National Human Genome Research Institute

National Institutes of Health

U.S. Department of Health and Human Services

Evaluating Potential Biases in and Interpreting Results from Epidemiologic Studies

U.S. Department of Health and Human Services
National Institutes of Health
National Human Genome Research Institute

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24, 2007

Nothing to Disclose

Essay

Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, outcomes, and analytical modes; when

factors that influence this problem and some corollaries thereof.

Modeling the Framework for False Positive Findings

Several methodologists have pointed out [9–11] that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a *p*-value less than 0.05. Research is not most appropriately represented and summarized by *p*-values, but, unfortunately, there is a widespread

is characteristic of the field and can vary a lot depending on whether the field targets highly likely relationships or searches for only one or a few true relationships among thousands and millions of hypotheses that may be postulated. Let us also consider, for computational simplicity, circumscribed fields where either there is only one true relationship (among many that can be hypothesized) or the power is similar to find any of the several existing true relationships. The pre-study probability of a relationship being true is $R/(R+1)$. The probability of a study finding a true relationship reflects the power $1 - \beta$ (one minus the Type II error rate). The probability

PLoS Med. 2005 Aug;2(8):e124.

WSJ.com THE WALL STREET JOURNAL. ONLINE

SCIENCE JOURNAL

By ROBERT LEE HOTZ

Most Science Studies Appear to Be Tainted By Sloppy Analysis

September 14, 2007; Page B1

We all make mistakes and, if you believe medical scholar John Ioannidis, scientists make more than their fair share. By his calculations, most published research findings are wrong.

Dr. Ioannidis is an epidemiologist who studies research methods at the University of Ioannina School of Medicine in Greece and Tufts University in Medford, Mass. In a series of influential analytical reports, he has documented how, in thousands of peer-reviewed research papers published every year, there may be so much less than meets the eye.

WSJ. 2004Sep14.

A Common Genetic Variant Is Associated with Adult and Ch

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Comment on "A Common Genetic Variant Is Associated with Adult and Childhood Obesity"

Ruth J. F. Loos,[1]* Inês Barroso,[2] Stephen O'Rahilly,[3] Nicholas J. Wareham[1]

Herbert *et al.* (Reports, 14 April 2006, p. 279) found that the rs7566605 genetic variant, located upstream of the *INSIG2* gene, was consistently associated with increased body mass index. However, we found no evidence of association between rs7566605 and body mass index in two large ethnically homogeneous population-based cohorts. On the contrary, an opposite tendency was observed.

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Science, 14 Apr 2006

Science, 12 Jan 2007

The Association of a SNP Upstream of *INSIG2* with Body Mass Index is Reproduced in Several but Not All Cohorts

Helen N. Lyon[1,2,3☯*], Valur Emilsson[4☯], Anke Hinney[5☯], Iris M. Heid[6,7☯], Jessica Lasky-Su[8,9☯], Xiaofeng Zhu[10☯], Gudmar Thorleifsson[4], Steinunn Gunnarsdottir[4], G. Bragi Walters[4], Unnur Thorsteinsdottir[4], Augustine Kong[4], Jeffrey Gulcher[4], Thuy Trang Nguyen[11,12], André Scherag[11,12], Arne Pfeufer[13,14], Thomas Meitinger[13,14], Günter Brönner[5], Winfried Rief[11,12], Manuel E. Soto-Quiros[15], Lydiana Avila[15], Barbara Klanderman[8], Benjamin A. Raby[8], Edwin K. Silverman[8], Scott T. Weiss[8], Nan Laird[8], Xiao Ding[8], Leif Groop[16,17,18], Tiinamaija Tuomi[17,18,19], Bo Isomaa[19], Kristina Bengtsson[17,18], Johannah L. Butler[1,2], Richard S. Cooper[20], Caroline S. Fox[21], Christopher J. O'Donnell[21], Caren Vollmert[6], Juan C. Celedón[8], H. Erich Wichmann[6,7], Johannes Hebebrand[5], Kari Stefansson[4], Christoph Lange[8], Joel N. Hirschhorn[1,2,22]

A SNP upstream of the *INSIG2* gene, rs7566605, was recently found to be associated with obesity as measured by body mass index (BMI) by Herbert and colleagues. The association between increased BMI and homozygosity for the minor allele was first observed in data from a genome-wide association scan of 86,604 SNPs in 923 related individuals from the Framingham Heart Study offspring cohort. The association was reproduced in four additional cohorts, but was not seen in a fifth cohort. To further assess the general reproducibility of this association, we genotyped rs7566605 in nine large cohorts from eight populations across multiple ethnicities (total $n = 16{,}969$). We tested this variant for association with BMI in each sample under a recessive model using family-based, population-based, and case-control designs. We observed a significant ($p < 0.05$) association in five cohorts but saw no association in three other cohorts. There was variability in the strength of association evidence across examination cycles in longitudinal data from unrelated individuals in the Framingham Heart Study Offspring cohort. A combined analysis revealed significant independent validation of this association in both unrelated ($p = 0.046$) and family-based ($p = 0.004$) samples. The estimated risk conferred by this allele is small, and could easily be masked by small sample size, population stratification, or other confounders. These validation studies suggest that the original association is less likely to be spurious, but the failure to observe an association in every data set suggests that the effect of SNP rs7566605 on BMI may be heterogeneous across population samples.

Lyon HN et al, *PLoS Genet*; 2007 Apr 27;3(4):e61.

The Association of a SNP Upstream of *INSIG2* with Body Mass Index is Reproduced in Several but Not All Cohorts

- Nine large cohorts from eight populations across multiple ethnicities
- Family-based, population-based, case-control designs
- Association at $p < 0.05$ in five cohorts but none in three cohorts
- Variability in strength of association over time
- Replication both in unrelated ($p = 0.046$) and family-based ($p = 0.004$) samples
- Suggests initial finding unlikely to be spurious but effect likely to be heterogeneous

Lyon HN et al, *PLoS Genet*; 2007 Apr 27;3(4):e61.

rs7566605 C/C Genotype and BMI ≥ 30 kg/m^2 in Unrelated Individuals (Lyon et al, *PLoS Gen* 2007)

	Obesity Association			Frequency C/C	
Cohort	OR	95% CI	P-value	Cases	Controls
Essen	1.75	[1.15-2.67]	0.008	0.05	0.05
FHS 1	1.26	[0.78-2.01]	0.06	0.14	0.11
FHS 2	1.52	[0.95-2.43]	0.08	0.16	0.11
FHS 3	1.81	[1.22-2.70]	0.003	0.18	0.11
FHS 4	1.18	[0.80-1.74]	0.4	0.13	0.11
FHS 5	1.14	[0.79-1.65]	0.5	0.12	0.11
FHS 6	1.12	[0.79-1.59]	0.5	0.13	0.11
Iceland	1.29	[1.06-1.57]	0.007	0.13	0.11
KORA S3	0.90	[0.70-1.16]	0.4	0.10	0.11
Maywood	0.88	[0.49-1.59]	0.7	0.06	0.06
Scandinavia	1.25	[0.69-2.24]	0.5	0.13	0.10

rs7566605 Genotype and BMI ≥ 30 kg/m^2 in Family Cohorts (Lyon et al, *PLoS Gen* 2007)

