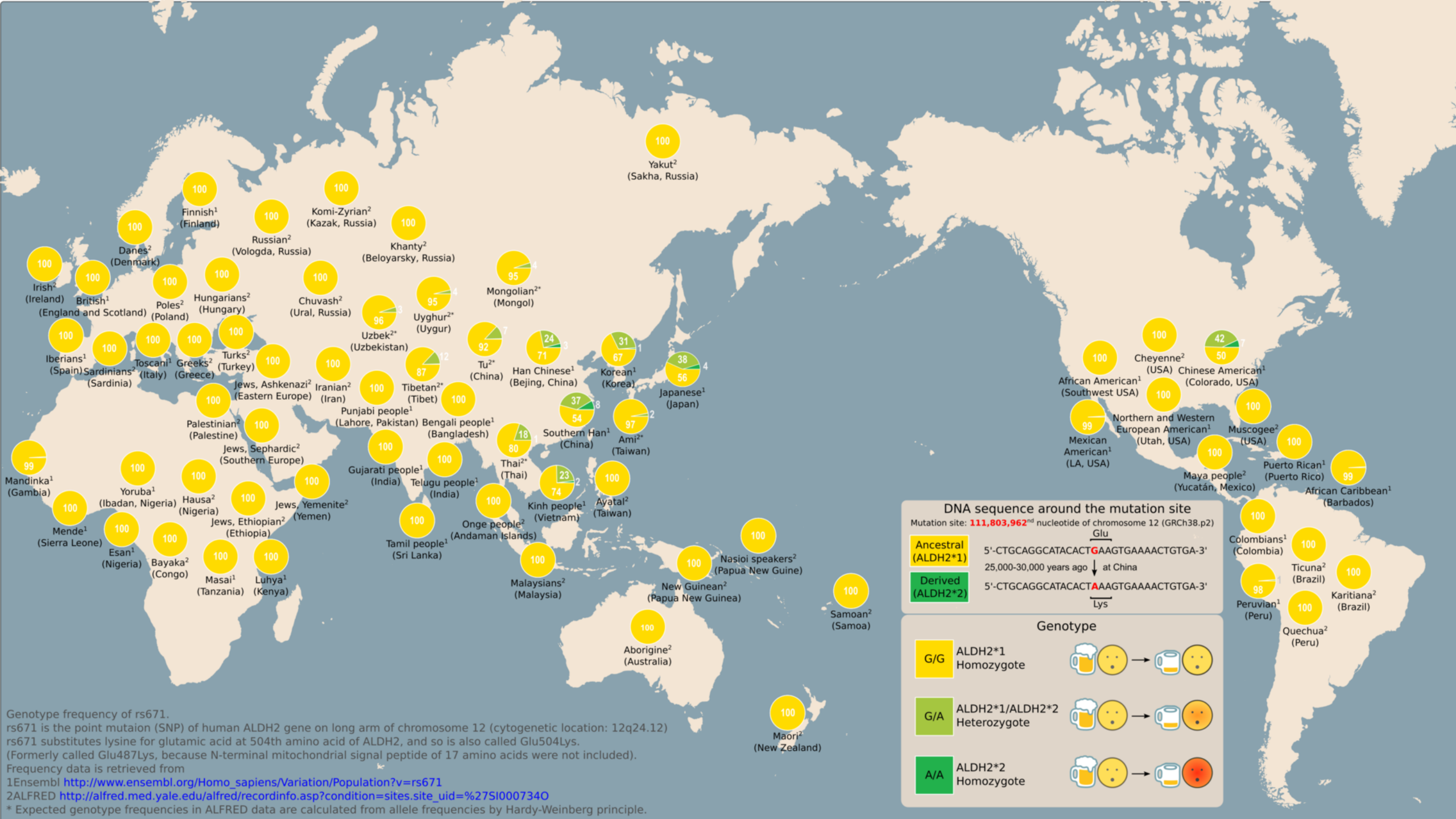




Metabolism of alcohol (ethanol) to acetaldehyde (ethanal) and then acetic acid (ethanoic acid)



Accumulation of acetaldehyde leads to alcohol flush reaction



Genotype frequency of rs671.
rs671 is the point mutaion (SNP) of human ALDH2 gene on long arm of chromosome 12 (cytogenetic location: 12q24.12)
rs671 substitutes lysine for glutamic acid at 504th amino acid of ALDH2, and so is also called Glu504Lys.
(Formerly called Glu487Lys, because N-terminal mitochondrial signal peptide of 17 amino acids were not included).
Frequency data is retrieved from
1Ensembl http://www.ensembl.org/Homo_sapiens/Variation/Population?v=rs671
2ALFRED http://alfred.med.yale.edu/alfred/recordinfo.asp?condition=sites.site_uid=%27SI000734O
* Expected genotype frequencies in ALFRED data are calculated from allele frequencies by Hardy-Weinberg principle.

Interaction between alcohol intake and *ADH1B* and *ALDH2* genotypes in Esophageal squamous-cell carcinoma

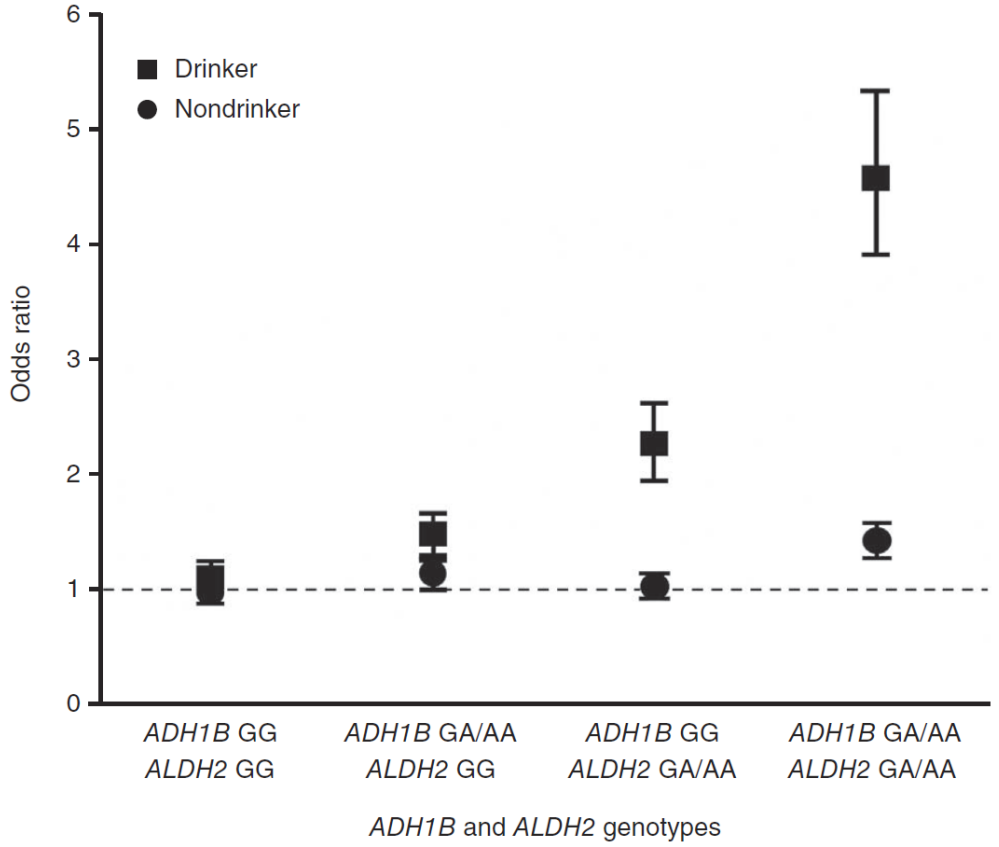


Figure 2 Plots showing the ORs for ESCC in alcohol drinkers and nondrinkers with different *ADH1B* rs1042026 and *ALDH2* rs11066015 genotypes. The vertical bars represent the 95% CIs. The horizontal dashed line indicates the null value (OR = 1.0).

Wu et al. (2012) Nat Genet

GE interactions and statistical power

- Rule of thumb:

You need **four** times as many individuals to detect an interaction effect compared to main effect analysis

Non-parametric analysis: The 4-by-2 table

	Case	Control
G=0,E=0	N_{100}	N_{000}
G=1,E=0	N_{110}	N_{010}
G=0,E=1	N_{101}	N_{001}
G=1,E=1	N_{111}	N_{011}

This presentation is “closest to the data” and makes no assumption about genetic model or how the gene and exposure jointly influence risk

For prospective data, yields estimates of relative risks.

For retrospective data, yields estimates of odds ratios.

For rare SNPs or exposures, the GxE-stratified estimates of risks/odds ratios from this table can be very noisy

Interaction on the multiplicative scale

	Case	Control	OR	
G=0,E=0	N_{100}	N_{000}	1	Reference
G=1,E=0	N_{110}	N_{010}	$\frac{N_{110}N_{000}}{N_{010}N_{100}}$	Risk among unexposed carriers
G=0,E=1	N_{101}	N_{001}	$\frac{N_{101}N_{000}}{N_{001}N_{100}}$	Risk among exposed non-carriers
G=1,E=1	N_{111}	N_{011}	$\frac{N_{111}N_{000}}{N_{011}N_{100}}$	Risk among exposed carriers

Often when people talk about interaction, they talk about departure from the multiplicative scale

$$OR_{INT} = \frac{OR_{11}}{OR_{10}OR_{01}}$$

Interaction exists when observed effect of G & E together is not a simple function of their individual effects

H_0: $OR_{GE}=OR_G OR_E$ vs. H_A: $OR_{GE}\neq OR_G OR_E$

In practice, we often test for interaction on the multiplicative scale

$$\text{logit}\,\mathrm{P}(D=1) = \beta + \beta_g G + \beta_e E + \beta_{ge} GE$$

$$Test: \beta_{ge} \neq 0$$

Thomas, 2004

Test for Interaction (jointly) – a tool for gene discovery

- Is this gene associated with disease risk in any of the exposure sub-groups?

