

INTRODUCTION TO GENETIC EPIDEMIOLOGY

(Antwerp Series)

Prof. Dr. Dr. K. Van Steen

FAMILY-BASED GENETIC ASSOCIATION STUDIES

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Pros and cons of FBAT

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1 Setting the scene

1.a Introduction to genetic associations

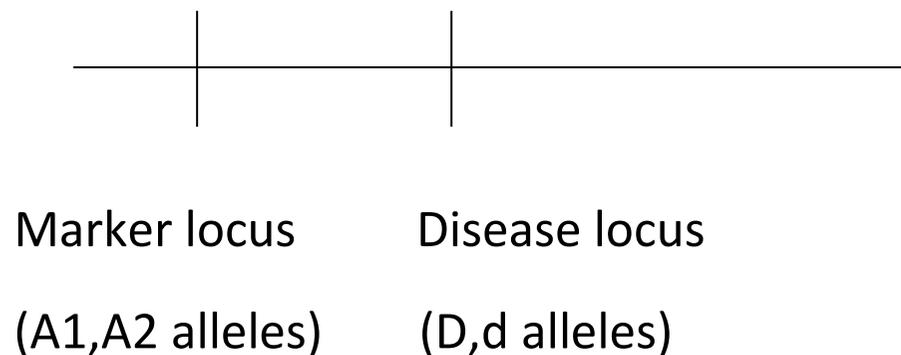
A genetic association refers to statistical relationships in a population between an individual's phenotype and their genotype at a genetic locus.

- Phenotypes:
 - Dichotomous
 - Measured
 - Time-to-onset
- Genotypes:
 - Known mutation in a gene (CKR5 deletion, APOE4)
 - Marker or SNP with/without known effects on coding

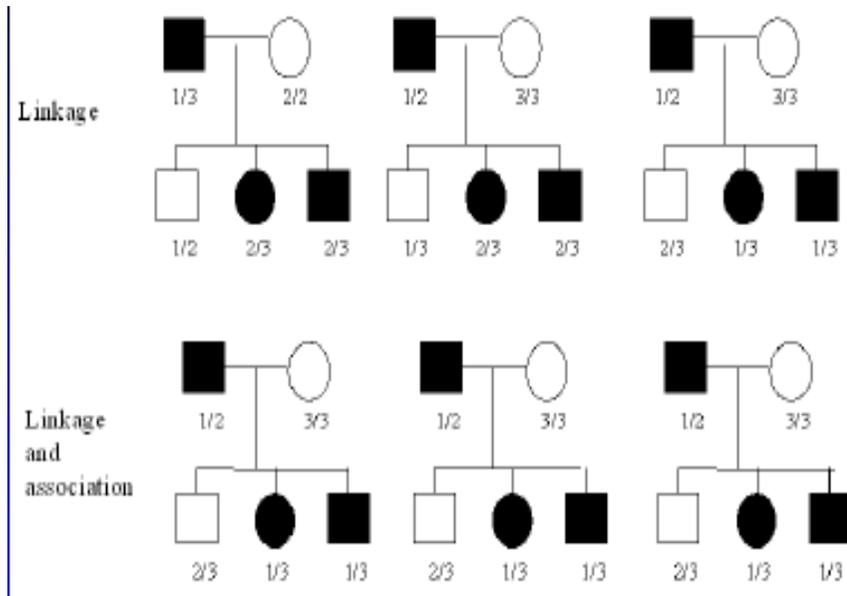
1.b Basic mapping strategies

Using families: linkage versus association

- Linkage is a physical concept: The two loci are “close’ together on the same chromosome. There is hardly any recombination between disease locus and marker locus
- Association is a population concept: The allelic values at the two loci are associated. A particular marker allele tends to be present with disease allele.



Features of linkage studies

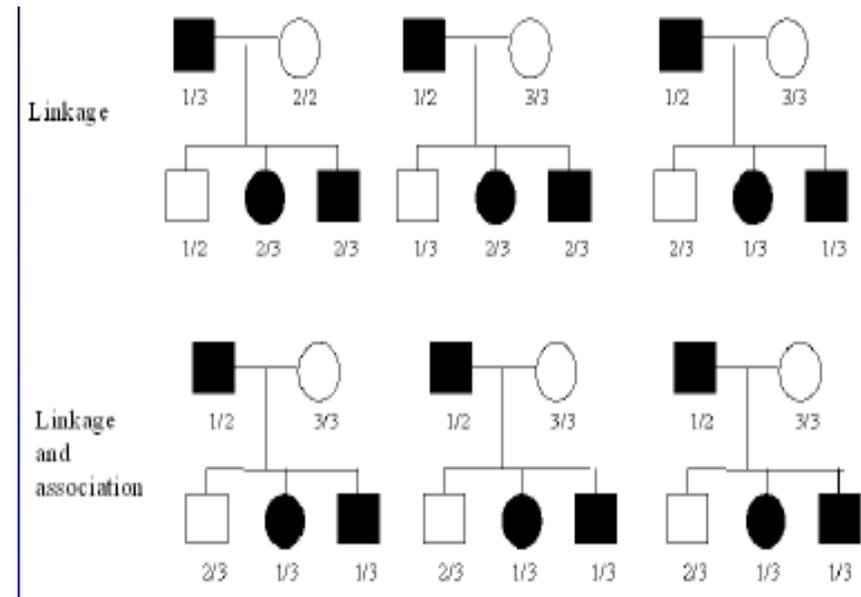


(Figure: courtesy of Ed Silverman)

- Linkage exists over a very broad region, entire chromosome can be done using data on only 400-800 DNA markers
- Broad linkage regions imply studies must be followed up with more DNA markers in the region
- Must have family data with more than one affected subject

Features of association studies

- Association exists over a narrow region; markers must be close to disease gene
 - The basic concept is linkage disequilibrium (LD)
- Used for candidate genes or in linked regions
- Can use population-based (unrelated cases) or family-based design



The Future of Genetic Studies of Complex Human Diseases

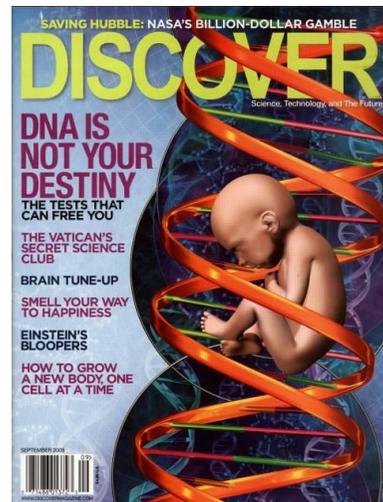
Neil Risch and Kathleen Merikangas

SCIENCE • VOL. 273 • 13 SEPTEMBER 1996

1.c Genome wide association analyses (GWAs)

Reasons for continuing popularity of GWAs

- The impact on medical care from genome-wide association studies could potentially be substantial. Such research is laying the groundwork for the era of personalized medicine, in which the current one size-fits-all approach to medical care will give way to more customized strategies.



... It will take more than SNPs alone

Genetic Risk Prediction — Are We There Yet?

Peter Kraft, Ph.D., and David J. Hunter, M.B., B.S., Sc.D., M.P.H.