	Mean Body Mass Index (kg/m^2)			
Cohort	C/C	C/G	G/G	P-value
CAMP	18.05	17.97	17.52	0.026
Costa Rica	18.19	17.46	17.72	0.027
Scandinavia	25.70	26.43	26.43	0.96
Combined				0.004

Possible Explanations of Heterogeneity of Results in Genetic Association Studies

- Biologic mechanisms
  - Genetic heterogeneity
  - Gene-gene interactions
  - Gene-environment interactions
- Spurious mechanisms
  - Selection bias
  - Information bias
  - Publication bias
  - Confounding (population stratification)
  - Cohort, age, period (secular) effects
  - Type I error

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- Spurious mechanisms
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  - Publication bias
  - Confounding (population stratification)
  - Cohort, age, period (secular) effects
  - Type I error

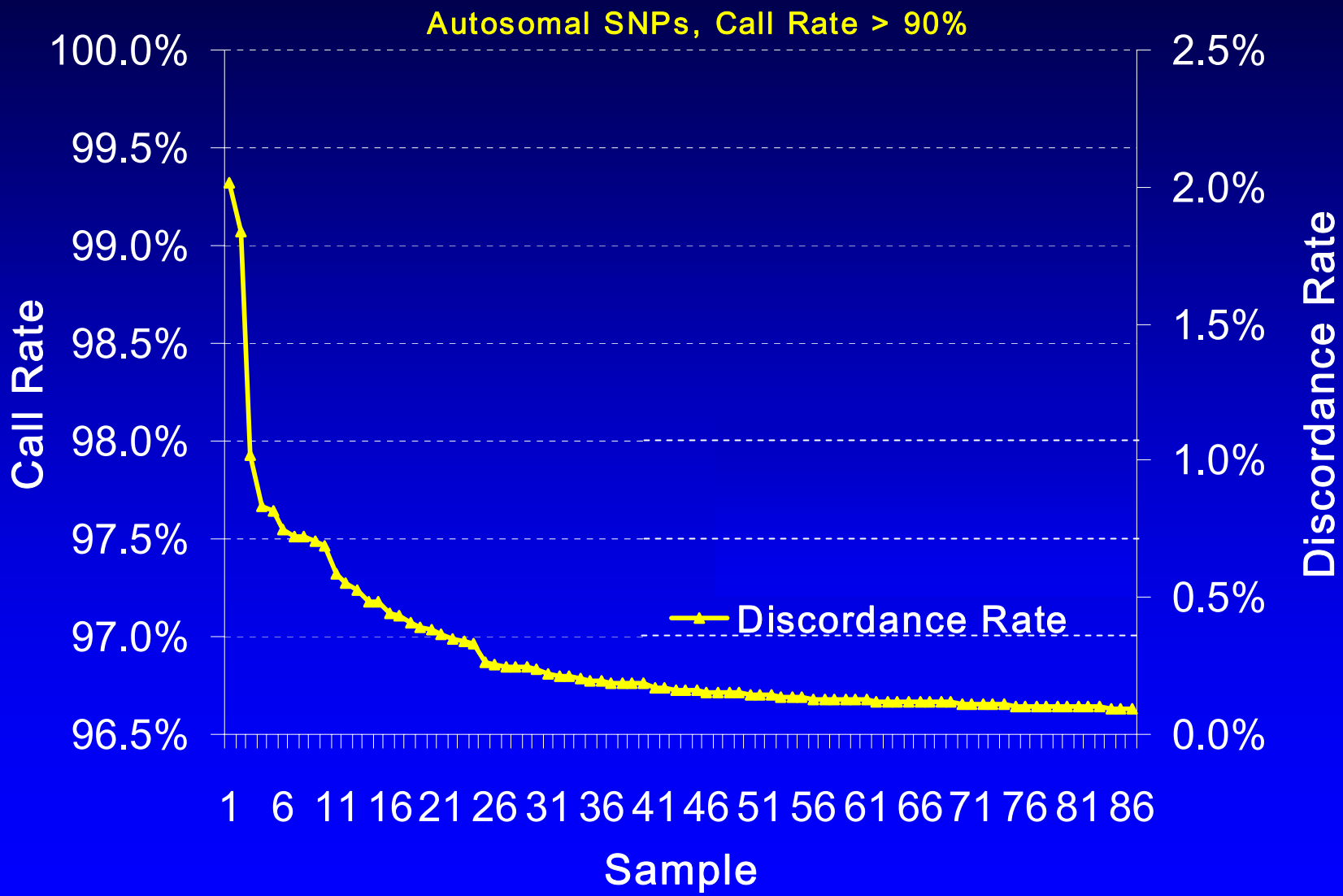
Definition of Bias

"Any process at any stage of inference which tends to produce results or conclusions that differ systematically from the truth."

To be distinguished from random error…

Sackett DL. Bias in analytic research. *J Chron Dis* 1979; 32:51-63.

Correlation between Discordance and Call Rate, Comparing ~250K SNPs in Common



Courtesy, K. Doheny

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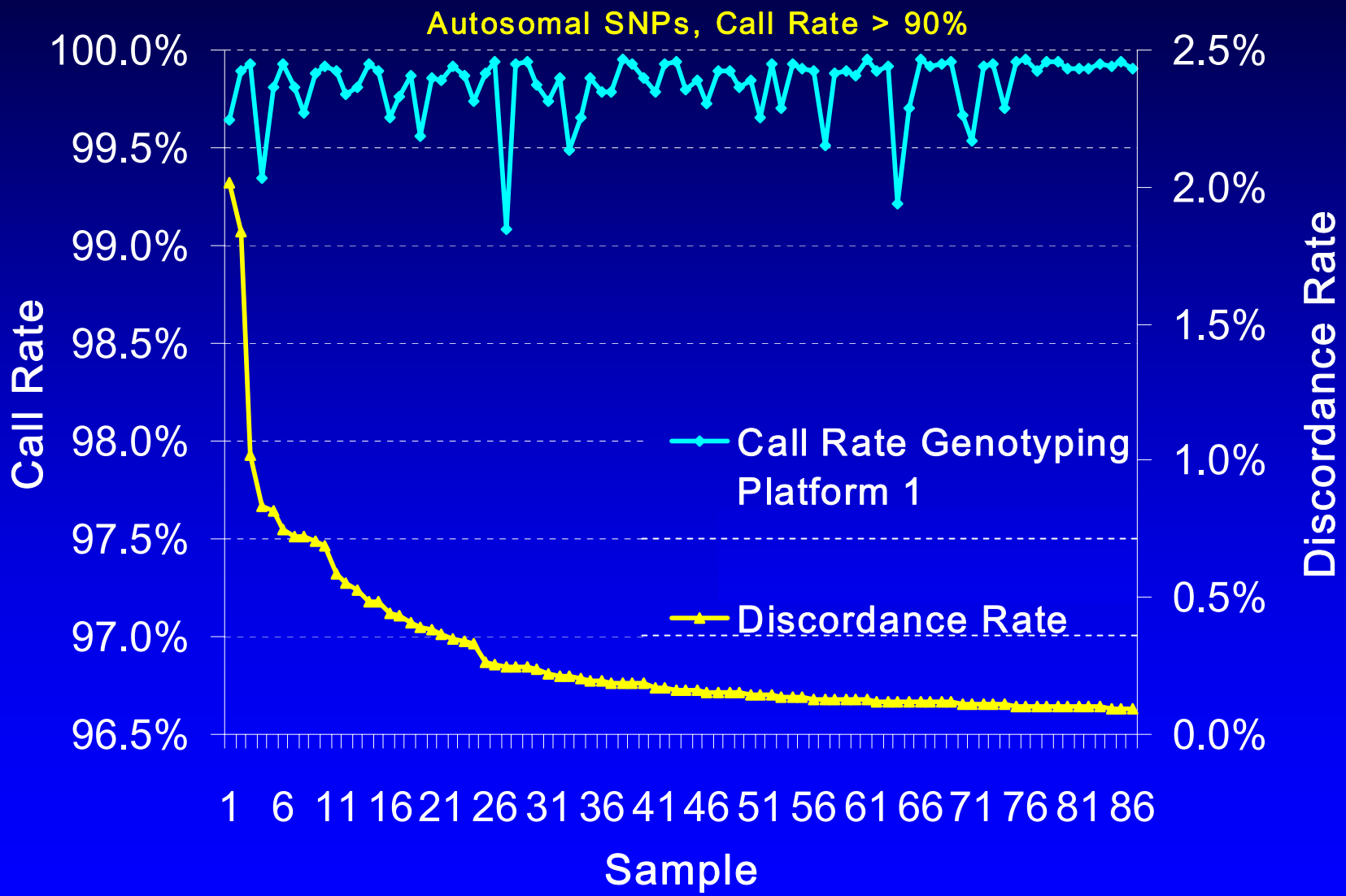