- Compare “main effect of E only” model to “main effects plus interaction” model in a 2 df test.

Null model: $\text{logit P}(D=1) = \beta + \beta_e E$

Alternative model: $\text{logit P}(D=1) = \beta + \beta_g G + \beta_e E + \beta_{ge} GE$

Kraft et al, Hum Hered. 2007

Case-Only Analysis

Based on genotype-exposure table in CASES

	Carrier	Non-carrier
Exposed	N_{11}	N_{12}
Unexposed	N_{21}	N_{22}

Assuming G and E are independent in the source population, then if G and E are associated in the cases, this indicates a departure from a multiplicative odds model. (i.e. regress E on G in cases—if correlated, there is an "interaction.")

Can be much more powerful than traditional logistic regression analysis!

Piegorsch et al, Stat Med 1994

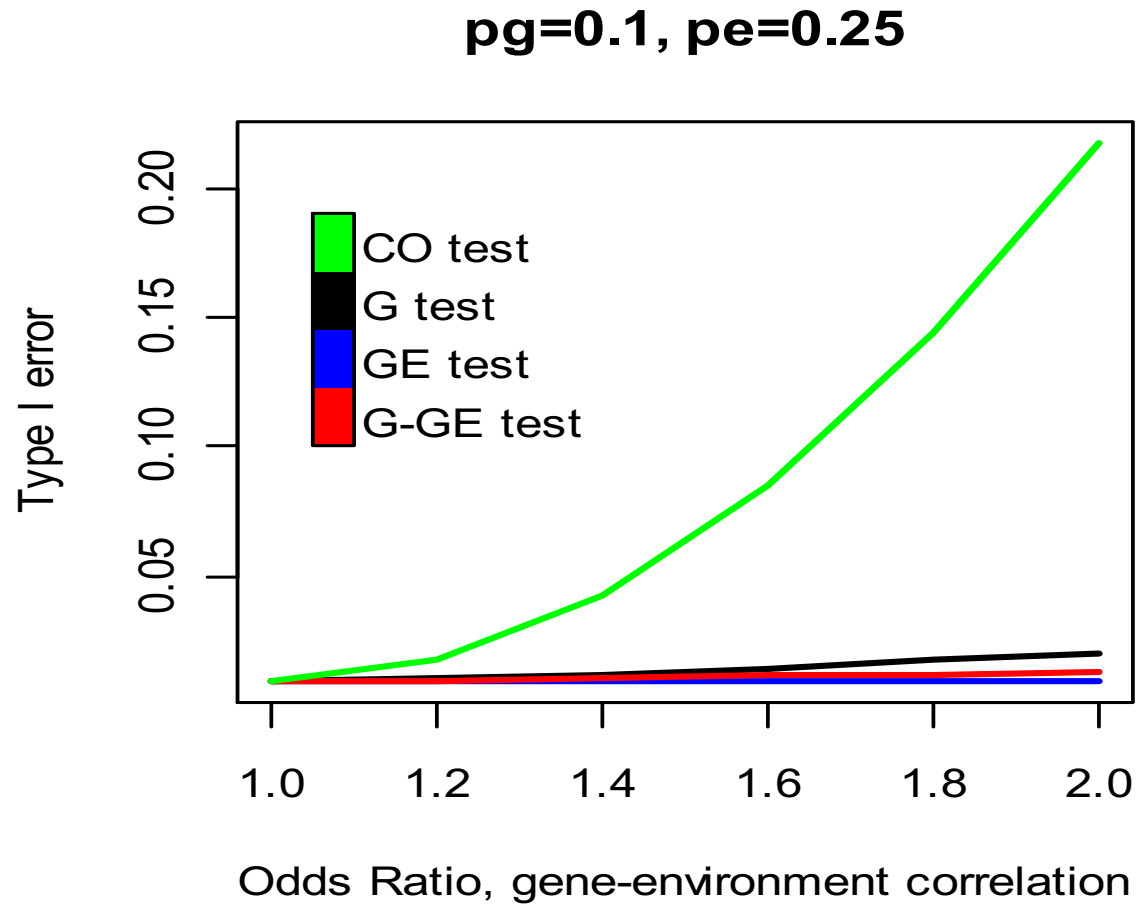
GREAT!!

Does this mean I can throw away all my controls (and decrease genotyping cost)?

- Well, the increase in power is not due to the restriction to cases per se, rather the additional assumption of G-E independence (which you can test in your controls)

- Controls allow for estimation of G and E main effects in addition to the interaction effect and will also allow for calculation of joint G-E-stratum-specific ORs

What if G and E are (positively) correlated?



Type I error rates as a function of GE dependence.

Sensitivity= 0.6

Specificity = 0.9

OR(E)= 1.6

Lindstrom et al, Hum Hered. 2009

Case-only analysis produces inflated results when there is a positive correlation between G and E
What if there is a negative correlation between G and E?

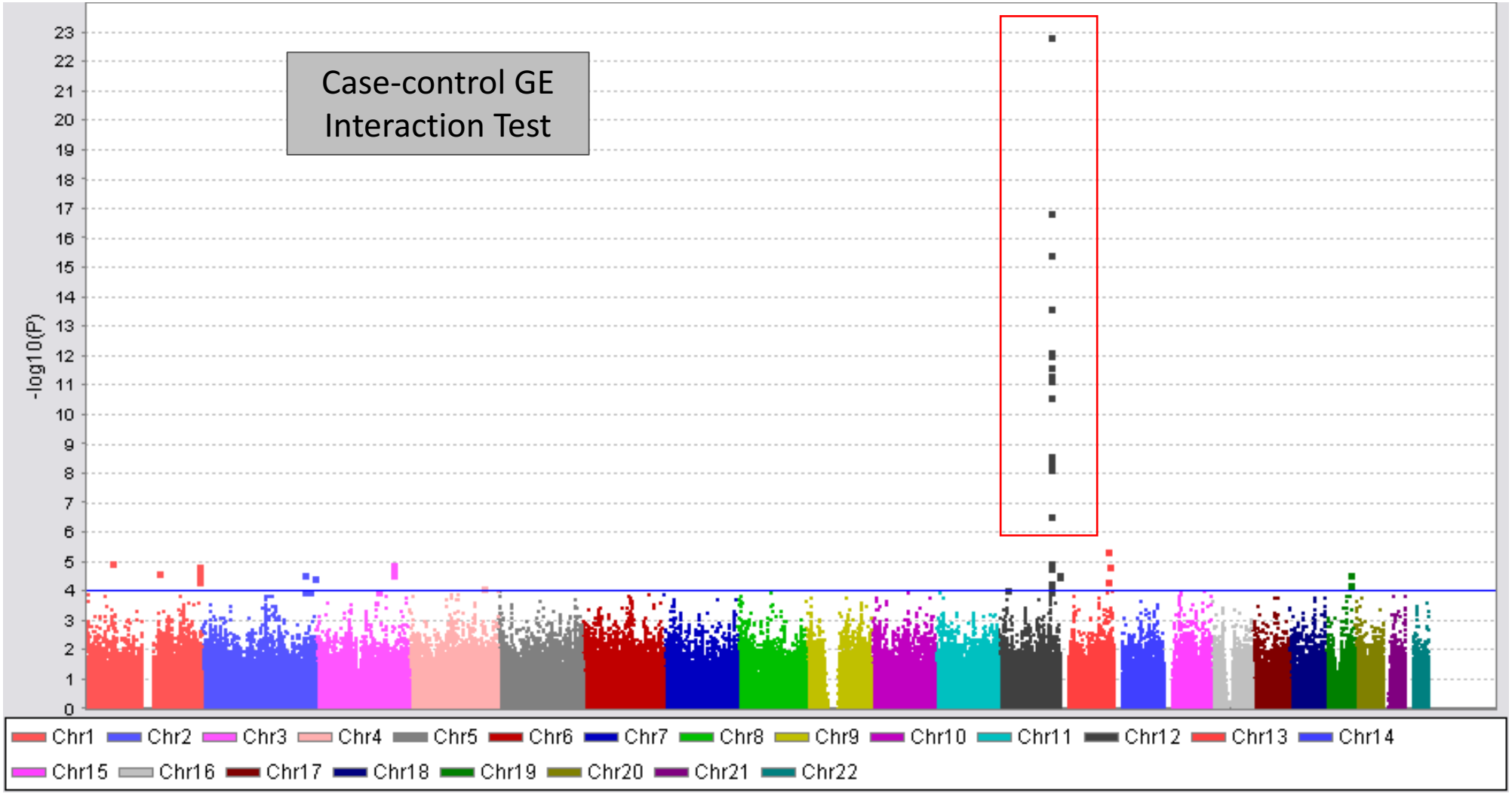
Example: ESCC, *ALDH2* and Alcohol Intake

The risk allele is associated with a decreased risk of heavy drinking in the general population, and an increase in the effect of alcohol on ESCC risk