A major goal of the Human Genome Project was to facilitate the identification of inherited genetic variants that increase or decrease the risk of complex diseases. The completion of the International HapMap Project and the development of new methods for genotyping individual DNA samples at 500,000 or more loci have led to a wave of discoveries through genomewide association studies. These analyses have identified common genetic variants that are associated with the risk of more than 40 diseases and human phenotypes. Several companies have begun offering direct-to-consumer testing that uses the

tests of genetic predisposition to important diseases would have major clinical, social, and economic ramifications. But the great majority of the newly identified risk-marker alleles confer very small relative risks, ranging from 1.1 to 1.5,² even though such analyses meet stringent statistical criteria (i.e., the identification of associations with disease that have very small P values and hence are unlikely to be false positives). However, even when alleles that are associated with a modest increase in risk are combined, they generally have low discriminatory and predictive ability.³

One argument in favor of us-

est relative risks are almost certainly overrepresented in the first wave of findings from genomewide association studies, since considerations of statistical power predict that they will be identified first. However, a striking fact about these first findings is that they collectively explain only a very small proportion of the underlying genetic contribution to most studied diseases. (Some exceptions exist — notably, age-related macular degeneration, for which a few alleles explain a substantial fraction of the genetic contribution.) Several lines of evidence support this overall conclusion.

(Kraft and Hunter 2009)

... It will take more than SNPs alone

PERSPECTIVES

GENETICS

Getting Closer to the Whole Picture

Uwe Sauer, Matthias Heinemann, Nicola Zamboni

A major challenge of biology is to unravel the organization and interactions of cellular networks that enable complex processes such as the biochemistry of growth or cell division. The underlying complexity arises from intertwined nonlinear and dynamic interactions among large numbers of cellular constituents, such as genes, proteins, and metabolites. As well, interactions among these components vary in nature (regulatory, structural, and catalytic), effect, and strength. The reductionist approach has successfully identified most of the components and many interactions but, unfortunately, offers no convincing concepts and methods to comprehend how system properties emerge. To understand how and why cells function the way they do, comprehensive and quantitative data on component concentrations are required to quantify component interactions. On page 593 of this issue, Ishii *et al.* (1) provide unsurpassed complete and quantitative data of components at the various constituent levels in a bacterial cell.

better addressed by observing, through quantitative measures, multiple components simultaneously, and by rigorous data integration with mathematical models (2). Such a systemwide perspective (so-called systems biology) on component interactions is required so that network properties, such as a particular functional state or robustness (3), can be quantitatively understood and rationally manipulated.

The technical challenges of the systems biological approach (4) are mainly along four lines (see the figure): (i) systemwide component identification and quantification (“omics” data) at the level of mRNA, proteins, and small molecular weight metabolites; (ii) experimental identification of physical component interactions, primarily for information processing networks; (iii) computational inference of structure, type, and quantity of component interactions from data; and (iv) rigorous integration of heterogeneous data. The last step is required to

A quantitative data set of RNA, proteins, and metabolites provides an unprecedented starting point to understand, at a systems level, the effects of perturbations on a cell.

ods relating to the first challenge has made tremendous advances in the past decade, but the level of sophistication and the associated costs have led to a situation where primarily single-component data—that is, data solely on genes, proteins, or metabolites—are available. Until the study by Ishii *et al.*, at best two different types of component data were reported for a given experiment, which severely limited progression along the iterative cycle between experiments and theory.

By joining forces among specialized labs, Ishii *et al.* report systemwide data on three main component layers of cells—transcriptome (mRNA), proteome (protein), and metabolome (metabolites)—with a particular focus on central carbon metabolism of the model bacterium *Escherichia coli*. Beyond component concentrations, the functional endpoint of gene, protein, and metabolite interactions—the intracellular metabolic fluxes—were quantified from ¹³C-labeling experiments (5). In a laborious procedure,

(Sauer et al 2007)

Reasons for continuing popularity of GWAs using SNPs

- There is a large compendium of validated SNP data
- SNP GWAs are able to potentially use *all* of the data
- They are more powerful for genes of small to moderate effect (see before)
- They allow for covariate assessment, detection of interactions, estimation of effect size, ...

BUT

ALL statistical issues cannot be ruled out

PERSPECTIVE

DRINKING FROM THE FIRE HOSE — STATISTICAL ISSUES IN GENOMEWIDE ASSOCIATION STUDIES

STATISTICS AND MEDICINE

Drinking from the Fire Hose — Statistical Issues in Genomewide Association Studies

David J. Hunter, M.B., B.S., and Peter Kraft, Ph.D.

[Related article, page 443](#)

The past 3 months have seen the publication of a series of studies examining the inherited genetic underpinnings of common diseases such as prostate cancer, breast cancer, diabetes, and in this issue of the *Journal*, coronary artery disease (reported by Samani et al., pages 443–453). These genomewide association studies have been able to examine interpatient differences in inherited genetic variability at an unprecedented level of resolution, thanks to the development of microarrays, or chips, capable of assessing more than 500,000 single-

ating the need for guessing which genes are likely to harbor variants affecting risk. Most of the robust associations seen in this type of study have not been with genes previously suspected of being related to the disease. Some of these associations have been found in regions not even known to harbor genes, such as the 8q24 region, in which multiple variants have been found to be associated with prostate cancer.² Such findings promise to open up new avenues of research, through both the discovery of new genes relevant to specific diseases and the

The main problem with this strategy is that, because of the high cost of SNP chips, most studies are somewhat constrained in terms of the number of samples and thus have limited power to generate P values as small as 10^{-7} . In addition, most variants identified recently have been associated with modest relative risks (e.g., 1.3 for heterozygotes and 1.6 for homozygotes), and many true associations are not likely to exceed P values as extreme as 10^{-7} in an initial study. On the other hand, a “statistically significant” finding in an underpowered study is more

(Hunter and Kraft 2007)

Using all of the data for case/control designs?

candidate gene approach

vs

genome-wide screening approach



Using all of the data for case/control designs ?

- There are many (single locus) tests to perform
- The multiplicity can be dealt with in several ways
 - clever multiple corrective procedures (see later)
 - adopting multi-locus tests (see later) or
 - haplotype tests,
 - pre-screening strategies (see later), or
 - multi-stage designs.

Which of these approaches are more powerful is
still under heavy debate...

2 Families versus unrelated cases and controls

2.a Every design has statistical implications

There are many possible designs for a genetic association study

	Details	Advantages	Disadvantages	Statistical analysis method
Cross-sectional	Genotype and phenotype (ie, note disease status or quantitative trait value) a random sample from population	Inexpensive. Provides estimate of disease prevalence	Few affected individuals if disease rare	Logistic regression, χ^2 tests of association or linear regression
Cohort	Genotype subsection of population and follow disease incidence for specified time period	Provides estimate of disease incidence	Expensive to follow-up. Issues with drop-out	Survival analysis methods
Case-control	Genotype specified number of affected (case) and unaffected (control) individuals. Cases usually obtained from family practitioners or disease registries, controls obtained from random population sample or convenience sample	No need for follow-up. Provides estimates of exposure effects	Requires careful selection of controls. Potential for confounding (eg, population stratification)	Logistic regression, χ^2 tests of association
Extreme values	Genotype individuals with extreme (high or low) values of a quantitative trait, as established from initial cross-sectional or cohort sample	Genotype only most informative individuals hence save on genotyping costs	No estimate of true genetic effect sizes	Linear regression, non-parametric, or permutation approaches
Case-parent triads	Genotype affected individuals plus their parents (affected individuals determined from initial cross-sectional, cohort, or disease-outcome based sample)	Robust to population stratification. Can estimate maternal and imprinting effects	Less powerful than case-control design	Transmission/disequilibrium test, conditional logistic regression or log-linear models
Case-parent-grandparent septets	Genotype affected individuals plus their parents and grandparents	Robust to population stratification. Can estimate maternal and imprinting effects	Grandparents rarely available	Log-linear models
General pedigrees	Genotype random sample or disease-outcome based sample of families from general population. Phenotype for disease trait or quantitative trait	Higher power with large families. Sample may already exist from linkage studies	Expensive to genotype. Many missing individuals	Pedigree disequilibrium test, family-based association test, quantitative transmission/disequilibrium test
Case-only	Genotype only affected individuals, obtained from initial cross-sectional, cohort, or disease-outcome based sample	Most powerful design for detection of interaction effects	Can only estimate interaction effects. Very sensitive to population stratification	Logistic regression, χ^2 tests of association
DNA-pooling	Applies to variety of above designs, but genotyping is of pools of anywhere between two and 100 individuals, rather than on an individual basis	Potentially inexpensive compared with individual genotyping (but technology still under development)	Hard to estimate different experimental sources of variance	Estimation of components of variance