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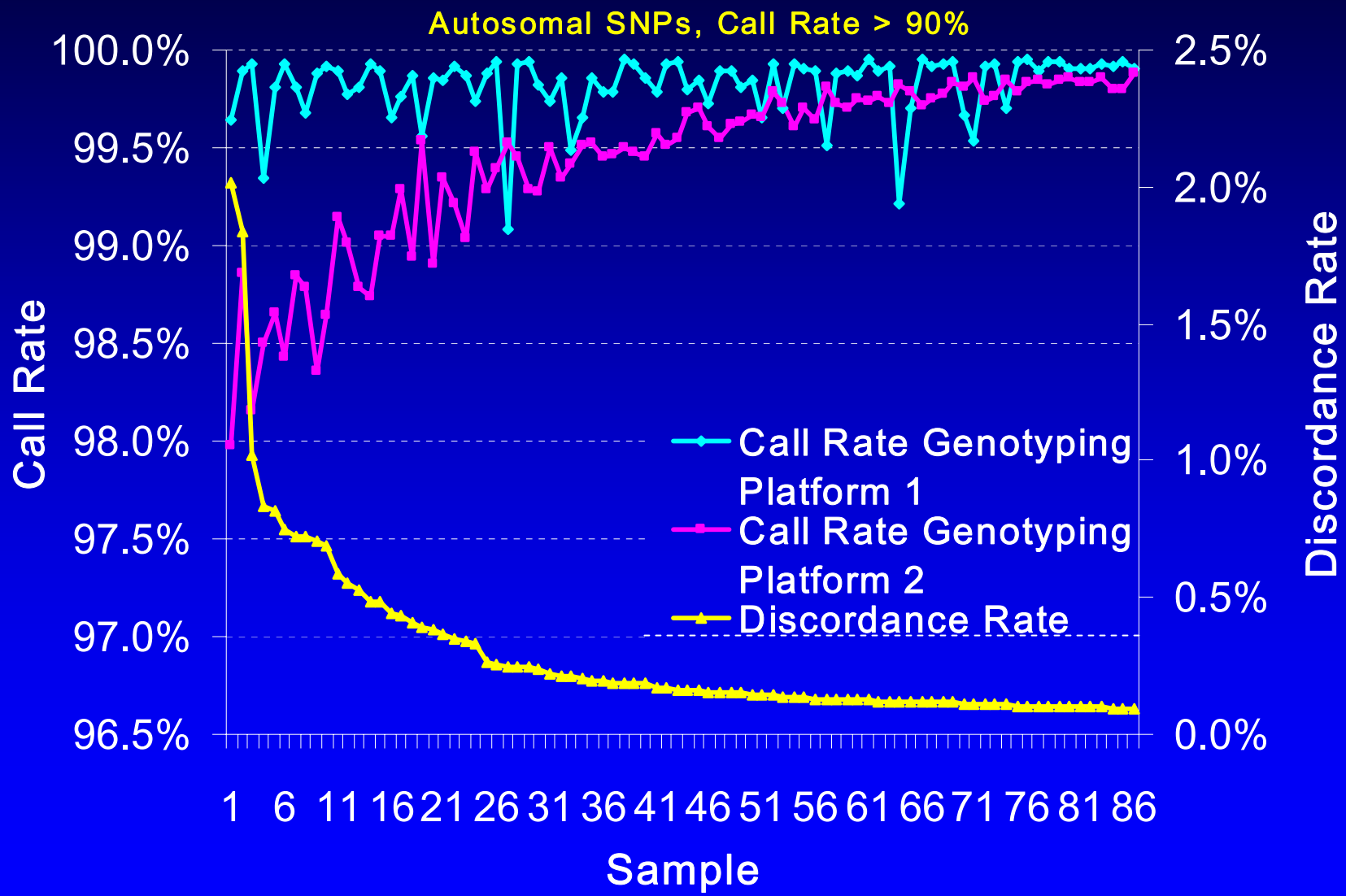

Courtesy, K. Doheny

Key Requirements for a Bias-Free Case-Control Study

- Cases are representative of all those in the study base who develop the disease
- Controls are representative of all those in the study base at risk of developing the disease and eligible to become cases and be detected in the study
- Collection of risk factor and exposure information is the same for cases and controls
- *Ancestral geographical origins and predominant environmental exposures of cases do not differ dramatically from controls*

Key Requirements for a Bias-Free Case-Control Study

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Selection Bias

- Collection of risk factor and exposure information is the same for cases and controls
- *Ancestral geographical origins and predominant environmental exposures of cases do not differ dramatically from controls*

Information Bias

Selection Bias: systematic differences between those who are selected for study and those who are not

- Prevalence-incidence or survival bias: Selection of currently available, existing cases will miss fatal and short episodes, and may miss mild or silent cases
- Non-response bias: Differential rates of non-response to inquiries between cases and controls
- Membership bias: Membership in a group (blood donors, Army recruits) may imply a degree of health differing systematically from the general population
- Referral or admission rate bias: Cases who are more likely to receive advanced treatment (those with greater access to health care or co-existing illness) may distort associations with other factors

Sackett D, *J Chron Dis* 1979; 32:51-63 and Schlesselman J, *Case-Control Studies*, 1982.

Are cases representative of all those who develop the disease?

- To assess representativeness and potential biases, need to know how cases defined
- Study of atrial fibrillation (Gudbjartsson et al, 2007)
  - Sample 1: hospital diagnosis of AF "confirmed by 12-lead ECG"
  - Sample 2: Patients with ischemic stroke or TIA, diagnosis of AF "based on 12-lead ECG"
  - Sample 3: Patients hospitalized with acute stroke "diagnosed with AF"
  - Sample 4: Patients with lone AF or AF plus hypertension referred to arrhythmia service, "AF documented by ECG"

Gudbjartsson et al, *Nature* 2007; 448:353-357.