	OR_{E-G}	OR_{GxE}
rs670 (*ALDH*2*)	0.23	2.69

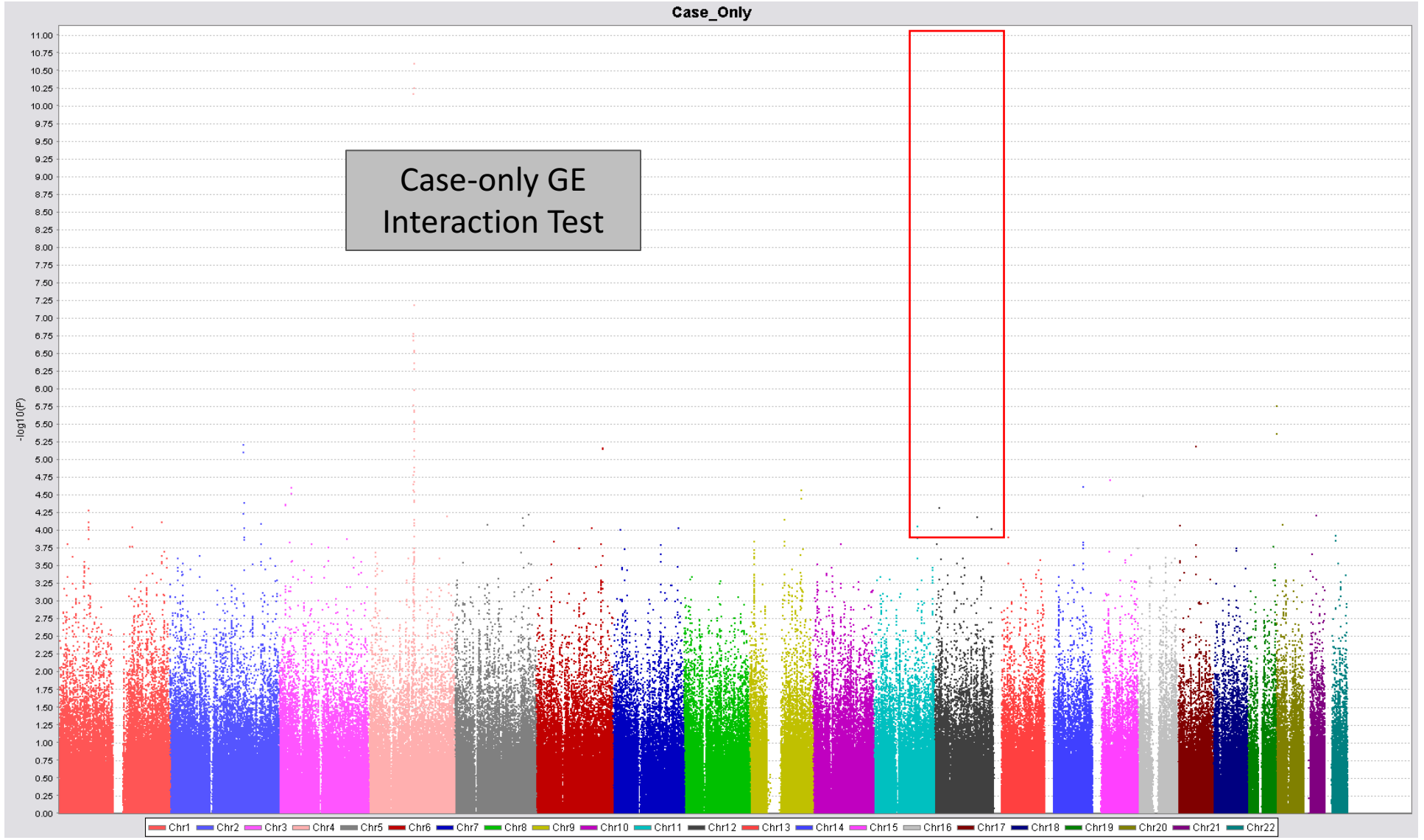
Wu et al, Genet Epi 2014

Example: ESCC, *ALDH2* and Alcohol Intake



Courtesy of Chen Wu

Example: ESCC, *ALDH2* and Alcohol Intake



Courtesy of Chen Wu

Empirical Bayes Estimator

- If G and E are independent– Case-only test. Otherwise GxE interaction tests in a case-control setting (1 df)

- Trade-off between bias and efficiency:

$$\hat{\beta}_{EB} = \frac{\hat{\sigma}^2_{CC}}{(\hat{\tau}^2 + \hat{\sigma}^2_{CC})}\hat{\beta}_{CO} + \frac{\hat{\tau}^2}{(\hat{\tau}^2 + \hat{\sigma}^2_{CC})}\hat{\beta}_{CC}$$

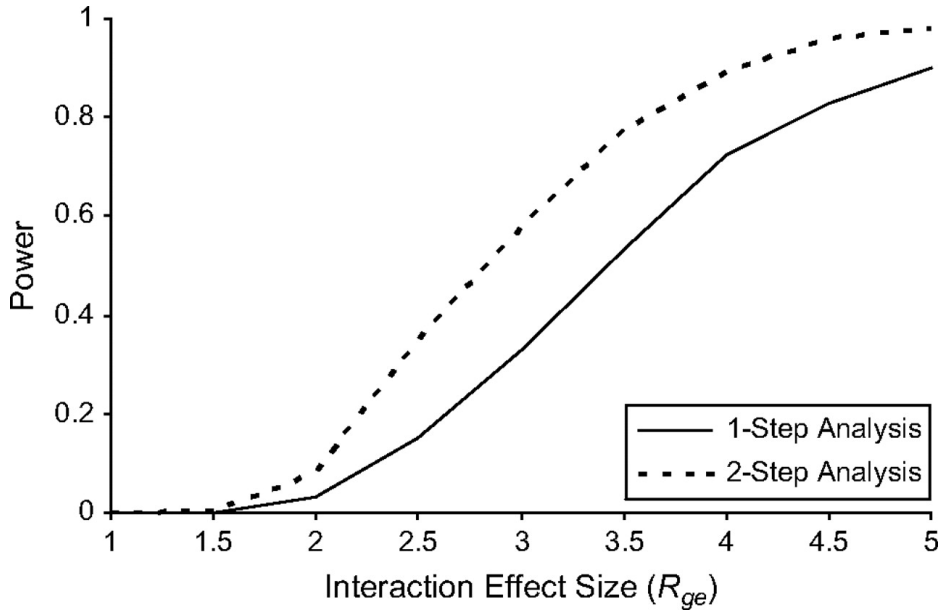
- $\hat{\tau}^2$ is an estimate of the G-E dependence θ^2_{GE}

Mukherjee & Chatterjee, Biometrics 2008

Genome-wide G-E Interaction analysis: 2-step approaches

1. Test for GxE dependence and/or associations between the SNP and your outcome in your entire dataset. Select SNPs with p<α_1

2. Take m SNPs from stage 1 and perform traditional GxE interaction tests in a case-control setting (1 df). All SNPs with p<α/m are declared significant



Murcray et al, Am J Epi 2009, Genet Epi 2011

Table 3. Genome-wide significance of tests for gene-environment interaction for rs11066015 (12q24) and rs3805322 (4q23)

	Genome-wide Significant? ($\alpha=5\times10^{-8}$)	
	ALDH2 rs11066015[a]	*ADH* rs3805322[b]
Standard case-control test	**Yes**	no
Case-only test	No	**Yes**
Empirical Bayes test	**Yes**	no
Hybrid two-step approach	**Yes**	no
Cocktail 1	**Yes**	**Yes**
Cocktail 2	**Yes**	**Yes**

[a] Empirical Bayes estimate of $OR_{G\times E}=3.66$ (2.79,4.80); for the screening stage of the hybrid test, both G-E association and marginal G-D tests were significant with $p_A=6.0\times10^{-14}<\alpha_A$ and $p_M=7.3\times10^{-8}<\alpha_M$, and the standard test of G×E interaction at the second stage was quite significant ($p<10^{-16}$); for the cocktail methods, $p^{screen}=p_M$ for cocktail 1 and $p^{screen}=p_A$ for cocktail 2, both of these pass the first stage threshold, and the second stage tests (the Empirical Bayes test for Cocktail 1 and standard case-control test for Cocktail 2) are both very significant ($p<10^{-16}$).

[b] Empirical Bayes estimate of $OR_{G\times E}=1.70$ (1.36,2.20), $p=5.4\times10^{-5}$; for the screening stage of the hybrid test, both G-E association and marginal G-D tests were significant with $p_A=1.1\times10^{-9}<\alpha_A$ and $p_M=9.3\times10^{-13}<\alpha_M$, however, the standard test of G×E interaction at the second stage did not meet the second stage threshold ($\sim 4.2\times10^{-4}$); for the cocktail methods, $p^{screen}=p_M$ for cocktail 1 and 2, which passes the first stage threshold, and the second stage test (the Empirical Bayes test for both) meets the second stage threshold ($\sim 4.2\times10^{-4}$).

Wu et al, Genet Epi 2014

GxE interaction studies require large sample sizes

- A common approach is to pool data from multiple studies within large international consortia.