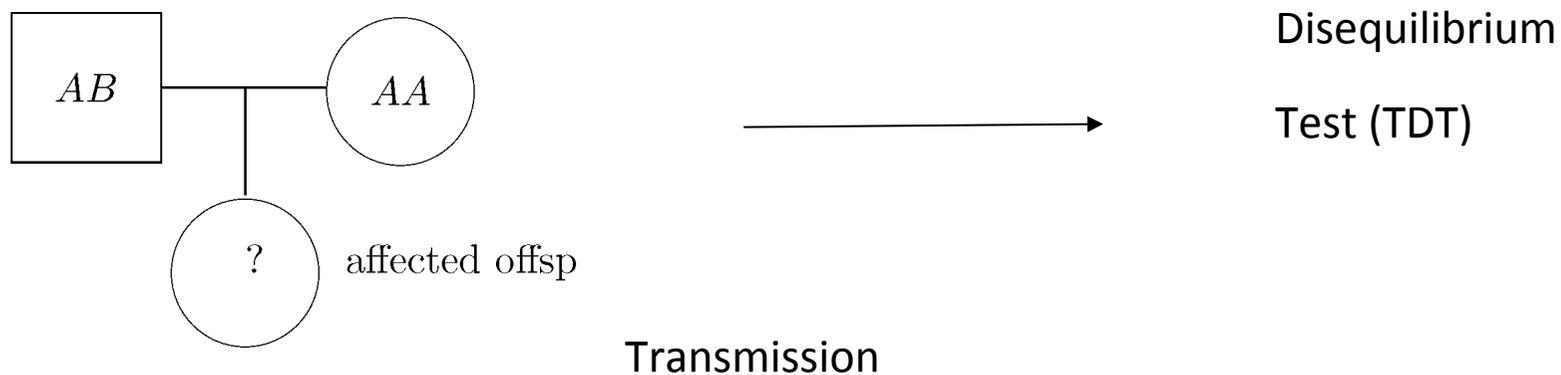
Table 2: Study designs for genetic association studies

(Cordell and Clayton, 2005)

Family-based designs

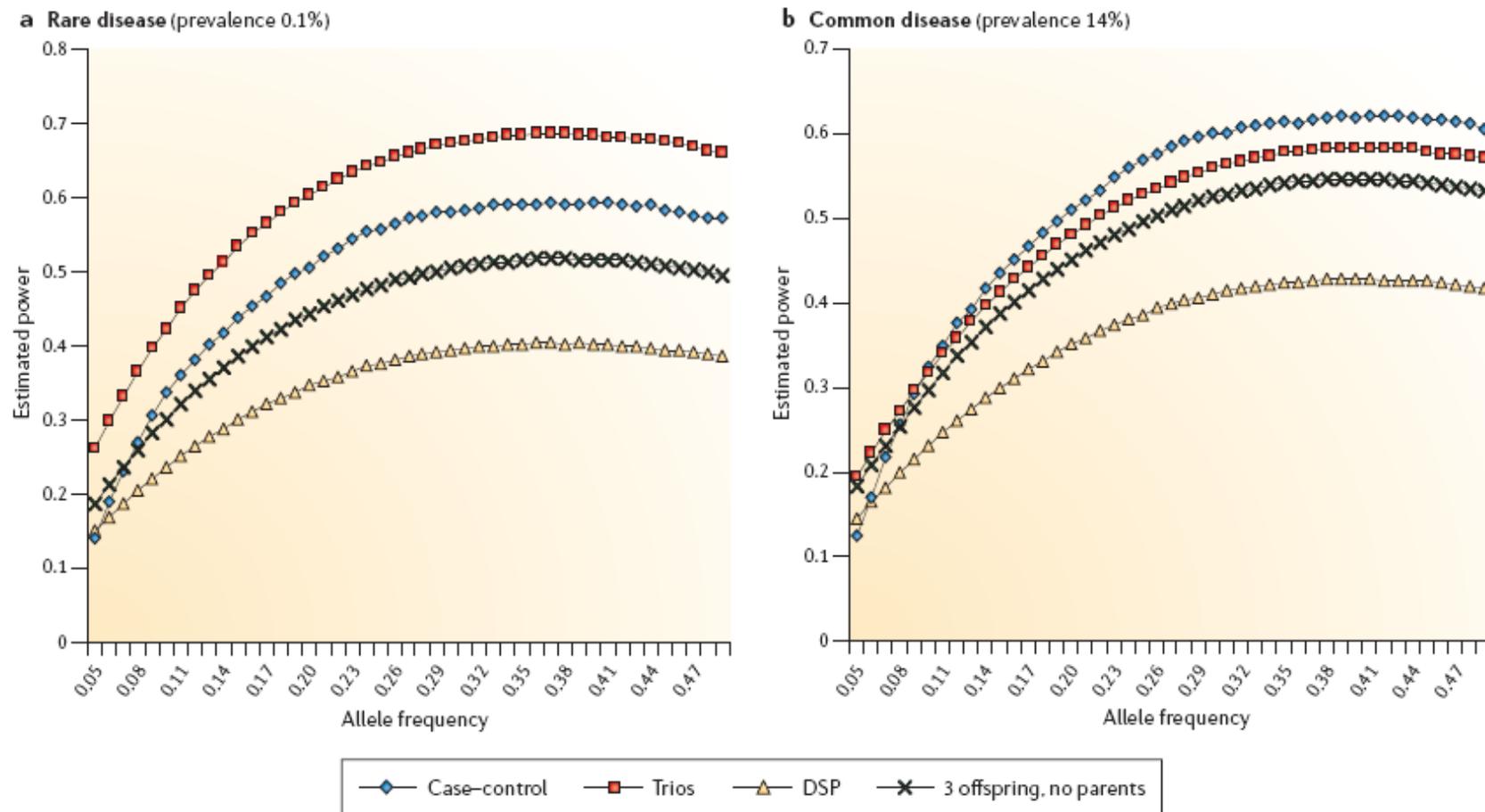
- Cases and their parents
- Test for both linkage and association
- Robust to population substructure: admixture, stratification, failure of HWE
- Offer a unique approach to handle multiple comparisons

Using trios



2.b Power considerations

Rare versus common diseases (Lange and Laird 2006)