Are controls representative of disease-free persons eligible to become cases in the study?

- Also need to know how controls selected and determined to be disease-free
- Study of gallstones (Buch et al, 2007)
  - Sample 1: Gallstone-free controls from single hospital (vs 9 hospitals providing cases defined as post-cholecystectomy for cholelithiasis) from records of routine ultrasound US tests
  - Sample 2: Local population register undergoing additional exam with negative US
  - Sample 3: Population sample undergoing abdominal US to determine either "gallstone carrier status or previous hx cholecystectomy"

Buch et al, *Nat Genet* 2007; 39:995-999.

Information Bias: systematic differences in data collection or reporting between cases and controls

- Recall bias: Questions about specific exposures may be asked more frequently of cases, or cases may search their memories more intensively
- Family information bias: The flow of family information about exposures or illnesses may be stimulated by, or directed to, a new case in its midst
- Exposure suspicion bias: Knowledge of a patient's disease status may influence the intensity and outcome of search for exposure to a putative cause
- Instrument bias: Defects in calibration or maintenance of measurement instruments may lead to systematic deviations from true values

Sackett D, *J Chron Dis* 1979; 32:51-63 and Schlesselman J, *Case-Control Studies*, 1982.

Is risk factor information collected the same way in cases and controls?

- Cases of schizophrenia ascertained through local treatment facilities, physician referrals, advocacy groups, Web sites, media announcements and ads
  - Personal interview for psychotic, mood, and substance-use disorders, medical history
  - Family informant interview for patient history and family psychiatric history
- Controls recruited by random-digit dialing, completed preliminary consent and clinical assessment online
  - Screen for lifetime common mood, anxiety and substance use disorders
  - Lifetime psychosis, bipolar disorder, nicotine dependence, neuroticism and extraversion

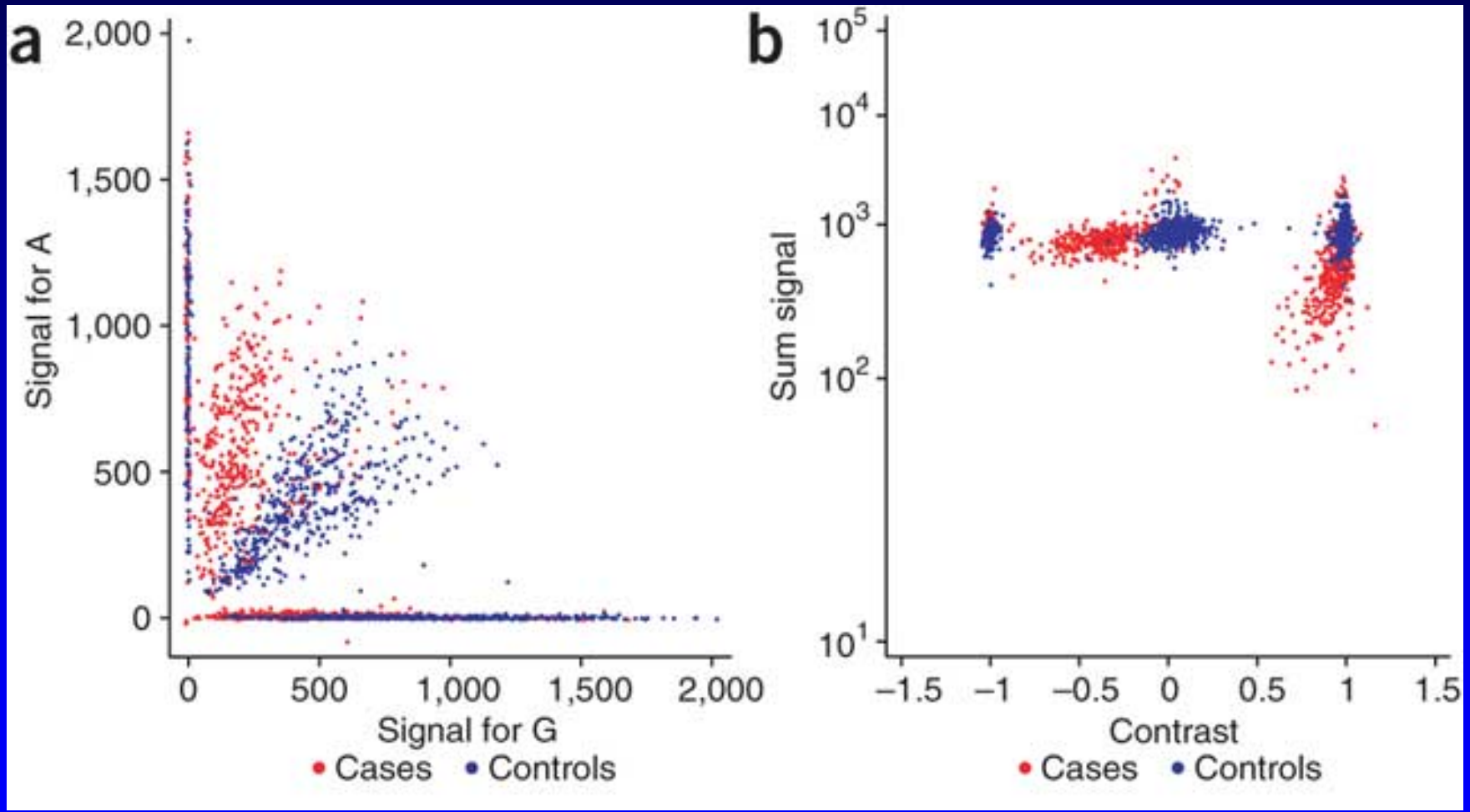
Suarez BK, *Am J Hum Genet* 2006; 78:315-333 and NIMH Genetics Initiative.

Is DNA collected and handled the same way in cases and controls?

- 816 cases T1D from GRID study
- 877 controls from 1958 British Birth Cohort Study
- 6,322 nonsynonymous SNPs
- Samples from lymphoblastoid cell lines extracted using same protocol in two different labs
- Case and control DNAs arranged randomly, teams masked to case-control status
- Some extreme associations could not be replicated by second genotyping method
- Four rather than three data clouds for some nsSNPs

Clayton DG et al, *Nat Genet* 2005; 37:1243-1246.