- Although this will result in greatly increases sample size, it introduces challenges for harmonizing data across studies. This is often the most difficult and time-consuming part of a multi-study GxE interaction study

Harmonizing E

N=25,050

(a)

Study (*N*)	Smoking-related questions	Possible responses
Study 1 (2,500)	1. Do you currently smoke cigarettes?	Y/N
	2. If yes, how many cigarettes per day?	###
Study 2 (1,200)	1. Have you smoked more than 100 cigarettes in your lifetime?	Y/N
	2. If yes, do you currently smoke?	Y/N
	3. If yes, how many packs per day do you smoke?	###
Study 3 (8,500)	1. Have you ever smoked?	Y/N
Study 4 (1,250)	1. Do you currently smoke?	Y/N
Study 5 (4,200)	1. Do you smoke?	Y/N
	2. When did you first start smoking regularly?	Past year; 1–5 years ago; >5 years ago
Study 6 (6,600)	1. Have you smoked tobacco in the past month?	Y/N
Study 7 (800)	1. Have you ever smoked regularly?	Y/N
	2. If yes, do you still smoke?	Y/N
	3. If yes, how much do you smoke a day?	1–10 cigarettes, 11–20 cigarettes, 21–30 cigarettes, >30 cigarettes

Bennett SN, et al. Genet Epidemiol. 2011

Harmonizing E

What are the sample sizes for these derived variables?

- Cigarettes per day
- Packs per day
- Former smoker
- ***Ever smoker***
- Current smoker

Table II. Examples of possible (a) smoking-related questions and (b) new variables for cross-study analyses

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Harmonizing E

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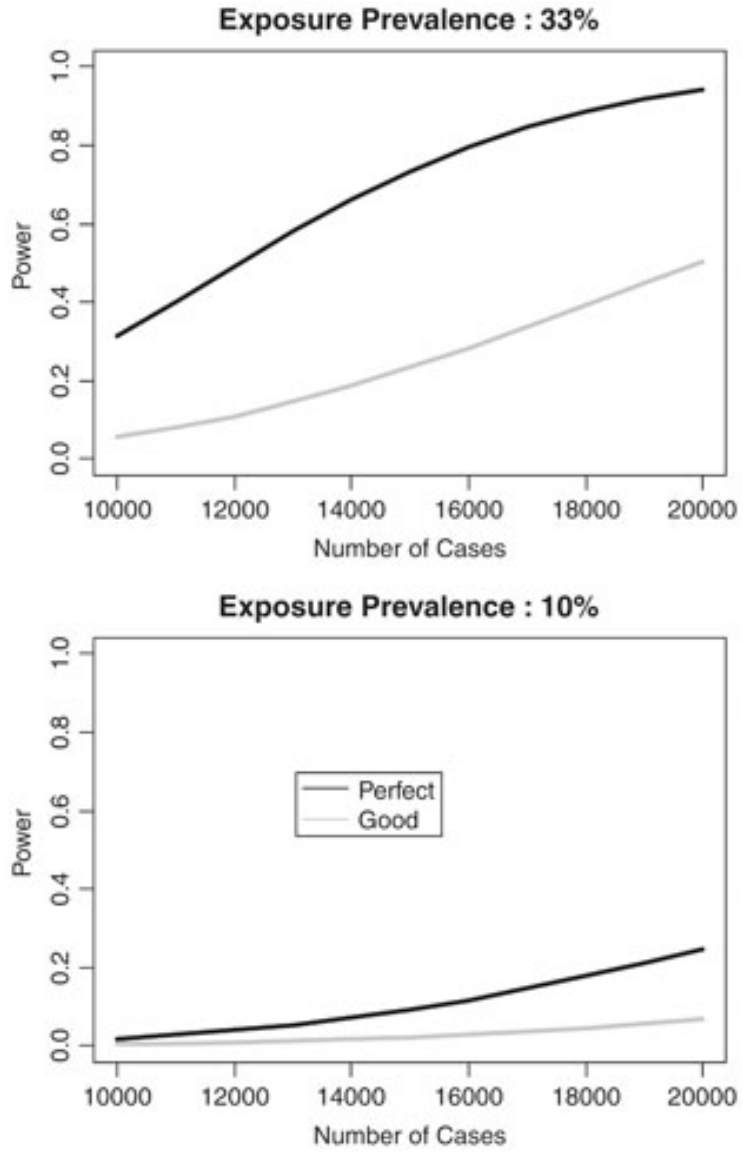
(b)

New variable	Studies that could contribute data	Total N	Comment
Cigarettes per day	Study 1	2,500	Data from Study 7 might also be included if specific values were assigned to each response category, e.g. 5 for category '1–10 cigarettes', 15 for category '11–20 cigarettes', and so on
Packs per day	Study 1 (if convert cigarettes/day to packs/day)	4,500	
	Study 2		
	Study 7 (if convert categories to packs/day)		
Former smoker	Study 2	2,000	
	Study 7		
Ever smoker	Study 2	10,500	Requires ability to determine if subjects are
	Study 3		former smokers
	Study 7		
Current smoker	Study 1	16,550	
	Study 2		
	Study 4		
	Study 5		
	Study 6 (if current smoker is defined as having smoked in the past month)		
	Study 7		

Bennett SN, et al. Genet Epidemiol. 2011

Even small errors in measurement can greatly decrease power to detect gene-environment interaction



"Good"
Sensitivity=77%
Specificity=99%

Bennett SN, et al. Genet Epidemiol. 2011

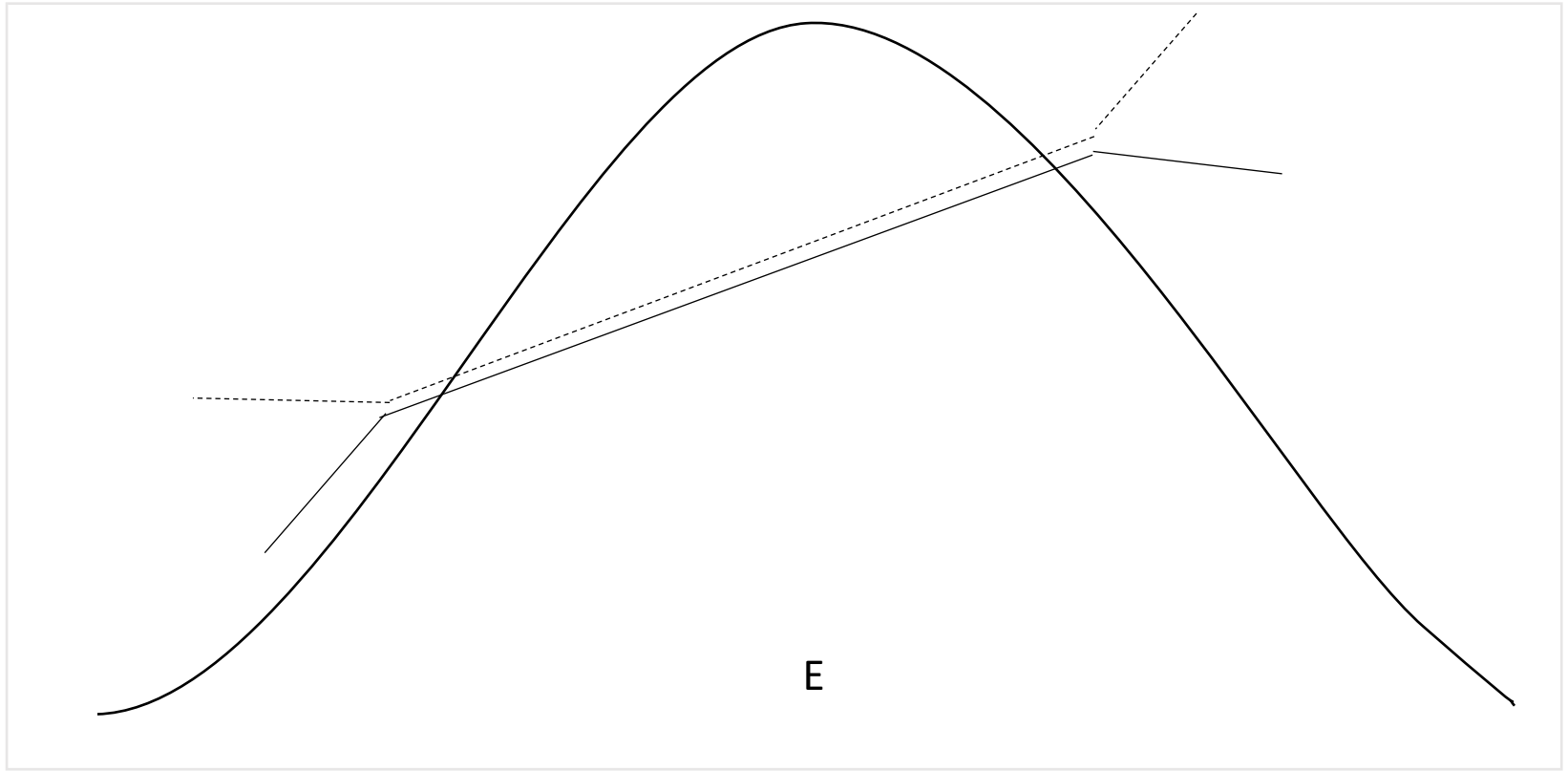
How, where, and when you measure exposure have consequences for evaluating gene-environment interactions

Different distribution of the exposure across studies

G=0

G=1

Discovery Sample



Kraft and Hunter (2010)

How, where, and when you measure exposure have consequences for evaluating gene-environment interactions