Power

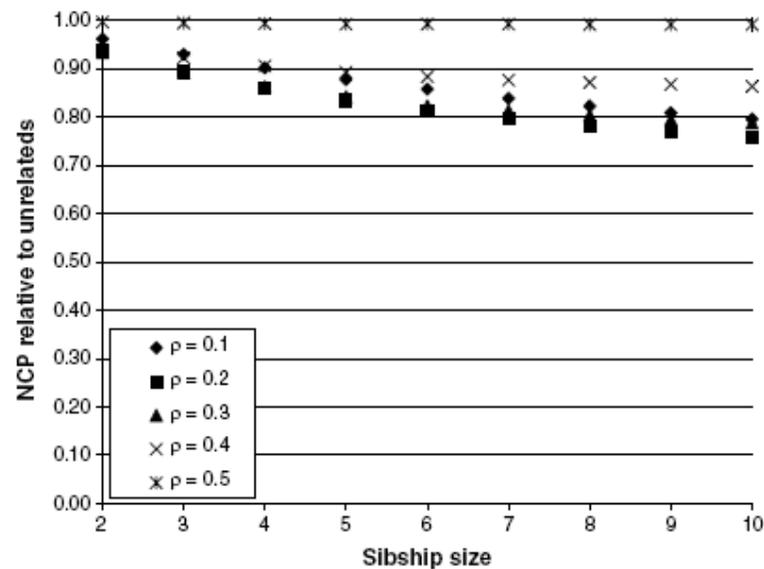


Figure 1 Relative power of GWAS for sibships versus unrelated individuals, for the same cost of genotyping. ρ is the phenotypic correlation between siblings.

- Little power lost by analysing families relative to singletons
- It may be efficient to genotype only some individuals in larger pedigrees
- Pedigrees allow error checking, within family tests, parent-of-origin analyses, joint linkage and association, ...

(Visscher et al 2008)

Power of GWAs (whether or not using related individuals)

- Critical to success is the development of robust study designs to ensure high power to detect genes of modest risk while minimizing the potential of false association signals due to testing large numbers of markers.
- Key components include
 - sufficient sample sizes,
 - rigorous phenotypes,
 - comprehensive maps,
 - accurate high-throughput genotyping technologies,
 - sophisticated IT infrastructure,
 - rapid algorithms for data analysis, and
 - rigorous assessment of genome-wide signatures.

The role of population resources

- Critical to success is the collection of sufficient numbers of rigorously phenotyped cases and matched control groups or family trios to have sufficient power to detect disease genes conferring modest risk.
- Power studies have shown that at least 2,000 to 5,000 samples for both cases and controls groups are required when using general populations.
- This large number of samples makes the collection of rigorously consistent clinical phenotypes across all cases quite challenging.
- In addition, matching of cases and controls with respect to geographic origin and ethnicity is critical for minimizing false positive signals due to population substructure (especially when non-family specific tests are used).

The role of SNP Maps and Genotyping

- A second key success factor is having a comprehensive map of hundreds of thousands of carefully selected SNPs.
- Currently there are several groups offering SNP arrays for genotyping, with **Affymetrix** (www.affymetrix.com) and **Illumina** (www.illumina.com) both providing products containing more than 500,000 SNPs.
- Achieving high call rates and genotyping accuracy are also critically important, because small decreases in accuracy or increases in missing data can result in relatively large decreases in the power to detect disease genes.

(http://www.genengnews.com/articles/chitem_print.aspx?aid=1970&chid=0)

The role of IT and Analytic Tools

- Genotyping instruments now have sufficient capacity to enable genotyping of thousands of subjects in only a few weeks.
- A study of 1,000 cases and 1,000 control subjects using a 550,000 SNP array produces over 1 billion genotypes.
- To properly store, manage, and process the enormous data sets arising from GWAS, a highly sophisticated IT infrastructure is needed, including computing clusters with sufficient CPUs and automated, robust pipelines for rapid data analysis.
- Given this wealth of genotypic data, the availability of efficient analytical tools for performing association analyses is critical to the successful identification of disease-associated signals.

(http://www.genengnews.com/articles/chitem_print.aspx?aid=1970&chid=0)

The role of IT and Analytic Tools

- Primary genome-wide analyses include a comparison of allele and genotype frequencies between case and control cohorts or for child-affected trios, a comparison of the frequencies of transmitted (case) and nontransmitted (control) alleles.
- An alternative test of association when using child-affected trios is the transmission disequilibrium test for the overtransmission of alleles to affected offspring (see next section).
- Since these analyses require considerable computing power to handle terabytes of data, genome-wide analyses are often limited to single SNPs with haplotype analyses performed once candidate regions are identified.
- But the field is changing ... STAY TUNED !!!

(http://www.genengnews.com/articles/chitem_print.aspx?aid=1970&chid=0)

Software

- With recent technical advances in high-throughput genotyping technologies the possibility of performing GWAs becomes increasingly feasible for a growing number of researchers.
- A number of packages are available in the R Environment to facilitate the analysis of these large data sets.
 - **GenABEL** is designed for the efficient storage and handling of GWAS data with fast analysis tools for quality control, association with binary and quantitative traits, as well as tools for visualizing results.
 - **pbatR** provides a GUI to the powerful PBAT software which performs family and population based family and population based studies. The software has been implemented to take advantage of parallel processing, which vastly reduces the computational time required for GWAS.

Software

- A number of packages are available in the R Environment to facilitate the analysis of these large data sets.
 - **SNPassoc** provides another package for carrying out GWAS analysis. It offers descriptive statistics of the data (including patterns of missing data) and tests for Hardy-Weinberg equilibrium. Single-point analyses with binary or quantitative traits are implemented via generalized linear models, and multiple SNPs can be analysed for haplotypic associations or epistasis.
- Check out Zhang 2008: R Packages for Genome-Wide association Studies

2.c The Transmission Disequilibrium Test

- Assumptions:
 - Parents' and offspring genotypes known
 - dichotomous phenotype, only affected offspring
- Count transmissions from heterozygote parents, compare to expected transmissions
- Expected computed using parents' genotypes and Mendel's laws of segregation (differ from case-control)
 - Conditional test on offspring affection status and parents' genotypes
- Special case of McNemar's test (columns: alleles not transmitted; rows: alleles transmitted)

(Spielman et al 1993)

Recall for binary outcomes

Control exposed	Case exposed	
	No	Yes
No	<i>a</i>	<i>b</i>
Yes	<i>c</i>	<i>d</i>

- For a single binary exposure, the relevant data may be presented in the table above, which counts sets not subjects.
- Estimation of odds ratio:

$$\hat{\theta} = \frac{b}{c}, \quad SE(\log \hat{\theta}) = \sqrt{\frac{1}{b} + \frac{1}{c}}$$

McNemar's test

- Score test of the null hypothesis, $\theta = 1$

$$U = b - \frac{b + c}{2} = \frac{b - c}{2},$$

$$V = \frac{b + c}{4}$$

- $\frac{U^2}{V} = \frac{(b-c)^2}{b+c}$ is distributed as chi-square (1 df) in large samples
- This test discards concordant pairs and tests whether discordant sets split equally between those with case exposed and those with control exposed
- McNemar's test is a special case of the Mantel-Haenszel test

Attraction of TDT

- H_0 relies on Mendel's laws, not on control group
- H_A linkage disequilibrium is present: DSL and marker loci are linked, and their alleles are associated

- Intuition:

If no linkage but association at population level, no systematic transmission of a particular allele. If linkage, but no association, different alleles will be transmitted in different families.