Signal Intensity Plots for *CD44* SNP rs9666607



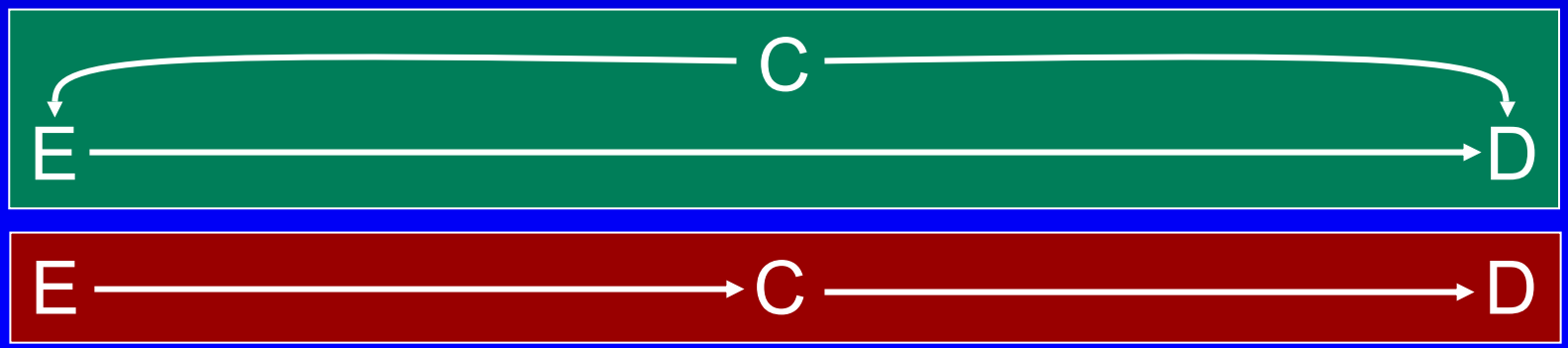
Clayton DG et al, *Nat Genet* 2005; 37:1243-1246.

Information Bias: systematic difference in ancestral geographical origins and predominant environmental exposures between cases and controls

- Population structure: confounding by ancestral origin (stay tuned)
- Confounding by demographics or environmental exposures

Confounding

- Confounder: “A factor that distorts the apparent magnitude of the effect of a study factor on risk. Such a factor is a determinant of the outcome of interest and is unequally distributed among the exposed and the unexposed” (Last, 1983).
  - Associated with exposure
  - Independent cause or predictor of disease
  - Not an intermediate step in causal pathway



Aschengrau and Seage, *Essentials of Epidemiology in Public Health,* 2003.

FTO Variants, Type 2 Diabetes, and Obesity (Frayling 2007 and Zeggini 2007)

	Diabetes Association		
Cohort	OR	95% CI	P-value
WTCCC phase 1	1.27	[1.16-1.37]	2×10^{-8}
WTCCC phase 2	1.22	[1.12-1.32]	5×10^{-7}
DGI	1.03	[0.91-1.71]	0.25

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	BMI Association (kg/m^2)		
	TT	AT	AA
WTCCC Cases	30.2	30.5	32.0
WTCCC Controls	26.3	26.3	27.1

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	BMI Association (kg/m^2)		
	TT	AT	AA
WTCCC Cases	30.2	30.5	32.0
WTCCC Controls	26.3	26.3	27.1

	Diabetes Association Adjusted for BMI		
	OR	95% CI	P-value
WTCCC phase 2	1.03	[0.96-1.10]	0.44

Identifying Confounders

- Conduct literature review to ascertain currently known risk factors
- Collect data on known risk factors and other potential confounders
- Identify differences between cases and controls in prevalence of potential confounders: “Table 1,” comparing cases and controls, is crucial!
- Identify associations of potential confounders with risk factor of interest
- Adjust associations for confounders and compare estimates, look for ~10-20% difference

Aschengrau and Seage, *Essentials of Epidemiology in Public Health,* 2003.

Distribution of Four Covariates in Case-Control Study of Nicotine Dependence

Covariate	Cases (n = 1,050)	Controls (n=879)
Male sex (%)	44	30
Age (yrs)	38	37
Fagerström (score)	6.3	0
Site		
US (n)	797	713
Australia (n)	253	66

Do determinants of dependence differ in men and women?
Do determinants of dependence differ in US and Australia?

Bierut LJ et al, *Hum Molec Genet* 2007; 16:24-35.

Distribution of Three Covariates in Case-Control Study of Neovascular AMD

Covariate	Cases (n = 96)	Controls (n = 130)
Male sex (%)	68	33
Age (yrs)	75	74
Smokers (%)	63	26

Do determinants of AMD differ in men and women?

Do determinants of AMD differ in smokers and non-smokers?

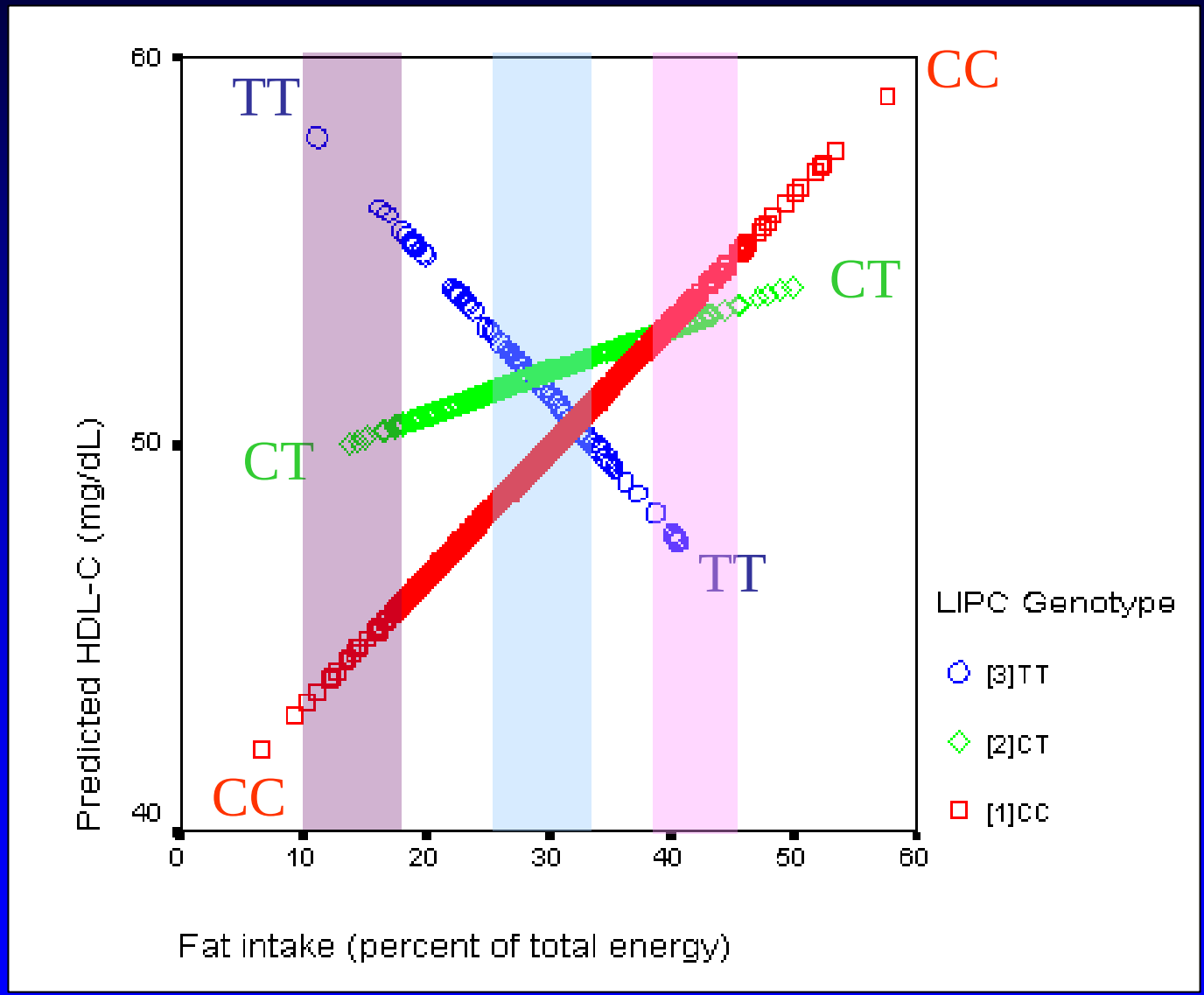
DeWan A et al, *Science* 2006; 314:989-992.