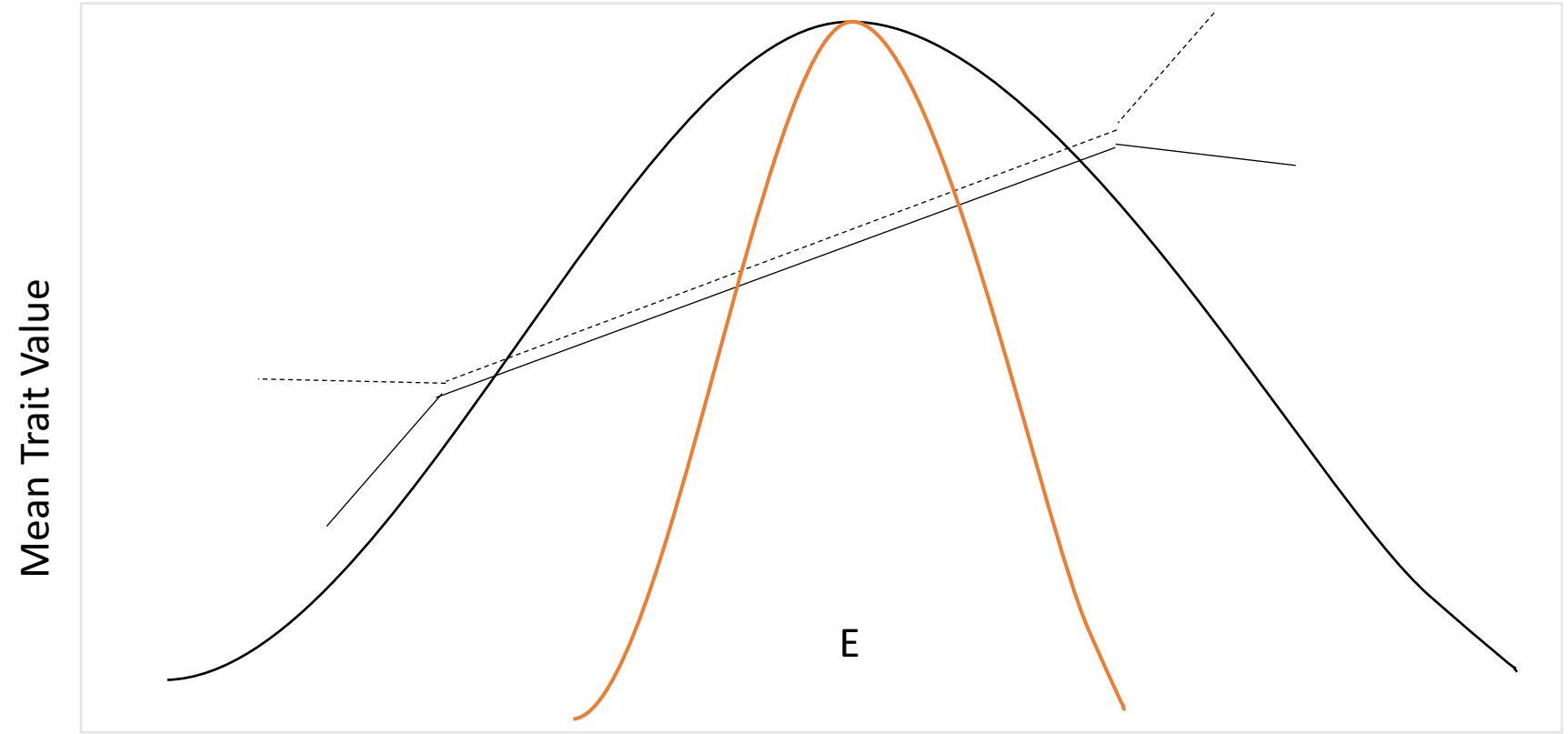
Different distribution of the exposure across studies

G=0

G=1

Discovery Sample

Replication Sample



Kraft and Hunter (2010)

Slide courtesy of L Mechanic

FTO, Physical Activity and Obesity

- Meta-analysis of 218,166 European-ancestry subjects
- Risk of Obesity (BMI ≥ 30 vs. BMI < 25 kg/m^2) for *FTO* rs9939609

	OR (95% CI)
Inactive	1.30 (1.24-1.36)
Active	1.22 (1.19-1.25)
Rs9939609 x Physical activity interaction	0.92 (0.88-0.97)
	P-value = 0.0010

Kilpelainen, 2011

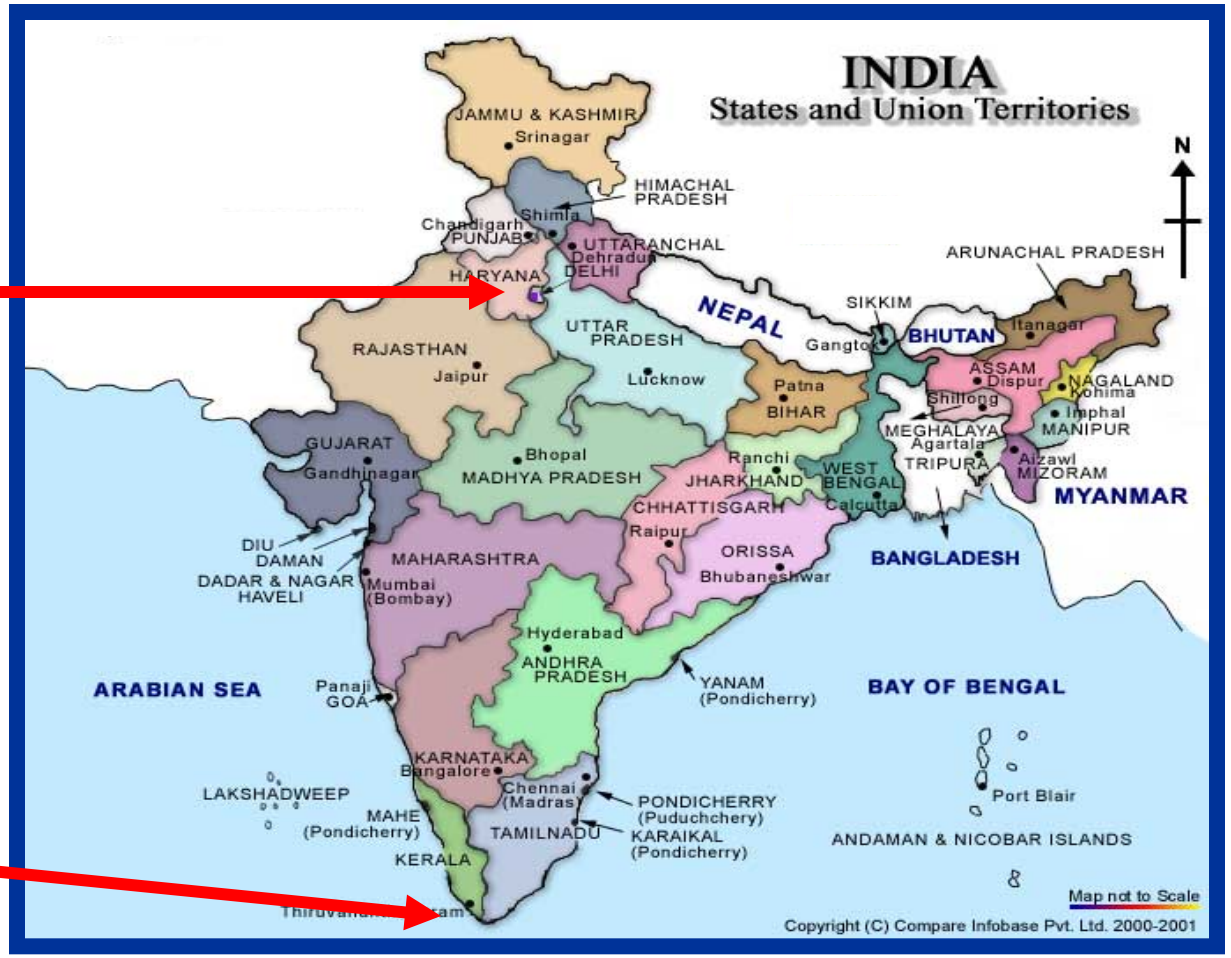
Slides courtesy of N Chatterjee

India health study

Interaction between FTO genotype, physical activity and obesity

New Delhi

Trivandrum



Participant characteristics by region

Characteristic	New Delhi	Trivandrum
Total (n=1,313)	n=619	n=694
Age, years (mean, SD)	47.4 ± 10.0	48.8 ± 9.2
Household monthly income, %		
<5,000 rupees	7.1	71.9
>10,000 rupees	76.7	3.1
Household items, %		
Car	25	7
Refrigerator	87	58
Washing machine	79	14
Total physical activity, MET-hr/wk	42.5 ± 43.8	147.3 ± 85.2
Vigorous physical activity, MET-hr/wk	0.6 ± 6.8	26.2 ± 51.4
Sitting, hr/day	10.4 ± 2.0	5.0 ± 2.3
Centrally obese, %	82.1	60.2