- Consequence:

TDT is robust to population stratification, admixture, other forms of confounding

(model free). The same properties hold for FBAT statistics of which the TDT is a special case.

Human
Heredit

Advances in Family-Based Association Analysis

15 Years of Practical Experience with the Original Transmission Disequilibrium Test

Guest Editor
Derek Gordon, Piscataway, N.J.

13 figures and 19 tables, 2008

(Spielman et al 1993)

Disadvantages of TDT

- Only affected offspring
- Only dichotomous phenotypes
- Biallelic markers
- Single genetic model (additive)
- No allowance for missing parents/pedigrees
- Method for incorporating siblings is limited
- Does not address multiple markers or multiple phenotypes

Generalization of the TDT

Need for a unified framework that flexible enough to encompass:

- standard genetic models
- other phenotypes, multiple phenotypes
- multiple alleles
- additional siblings; extended pedigrees
- missing parents
- multiple markers
- haplotypes

(Horvath et al 1998, 2001; Laird et al 2000, Lange et al 2004)

2.d FBAT test statistic

T : code trait, based on phenotype Y and offset μ

X : code genotype (harbors genetic inheritance model)

P : parental genotypes

$$U = \sum T(X - E(X|P))$$
$$U = \sum (Y - \mu)(X - E(X|P))$$

- \sum is *sum* over all offspring ,
- $E(X|P)$ is the expected marker score computed under H_0 , conditional on P
- $Var(U) = \sum T^2 Var(X|P)$
- $Var(X|P)$ computed from offspring distribution, conditional on P and T .

FBAT test statistic

$$Z = U / \sqrt{\text{Var}(U)}$$

- Asymptotic distributions
 - $Z \sim N(0,1)$ under H_0
 - $Z^2 \sim \chi^2$ on 1 df under H_0
- $Z^2_{FBAT} = \chi^2_{TDT}$ when
 - $Y=1$ if child is affected, $Y=0$ if child is unaffected in a trio design
 - $T=Y$
 - X follows an additive coding
 - no missing data

(Horvath et al 1998, 2001; Laird et al 2000)

General theory on FBAT testing

- Test statistic:

- works for any phenotype, genetic model
- use covariance between offspring trait and genotype

$$U = \sum (Y - \mu)(X - E(X|P))$$

- Test Distribution:

- computed assuming H_0 true; random variable is offspring genotype
- condition on parental genotypes when available, extend to family configurations (avoid specification of allele distribution)
- condition on offspring phenotypes (avoid specification of trait distribution)

(Horvath et al 1998, 2001; Laird et al 2000)

Key features of TDT are maintained

- Random variable in the analysis is the offspring genotype
- Parental genotypes are fixed (condition on the parental genotypes)
- Trait is fixed (condition on all offspring being affected)

Missing genotypes revisited

- We have already given evidence about additional advantages to impute missing marker data, whenever possible
- This imputation process generally becomes more complicated when genotypes need to be imputed in studies of related individuals.
- Two important packages that allow for proper genotype imputation in family-based designs include MERLIN and MENDEL
- The latest developments can be retrieved from Gonçalo Abecasis or Jonathan Marchini
 - <http://www.sph.umich.edu/csg/abecasis/>
 - <http://www.stats.ox.ac.uk/~marchini/>

(Li et al 2009)

3 From complex phenomena to models

3.a Introduction

- There are likely to be ***many*** susceptibility genes each with combinations of ***rare and common*** alleles and genotypes that impact disease susceptibility primarily through ***nonlinear interactions*** with ***genetic and environmental*** factors
- Analytically, it can be difficult to distinguish between ***interactions*** and ***heterogeneity***.

3.b When the number of tests grows

Multiple testing revisited

- Multiple testing is a thorny issue, the bane of statistical genetics.
 - The problem is not really the number of tests that are carried out: even if a researcher only tests one SNP for one phenotype, if many other researchers do the same and the nominally significant associations are reported, there will be a problem of false positives.

(Balding 2006)

Multiple testing (continued)

- With too many SNPs
 - Family-wise error rate (FWER)
 - Bonferroni Threshold: $< 10^{-7}$
 - Permutation data sets
 - Enough compute capacity?
 - False discovery rate (FDR) and variations thereof
 - it starts to break down
 - the power over Bonferroni is minimal
 - Bayesian methods such as false-positive report probability (FPRP)
 - Could work but for now not yet well documented
 - What are the priors?

3.c When the number of SNPs grows

Variable selection (reduces multiple testing burden)

- Pre-screening for subsequent testing:
 - Independent screening and testing step (PBAT screening)
 - Dependent screening and testing step
- Identify linkage disequilibrium blocks according to some criterion and infer and analyze haplotypes within each block, while retaining for individual analysis those SNPs that do not lie within a block
- Multi-stage designs ...

4 Family-based screening strategies

4.a PBAT screening

Addressing GWA's multiple testing problems

- Adapted from Fulker model with "between" and "within" component (1999):

$$E[Y] = \mu + \boxed{a_w(X - E[X|P])} + \boxed{a_b(E[X|P])}$$

Family-based Population-based
association

X : coded genotype

P : parental genotypes

Screen

- Use 'between-family' information $[f(S, Y)]$
- Calculate conditional power (a_b, Y, S)
- Select *top N* SNPs on the basis of power