Dealing with Confounders

- In design:
  - Randomize
  - Restrict: confine study subjects to those within specified category of confounder
  - Match: select cases and controls so confounders equally distributed
- In analysis:
  - Standardize: for age, gender, time
  - Stratify: separate sample into subsamples according to specified criteria (binning?)
  - Multivariate analysis: adjust for many confounders

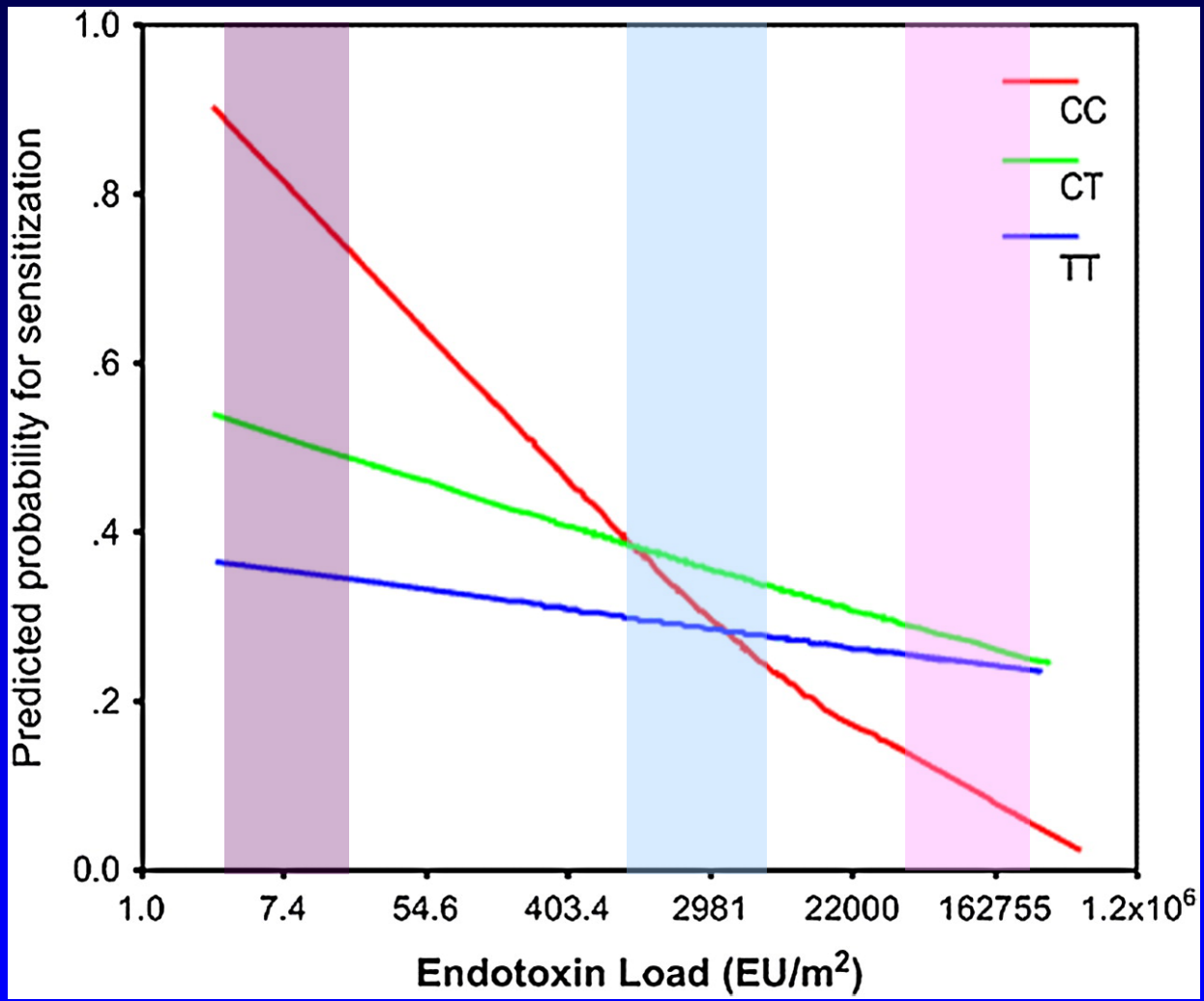
Aschengrau and Seage, *Essentials of Epidemiology in Public Health,* 2003.

Interaction: Is *LIPC* Genotype Related to HDL-C?



Ordovas et al, *Circulation* 2002; 106:2315-2321.

Inverse Relation between Endotoxin Exposure and Allergic Sensitization by CD14 Genotype



Simpson A et al, ***Am J Respir Crit Care Med*** **2006;174:386-392.**

Dealing with Interaction

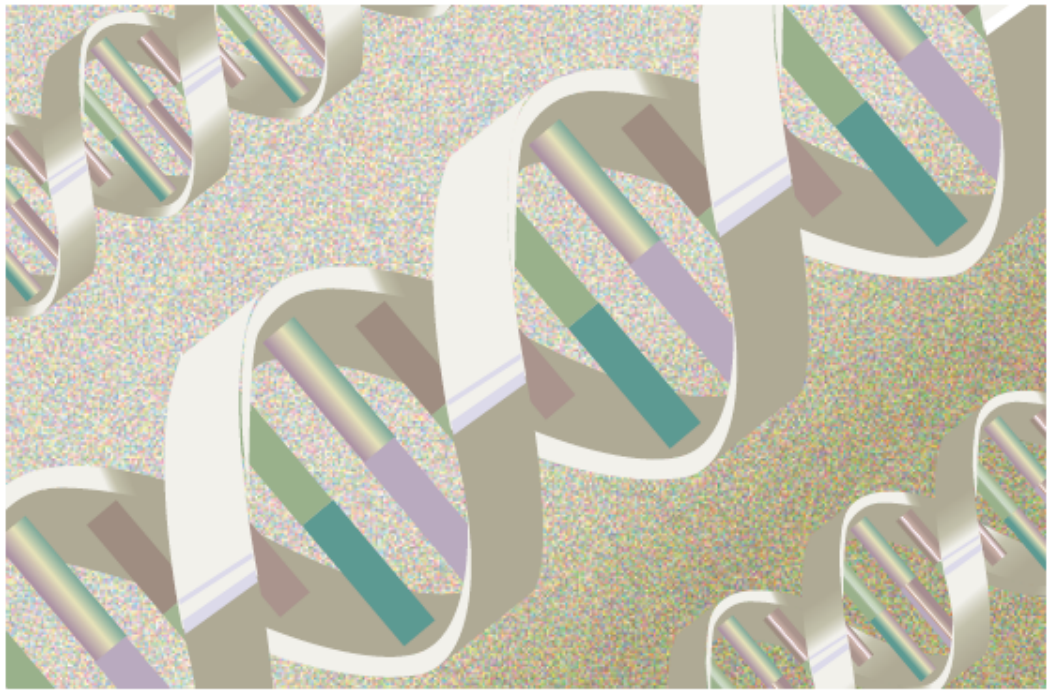
- Definition: differences in the association of one factor with a second factor according to the level of a third factor
- Beware: most studies are underpowered to identify interactions, formal interaction terms often not tested (Patsopoulos et al, *JAMA* 2007; 298:880-893)
- If it's really there, rejoice!
- Stratify, do NOT adjust!
- May provide clues to biologic mechanisms

FEATURE

Replicating genotype–phenotype associations

What constitutes replication of a genotype–phenotype association, and how best can it be achieved?