Association of *FTO* rs3751812 with waist circumference

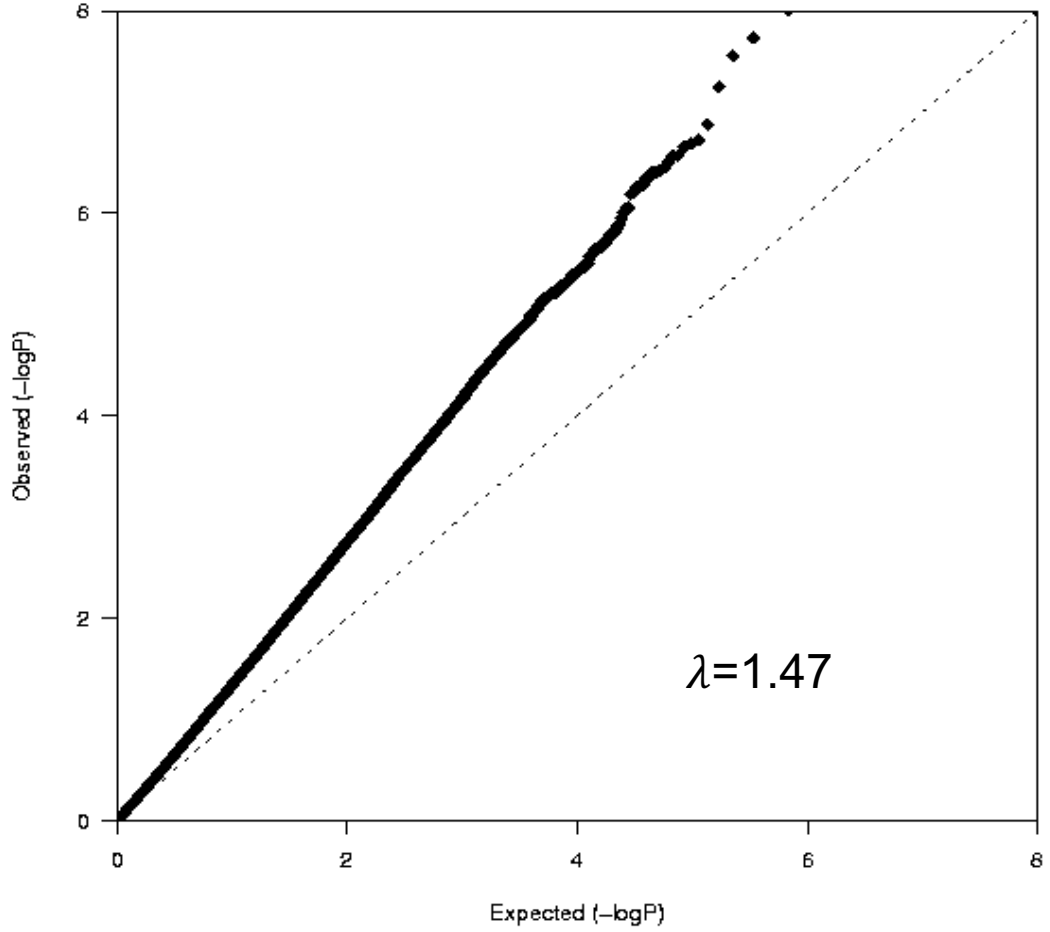
Characteristic	N	**Effect size per T allele (95% CI)**	P_{trend}	**Interaction by PA**
Overall	1,209	**+1.61 cm (0.67, 2.55)**	0.0008	**0.009**
New Delhi				
Overall	578	**+2.53 cm (1.08, 3.97)**	0.0006	**0.59**
By PA				
≤ 91 MET-hrs/wk	517	**+2.36 cm (0.82, 3.89)**	0.003	
92-151 MET-hrs/wk	32	**+6.39 cm (1.94, 10.85)**	0.005	
152-217 MET-hrs/wk	24	**-0.95 cm (-7.33, 5.42)**	0.77	
218+ MET-hrs/wk	5	**N/A**	N/A	
Trivandrum				
Overall	574	**+0.87 cm (-0.35, 2.08)**	0.16	**0.004**
By PA				
≤ 91 MET-hrs/wk	170	**+3.50 cm (0.90, 6.10)**	0.008	
92-151 MET-hrs/wk	132	**+1.13 cm (-1.08, 3.33)**	0.32	
152-217 MET-hrs/wk	141	**+1.04 cm (-1.63, 3.70)**	0.45	
218+ MET-hrs/wk	131	**-2.32 cm (-4.82, 0.18)**	0.07	

Moore, 2012

A note about modeling “E”

Genome-wide GxE Interaction study of BMI and Type II Diabetes

Standard case-control test for GxE Interaction



Slide courtesy Marilyn Cornelis

Tchetgen Tchetgen and Kraft, Epidemiology, 2011

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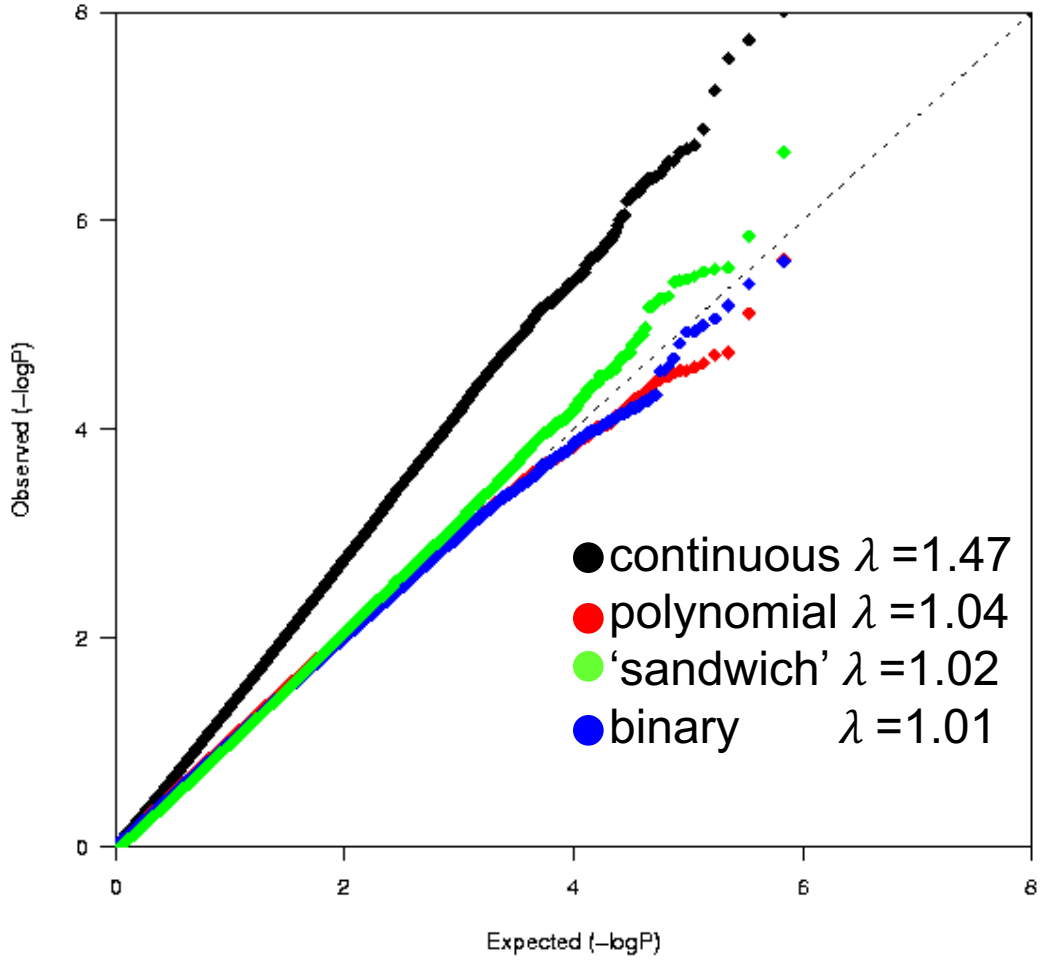