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$

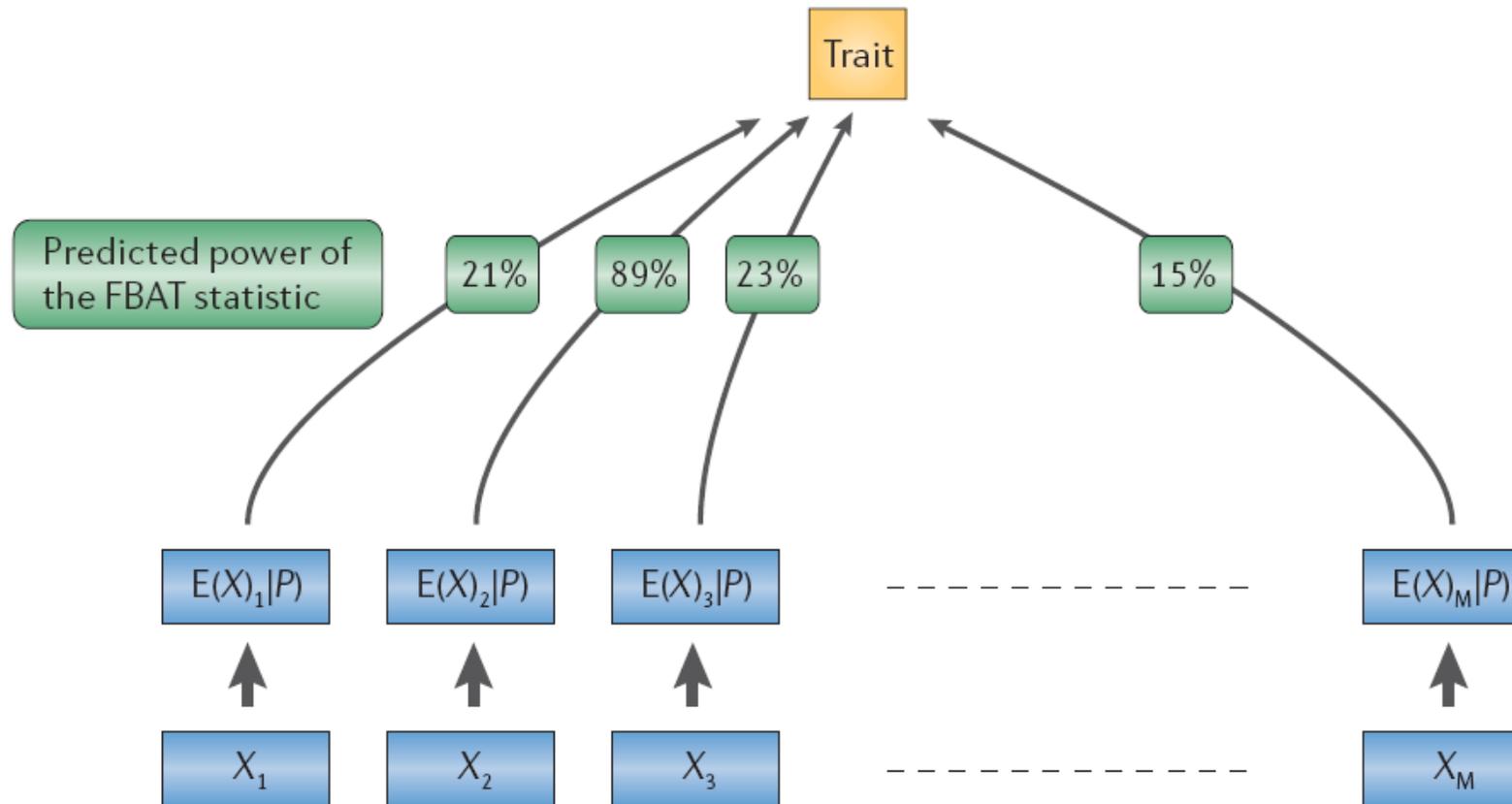
Test

- Use 'within-family' information $[f(X/S)]$ while computing the FBAT statistic
- This step is independent from the screening step
- Adjust for *N* tests (not 500K!)

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$

(Van Steen et al 2005)

PBAT screening



(Lange and Laird 2006)

Detection of 1 DSL

(Van Steen et al 2005)

- SNPChip 10K array on prostate cancer (467 subjects from 167 families) taken as genotype platform in simulation study (10,000 replicates)

		Causal mutation in Affymetrix block			
		SNP1	SNP2	SNP3	SNP4
Method I (top 1)	0.05	0.587 (0.268)	0.690 (0.264)	0.455 (0.091)	0.527 (0.054)
	0.07	0.771 (0.400)	0.841 (0.333)	0.783 (0.116)	0.794 (0.066)
	0.10	0.950 (0.511)	0.964 (0.379)	0.958 (0.125)	0.967 (0.069)
Method II (top 1)	0.05	0.406 (0.152)	0.460 (0.092)	0.318 (0.046)	0.365 (0.122)
	0.07	0.686 (0.293)	0.739 (0.116)	0.688 (0.130)	0.720 (0.241)
	0.10	0.957 (0.345)	0.950 (0.179)	0.958 (0.167)	0.937 (0.373)
Method III	0.05	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	0.07	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.008 (0.000)
	0.10	0.032 (0.032)	0.040 (0.032)	0.057 (0.057)	0.049 (0.041)
Method IV	0.05	0.024 (0.008)	0.008 (0.008)	0.008 (0.008)	0.000 (0.000)
	0.07	0.041 (0.041)	0.008 (0.008)	0.033 (0.033)	0.024 (0.016)
	0.10	0.153 (0.153)	0.113 (0.105)	0.098 (0.098)	0.146 (0.138)

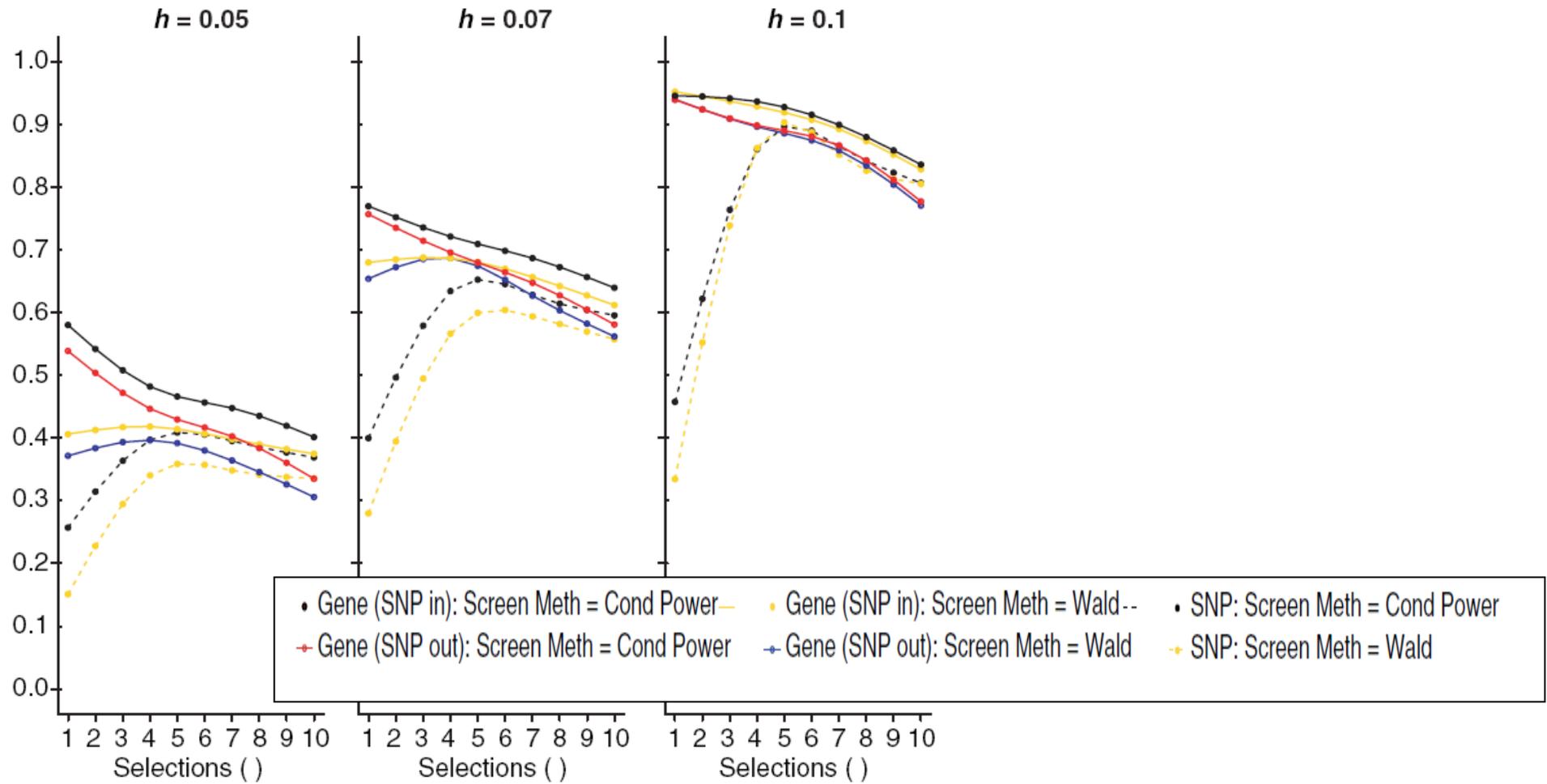
Method I: explained PBAT screening method

Method III: Benjamini-Yekutieli FDR control to 5% (general dependencies)

Method IV: Benjamini-Hochberg FDR control to 5%

Power to detect 1 DSL

(Van Steen et al 2005)



One stage is better than multiple stages?