NCI-NHGRI Working Group on Replication in Association Studies



The study of human genetics has recently undergone a dramatic transition with the completion of both the sequencing of the human genome and the mapping of human haplotypes of the most common form of genetic variation, the single nucleotide polymorphism (SNP)[1–3]. In concert with this rapid expansion of detailed genomic information, cost-effective genotyping technologies have been developed that can assay hundreds of thousands of SNPs simultaneously. Together, these advances have allowed a systematic, even 'agnostic', approach to genome-wide interrogation, thereby relaxing the requirement for strong prior hypotheses.

So far, comprehensive reviews of the published literature, most of which reports work based on the candidate-gene approach, have demonstrated a plethora of questionable genotype–phenotype associations, replication of which has often failed in independent studies[4–7]. As the transition to genome-wide association studies occurs, the challenge will be to separate true associations from the blizzard of false positives attained through attempts to rep-

studies because of issues in either the initial study or the attempted replication[4–6,32,33]. Small sample size is a frequent problem and can result

conclusion from the literature because follow-up studies have not consistently analysed the same markers or those in perfect linkage dis-

Chanock et al, *Nature* 2007; 447:655-660.

Replication,

Replication, Replication,

Replication, Replication, Replication

Initial study:

- Sufficient description to permit replication
- Suggested criteria for soundness of initial report

Replication study:

- Similar population, similar phenotype
- Same genetic model, same SNP, same direction
- Adequately powered to detect postulated effect

Chanock et al, *Nature* 2007; 447:655-660.

Information to be Included in Initial Report

- Study information:
  - Source of cases and controls
  - Methods used for defining affection status
  - Participation rates and flow chart of selection
  - Standard “Table 1,” including rates of missing data
  - Success rate of DNA acquisition, comparability
- Genotyping and quality control procedures
- Results
  - Analysis methods in sufficient detail to understand and reproduce what was done
  - Simple single-locus and multi-marker (haplotype) association analyses
  - Significance of any known 'positive controls'

Chanock S et al, *Nature* 2007; 447:655-660.

Essay

Why Mo…shed Research Fi…gs Are Fa…

John P. A. Ioanni…

Summary

There is incr…m that most current publish…findings are false. The prob…esearch claim is true may dep… power and bias, the numbe…dies on the same question, a…tly, the ratio of true to no rela…ng the relationships prob…entific field. In this framew…finding is less likely to be tru…dies conducted in a field ar… effect sizes are smaller; w… greater number and lesser … of tested relationships; where… greater flexibility in designs, def… outcomes, and analytical modes; wh…

fa…ce this problem and some…of.

Modeling…k for False Positive Fin…

Several methode… pointed out [9–11]… rate of nonreplication… confirmation) of researc… is a consequence of the co… yet ill-founded strategy of clai… conclusive research findings sole… the basis of a single study assessed b… formal statistical significance, typically for a *p*-value less than 0.05. Research …ot most appropriately represente… …arized by *p*-values…

is character…eld and can vary a lot de…whether the field targets …relationships or searches f…or a few true relation…, thousands and millions…es that may be postulate…o consider, for compu…licity, circumscr…here either there is only o…onship (among …ny t…pothesized) or …to find any of the …e relationships. The …bility of a relationship …/(*R* + 1). The probability …nding a true relationship …e power 1 – β (one minus …ype II error rate). The probability

Ioannidis J, *PLoS Med.* 2005 Aug;2(8):e124.

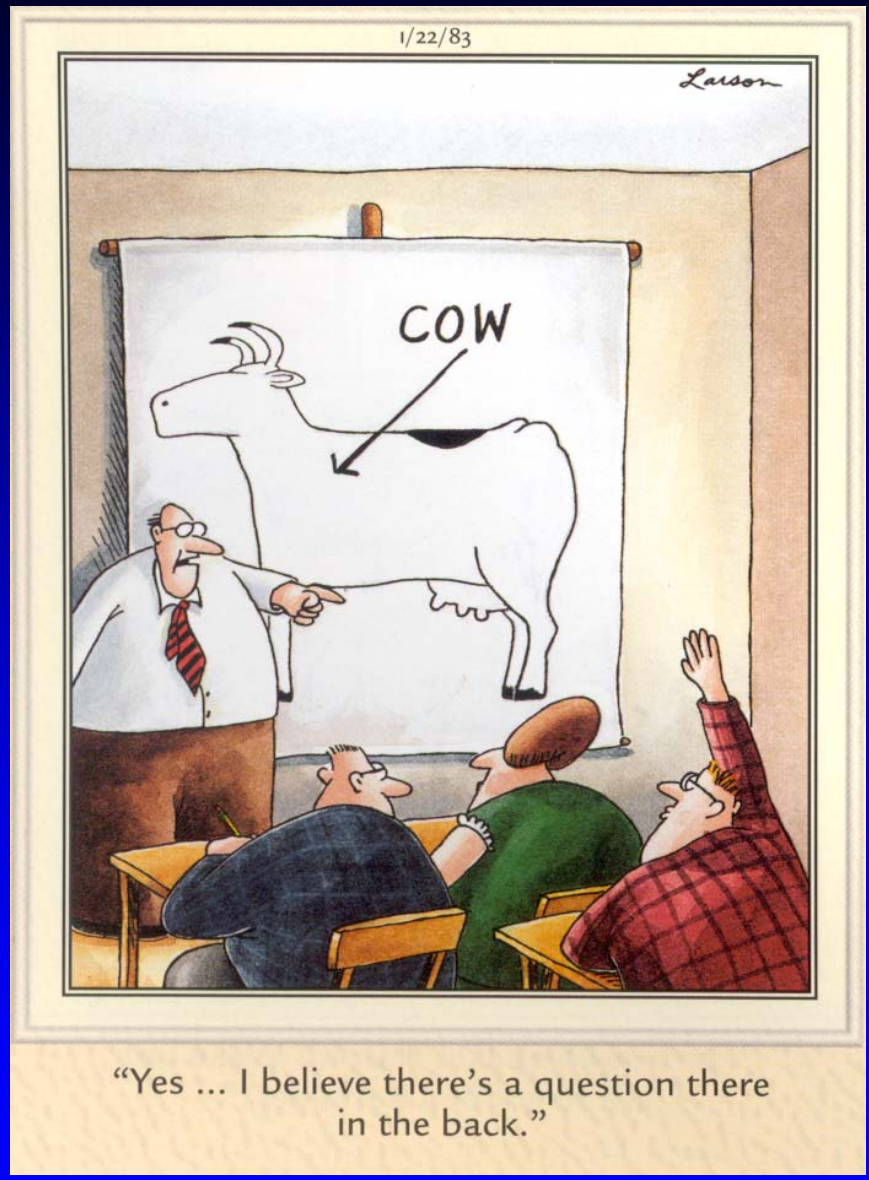
Controlling Bias in Genomic Research: Design

- Define population to be studied
- Maximize representativeness
- Use standard, reproducible meth
  assignment of case/control st
- Use incident cases
- Select control
  be
- Es
- Apply standard genotyping QC methods
- Replicate positive findings on different genotyping platform

Ask an Epidemiologist?