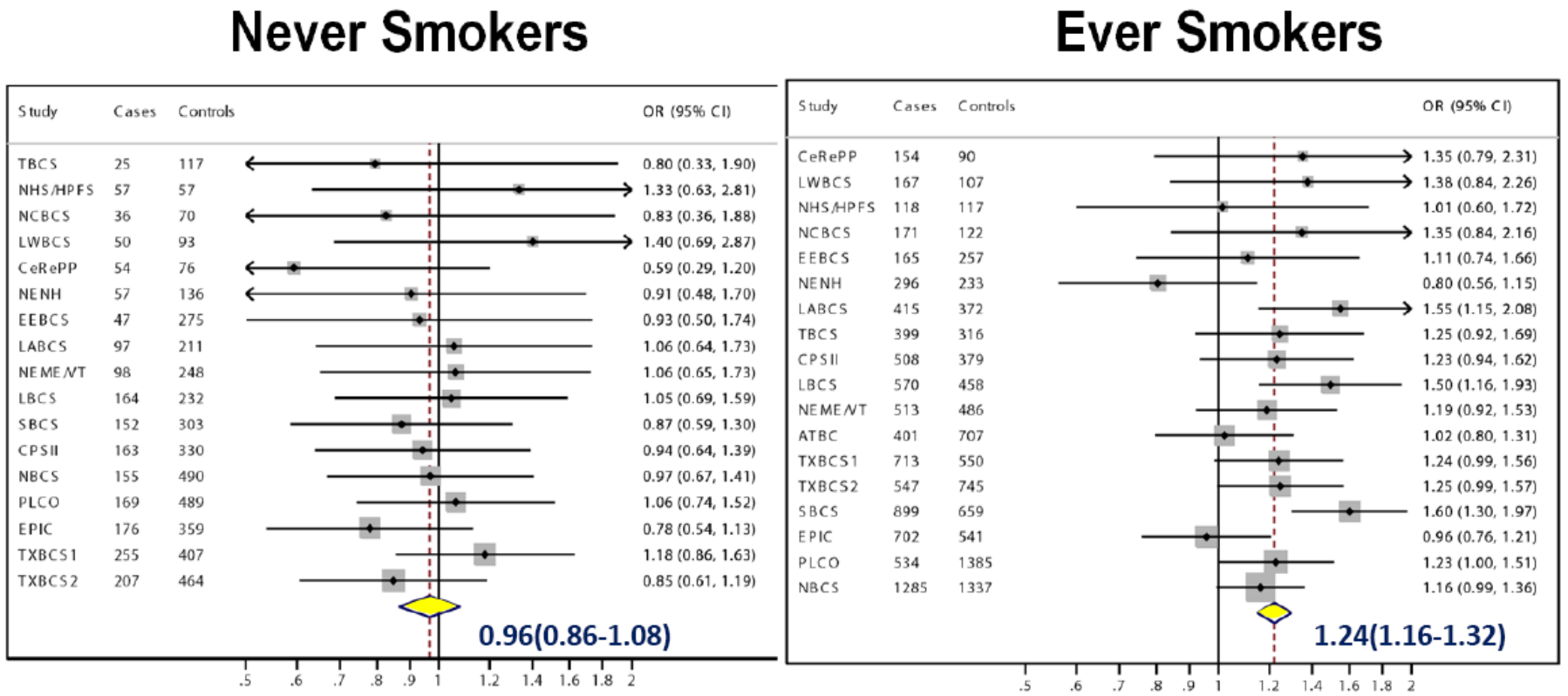
Slide courtesy Marilyn Cornelis

Tchetgen Tchetgen and Kraft, Epidemiology, 2011

Real data examples

Slide courtesy of N Rothman

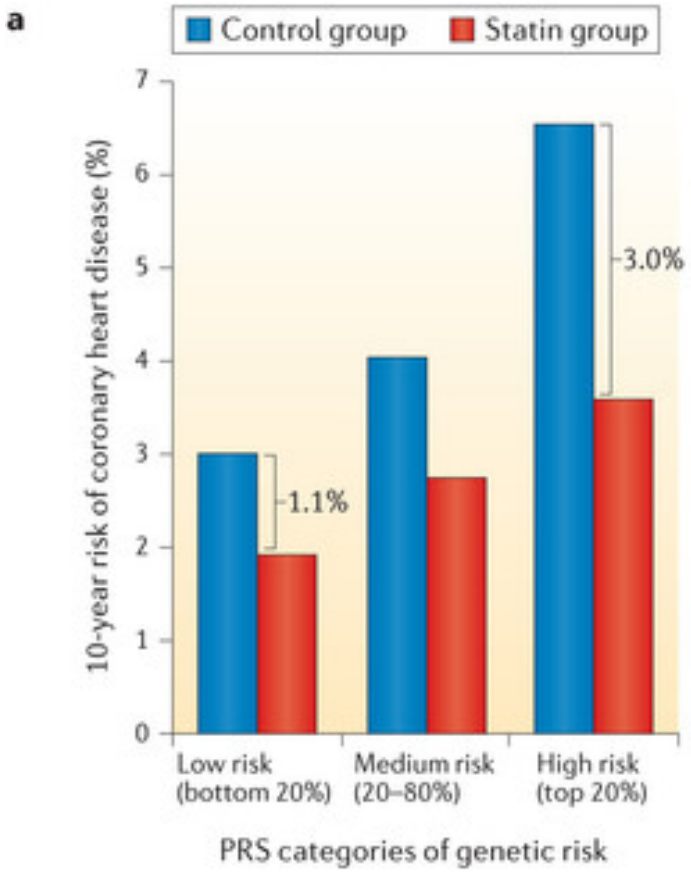
GE Interaction for Bladder Cancer Risk: NAT2 Slow Acetylation Increases Risk only for Smokers



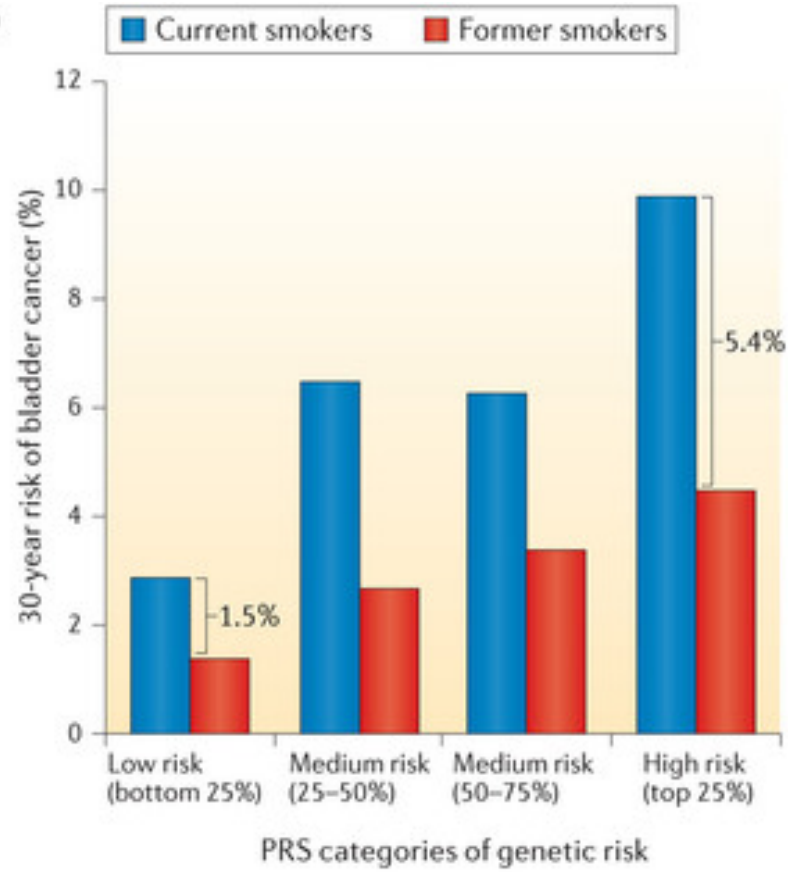
P-interaction = 2.8×10^{-4}

Rothman *et al.*, Nat Genet 2010

Intervention in high-risk groups is more efficient



ARR (%)	1.1	1.3	3.0
RRR	0.36	0.32	0.46

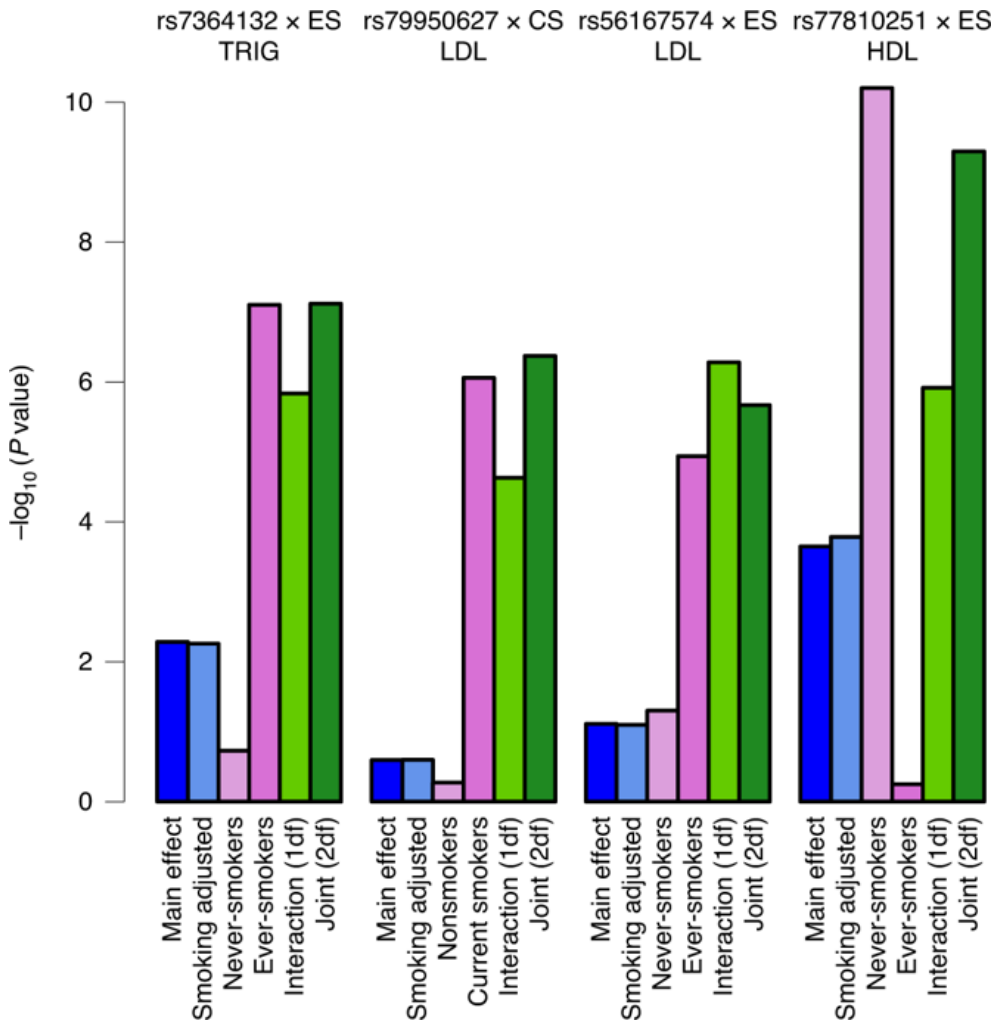


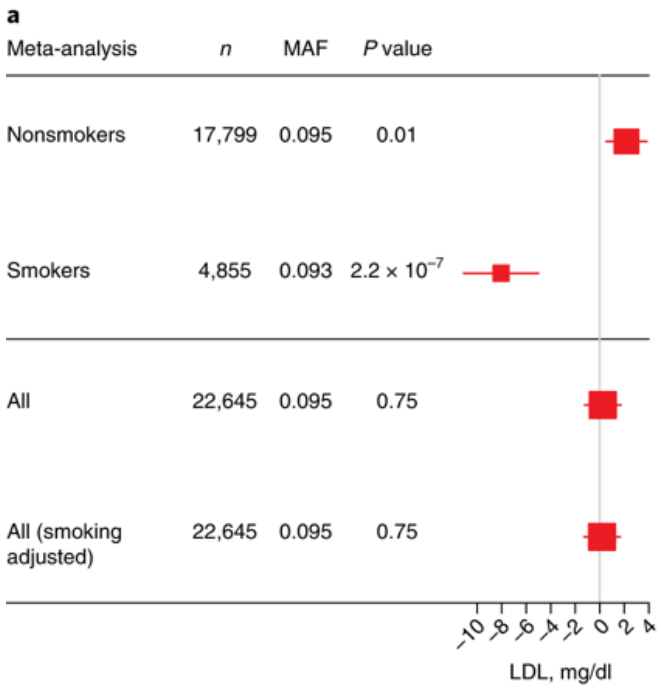
ARR (%)	1.5	3.8	2.9	5.4
RRR	0.52	0.60	0.46	0.55