- Macgregor (2008) claims that a total test for family-based designs should be more powerful than a two-stage design
- However, these and similar conclusions are restricted by the methods they include in the comparative study:
 - Ranking based conditional power versus ranking based on p -values (which is much less informative)
 - Summing the conditional mean model statistic (from PBAT pre-screening stage) and FBAT statistic (from PBAT testing stage) to obtain a single-stage procedure
 - The top K approach of Van Steen et al (2005) versus the even more powerful weighted Bonferroni approach of Ionita-Laza (2007)

Weighted Bonferroni Testing

Screen

- Compute, for all genotyped SNPs, the conditional power of the family-based association test (FBAT) statistic on the basis of the estimates obtained from the conditional mean model
- Since these power estimates are statistically independent of the FBAT statistics that will be computed subsequently, the overall significance level of the algorithm does not need to be adjusted for the screening step.

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$

Test

- The new method tests all markers, not just the 10 or 20 SNPs with the highest power ranking tested in the top K approach.
- Unlike a Bonferroni or FDR approach, the new method incorporates the extra information obtained in the screening step (conditional power estimate of the FBAT statistic)

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$

(Ionita-Laza et al. 2007)

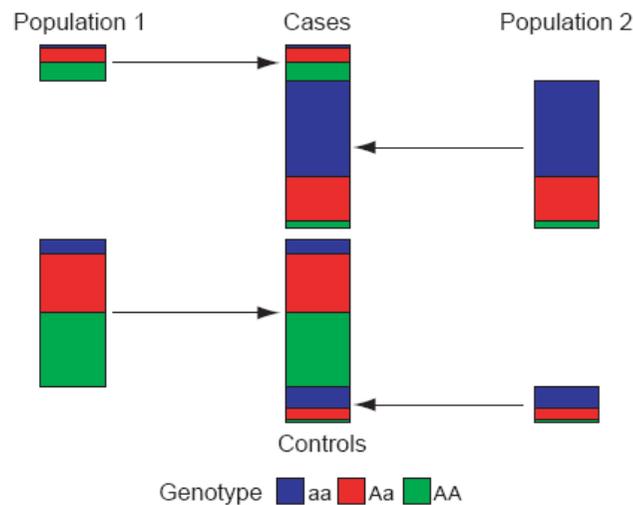
Motivation

- Markers that have a high power ranking are tested at a significance level that is far less stringent than that used in a standard Bonferroni adjustment.
- For SNPs with low power estimates, the evidence against the null hypothesis has to be extremely strong to overthrow the prior evidence against association from the screening step.
- This adjustment is made at the expense of the lower-ranked markers, which are tested using more-stringent thresholds.
- The adjustment follows the intuition that low conditional power estimates imply small genetic effect sizes and/or low allele frequencies, which makes such SNPs less desirable choices for the investment of relatively large parts of the significance level.

(Ionita-Laza et al. 2007)

4.b GRAMMAR screening

- Even though family-based design is adopted, when not conditioning on parental genotypes, a distinction should be made between:
 - Analysis of samples of relatives from genetically homogeneous population
 - Analysis of samples of relatives from genetically heterogeneous population



If we mix two populations that have both different disease prevalence and different marker distribution in each population, and there is no association between the disease and marker allele in each population, then there will be an association between the disease and the marker allele in the mixed population. (Marchini 2004)

Mixed model for families

- A conventional polygenic model of inheritance, which is a statistical genetics' "gold standard", is a mixed model

$$Y = \mu + G + e$$

with an overall mean μ , the vector of random polygenic effects G , and the vector of random residuals e

- For association testing, we need an additional term kg

$$Y = \mu + k g + G + e$$

where

G is random polygenic effect distributed as $MVN(0, \phi\sigma_G^2)$

ϕ is relationship matrix

σ_G^2 is polygenic variance

- This model is also known as the **measured genotype model (MG)**

GRAMMAR

- The MG approach, implemented using (restricted) maximum likelihood, is a powerful tool for the analysis of quantitative traits
 - when ethnic stratification can be ignored and
 - pedigrees are small or
 - when there are few dozens or hundreds of candidate polymorphisms to be tested.
- This approach, however, is not efficient in terms of computation time, which hampers its application in genome-wide association analysis.



Genomewide Rapid Association using Mixed Model And Regression

(Aulchenko et al 2007; Amin et al 2007)

GRAMMAR

- Step 1: Compute individual environmental residuals (r^*) from the additive polygenic model
- Step 2: Test the markers for association with these residuals using simple linear regression

$$r^* = \mu + k g + e$$

Note that family-effects have been “removed”!

- Step 3: Due to multiple testing, one could think of type I levels being elevated. However, GRAMMAR actually leads to a conservative test
- Step 4: A genomic-control like procedure, computing the deflation factor as a corrective factor, solves this problem

(Aulchenko et al 2007, Amin et al 2007)

GRAMMAR versus FBAT

- The GRAMMAR test becomes increasingly conservative and less powerful with the increase in number of large full-sib families and increased heritability of the trait.
- Interestingly, empirical power of GRAMMAR is very close to that of MG
- When no genealogical info on all generations, or when it is inaccurate, the most likely outcome for GRAMMAR (and GM) will be an inflated type I error.
- FBAT has increased power when heritability increases and uses “within” family information only from “informative” families
- FBAT does not explicitly rely on kinship matrices;
- FBAT is robust to population stratification

5 Validation

5.a Replication

- Replicating the genotype-phenotype association is the “gold standard” for “proving” an association is genuine
- Most loci underlying complex diseases will not be of large effect. It is unlikely that a single study will unequivocally establish an association without the need for replication
- SNPs most likely to replicate:
 - Showing modest to strong statistical significance
 - Having common minor allele frequency
 - Exhibiting modest to strong genetic effect size
- Note: Multi-stage design analysis results should not be seen as “evidence for replication” ...