Controlling Bias in Genomic Research: Analysis and Interpretation

- Describe sources of cases and controls
- Describe methods of disease ascertainment
- Compare participants and non-participants
- Compare cases and controls
- Stratify and adjust for important confounders (including population stratification)
- Stratify and test for important interactions
- Report results of genotyping QC
- Report results of prior known associations



Larson, G. *The Complete Far Side*. 2003.

Reasonable Person Test: Does the Finding Make Sense?

- Bova et al studied MTHFR C677T variant in 48 persons with > 75% carotid stenosis compared to 26 persons with < 25% stenosis
- Persons with severe stenosis more likely to carry T allele
- Difference significant only in those with neither coronary nor peripheral arterial disease
- Carotid stenosis and coronary disease share major risk factors and are highly correlated

Bova I, et al. *Stroke* 1999;30:2180-2182.

Is amyl nitrite associated with Kaposi's sarcoma in homosexual men?

Amyl Nitrite Use	Kaposi's Sarcoma				Odds Ratio [95% CI]
	Present		Absent		
	%	(n/N)	%	(n/N)	
High	60	(12/20)	15	(6/40)	8.5 [2.4-29.6]
Low	40	(8/20)	85	(34/40)	
Total	100	(40/40)	100	(40/40)	

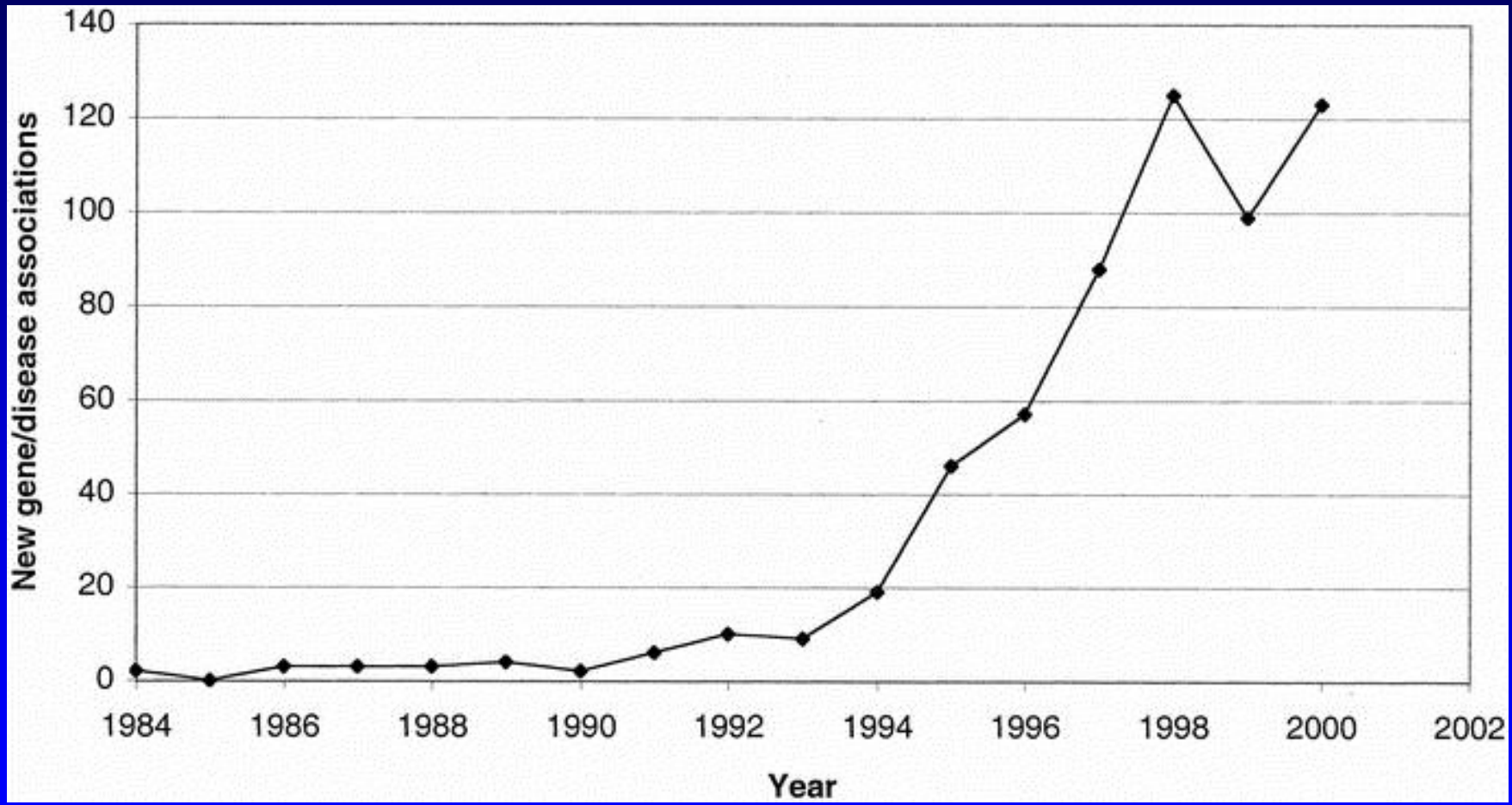
Morabia A. *Prev Med* 1995; 24:90-95.

Could an oncovirus explain some or all of the observed association?

HIV Status	Amyl Nitrite Use	Kaposi's Sarcoma				Odds Ratio [95% CI]
		Present		Absent		
		%	(n/N)	%	(n/N)	
Present	High	63	(12/19)	33	(3/9)	3.4 [0.6-15.6]
	Low	37	(7/19)	67	(6/9)	
Absent	High	0	(0/1)	10	(3/31)	4.7 [0.1, 170]
	Low	100	(1/1)	90	(28/31)	

Morabia A. *Prev Med* 1995; 24:90-95.

Number of New, Significant Gene-Disease Associations by Year, 1984 - 2000



Hirschhorn J et al, *Genet Med* 2002; 4:45-61.

Of 600 Gene-Disease Associations, Only 6 Significant in ≥ 75% of Identified Studies

Disease/Trait	Gene	Polymorphism	Frequency
DVT	F5	Arg506Gln	0.015
Graves' Disease	CTLA4	Thr17Ala	0.62
Type 1 DM	INS	5' VNTR	0.67
HIV/AIDS	CCR5	32 bp Ins/Del	0.05-0.07
Alzheimer's	APOE	Epsilon 2/3/4	0.16-0.24
Creutzfeldt-Jakob Disease	PRNP	Met129Val	0.37

Hirschhorn J et al, *Genet Med* 2002; 4:45-61.