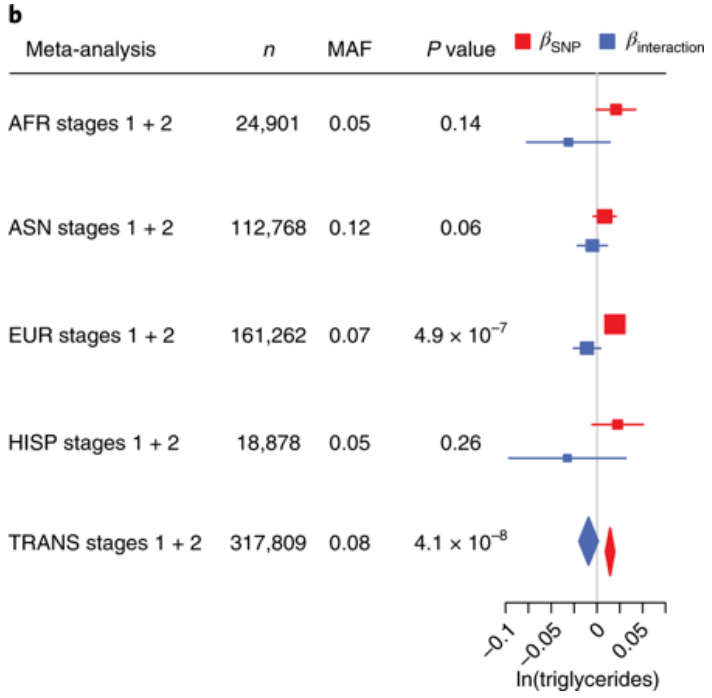
ARR=Absolute risk reduction

Nature Reviews | Genetics

Genome-wide gene–smoking interaction study of serum lipids in 387,272 individuals







Bentley et al. Nat Genet 2019

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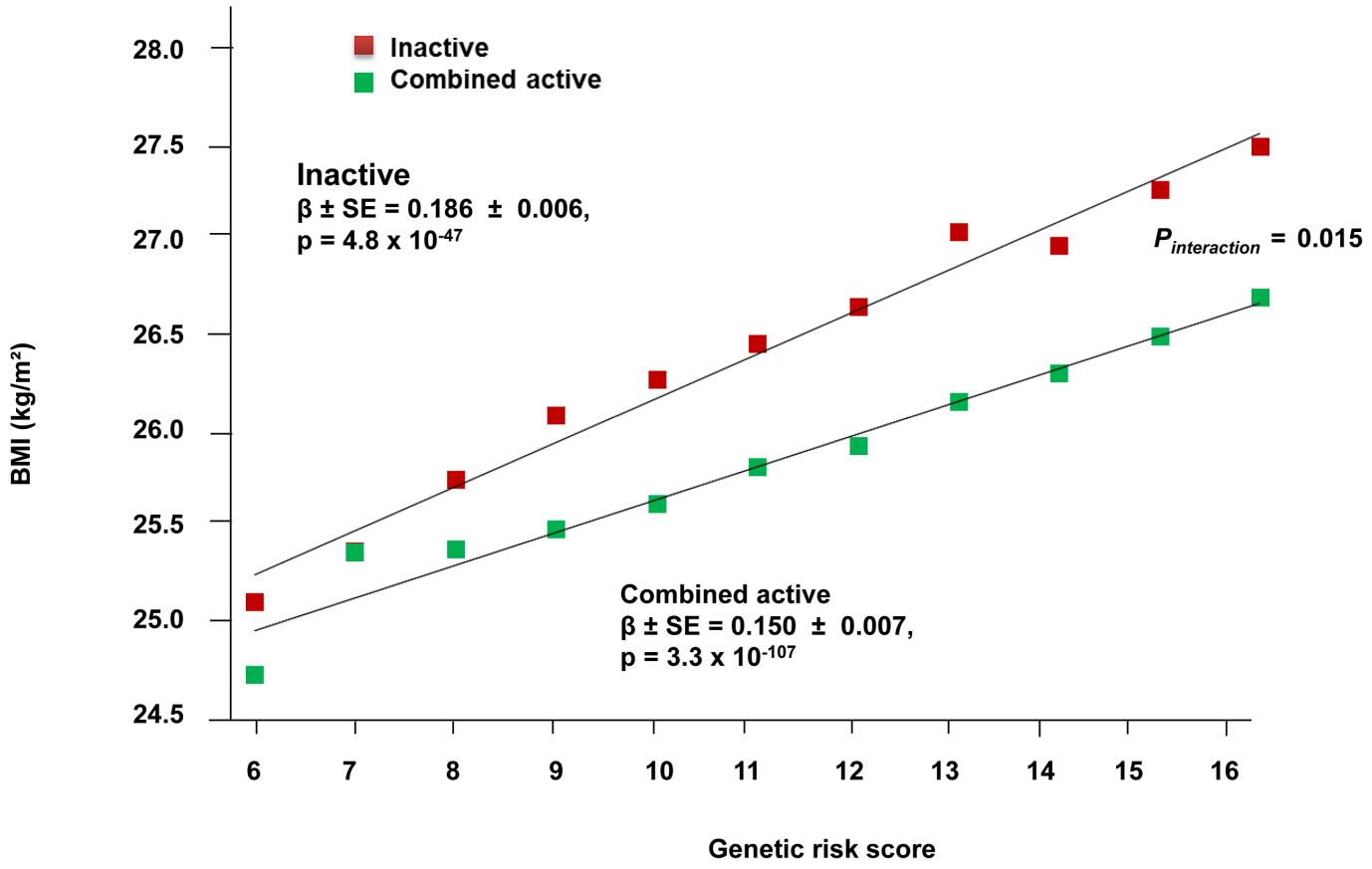

SNPs	Nearest gene	β_{GE}	(95% CI)	$P_{interaction}$
rs1121980*	*FTO*	−0.052	(−0.086, −0.018)	0.003
rs7498665*	*SH2B1*	−0.003	(−0.039, 0.033)	0.867
rs10913469*	*SEC16B*	−0.049	(−0.091, −0.006)	0.025
rs10838738*	*MTCH2*	−0.012	(−0.047, 0.023)	0.502
rs17782313*	*MC4R*	−0.029	(−0.069, 0.010)	0.147
rs3101336*	*NEGR1*	0.006	(−0.028, 0.040)	0.728
rs6548238*	*TMEM18*	0.002	(−0.043, 0.047)	0.936
rs10938397	*GNPDA2*	−0.001	(−0.036,0.034)	0.946
rs925946*	*BDNF*	−0.013	(−0.052, 0.025)	0.491
rs368794*	*KCTD15*	−0.001	(−0.037, 0.035)	0.969
rs7647305*	*ETV5*	0.024	(−0.018, 0.066)	0.267
rs7132908*	*FAIM2*	−0.024	(−0.059, 0.010)	0.164

Physical activity was expressed according to the Cambridge Physical Activity Index (CPAI) (4 level scale); further details for the construction of the CPAI can be found in the *Materials and Methods* section and Table S7.
*Some studies used proxies for these variants, as reported in Table S8.
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Ahmad, PLoS Genetics 2013

Software for analysis

Software	Good for	URL
PLINK	GWAS, data handling, GE test, joint test	http://pngu.mgh.harvard.edu/~purcell/plink/
GxEscan	R script incorporating multiple GWAS GxE tests	http://biostats.usc.edu/software
Multassoc	Test a group of SNPs taking interaction with other G, E into account	http://dceg.cancer.gov/tools/analysis/multassoc
R	Flexible, write your own scripts	http://www.r-project.org/
METAL	Meta-analysis	http://www.sph.umich.edu/csg/abecasis/metal/
CGEN	R package, additive interaction	https://rdrr.io/bioc/CGEN/man/additive.test.html

Software for power calculations

Software	Good for	URL
Quanto	Joint test, GE test, family-based designs, case-control, continuous outcome	http://hydra.usc.edu/gxe/
Power	Additive interaction	http://dceg.cancer.gov/tools/design/power
Gemis	Misclassification in E	http://www.hsph.harvard.edu/peter-kraft/software/