Guidelines for replication studies

- Replication studies should be of sufficient size to demonstrate the effect
- Replication studies should be conducted in independent datasets
- Replication should involve the same phenotype
- Replication should be conducted in a similar population
- The same SNP should be tested
- The replicated signal should be in the same direction
- Joint analysis should lead to a lower p -value than the original report
- Well-designed negative studies are valuable

5.b Proof of concept

A Common Genetic Variant Is Associated with Adult and Childhood Obesity

Alan Herbert,^{1*} Norman P. Gerry,¹ Matthew B. McQueen,² Iris M. Heid,^{3,4} Arne Pfeufer,^{5,6}
Thomas Illig,^{3,4} H.-Erich Wichmann,^{3,4,7} Thomas Meitinger,^{5,6} David Hunter,^{2,8,9} Frank B. Hu,^{2,8,9}
Graham Colditz,^{8,9} Anke Hinney,¹⁰ Johannes Hebebrand,¹⁰ Kerstin Koberwitz,^{6,10}
Xiaofeng Zhu,¹¹ Richard Cooper,¹¹ Kristin Ardlie,¹² Helen Lyon,^{13,14,15} Joel N. Hirschhorn,^{13,14,15}
Nan M. Laird,¹⁶ Marc E. Lenburg,¹ Christoph Lange,^{9,13} Michael F. Christman^{1*}

www.sciencemag.org **SCIENCE** VOL 312 14 APRIL 2006

Genome wide association study of BMI

- A surrogate measure for obesity
- $BMI = \text{weight} / (\text{height})^2$ in kg / m^2
- Classification
 - ≥ 25 = overweight
 - ≥ 30 = obese

Epidemiology of BMI

- Prevalence (US)
 - 65% overweight
 - 30% obese
- Seen as risk factor for
 - Diabetes, Stroke, ...
- Non-genetic risk factors
 - Sedentary lifestyle, dietary habits, etc
- Genetic risk factors
 - Heritability = 30-70%

Design

- Framingham Heart Study (FHS)
 - Public Release Dataset (NHLBI)
 - 694 offspring from 288 families
 - Longitudinal BMI measurements

- Genotypes
 - Affymetrix GeneChip 100K

Analysis technique

- FBAT screening methodology (Van Steen et al. 2005)
- Exploit longitudinal character of the measurements:
 - Principal Components (PC) Approach
 - Maximize *heritability*
 - Univariate test (one combined trait per obs)
 - PBAT algorithm
 - Find maximum *heritability* of trait without biasing the testing step

Ranking from screen	SNP	Chromosome	Frequency	Informative families	P value FBAT
1	rs3897510	20p12.3	0.36	30	0.2934
2	rs722385	2q32.1	0.16	15	0.1520
3	rs3852352	8p12	0.33	34	0.7970
4	rs7566605	2q14.1	0.37	39	0.0026
5	rs4141822	13q33.3	0.29	27	0.0526
6	rs7149994	14q21.1	0.35	31	0.0695
7	rs1909459	14q21.1	0.39	38	0.2231
8	rs10520154	15q15.1	0.36	38	0.9256
9	rs440383	15q15.1	0.36	38	0.8860
10	rs9296117	6p24.1	0.40	44	0.3652

(genomewide sign: 0.005; rec model)

“Replication”

Family-based design

STUDY	FAMILIES	TEST	P-VALUE
FHS (Original)	288	PBAT	0.003
Maywood (Dichotimous)	342	PBAT	0.009

Cohort design

Maywood (Quantitative)	342	PBAT	0.070
Essen (Children)	368	TDT	0.002

STUDY	SUBJECTS	TEST	P-VALUE
KORA (QT)	3996	Regression	0.008
NHS (QT)	2726	Regression	> 0.10

(Example on Framingham Study: courtesy of
Matt McQueen)



**“If you consider the wind-chill factor, adjust
for inflation and score on a curve,
I only weigh 98 pounds!”**

Why did this work so well?

- The Study Population
 - Unascertained sample
 - Family-based
 - Longitudinal measurements
- The Method
 - PBAT
- Good Fortune

5.c Unexplained heritability

What are we missing?

- Despite these successes, it has become clear that usually only a small percentage of total genetic heritability can be explained by the identified loci.
- For instance:
for inflammatory bowel disease (IBD), 32 loci significantly impact disease but they explain only 10% of disease risk and 20% of genetic risk (Barrett et al 2008).

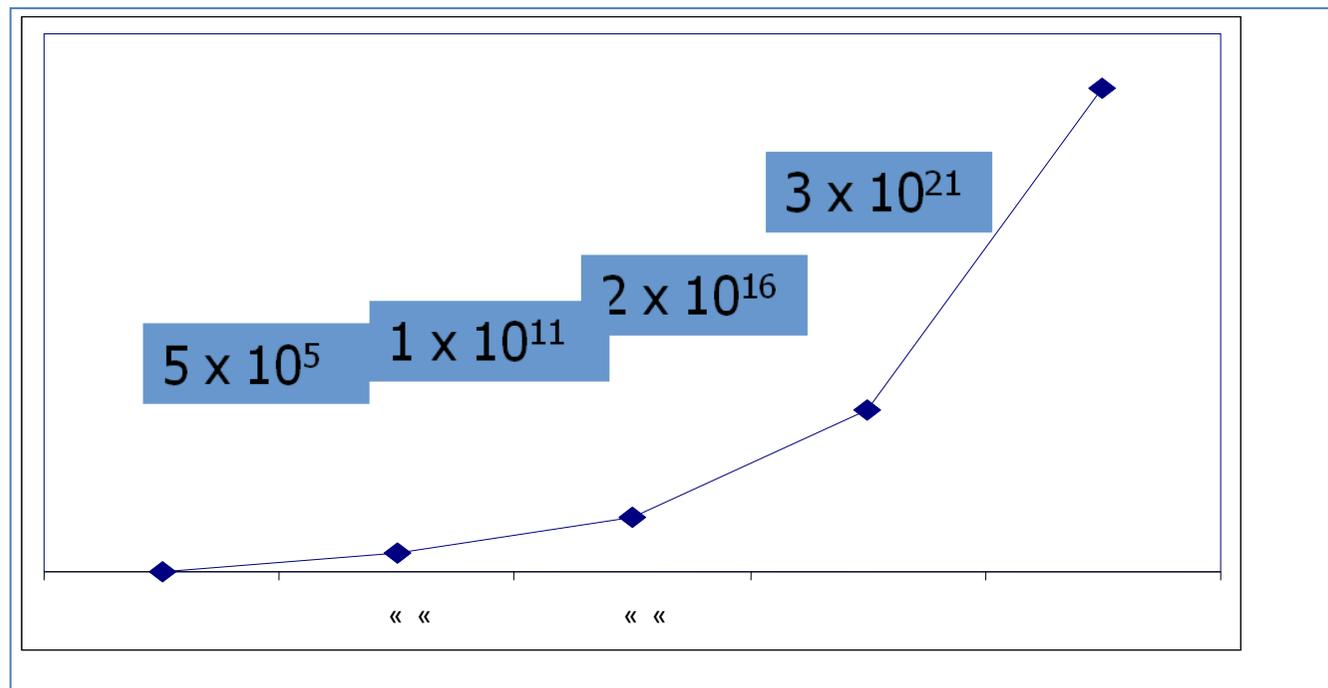
Possible reasons for poor “heritability” explanation

- This may be attributed to the fact that reality shows
 - multiple small associations (in contrast to statistical techniques that can only detect moderate to large associations),
 - dominance or over-dominance, and involves
 - non-SNP polymorphisms, as well as
 - epigenetic effects,
 - gene-environment interactions and
 - gene-gene interactions (Dixon et al 2000).

6 Beyond main effects

6.a Dealing with multiplicity

- Multiple testing explosion: ~500,000 SNPs span 80% of common variation in genome (HapMap)



Ways to handle multiplicity

Recall that several strategies can be adopted, including:

- clever multiple corrective procedures
- pre-screening strategies,
- multi-stage designs,
- adopting haplotype tests or
- multi-locus tests

Which of these approaches are more powerful is
still under heavy debate...

- The multiple testing problem becomes “unmanageable” when looking at multiple loci jointly?

6.b A bird's eye view on roads less travelled by

Multiple disease susceptibility loci (mDSL)

- Dichotomy between
 - Improving single markers strategies to pick up multiple signals at once (PBAT)
 - Testing groups of markers (FBAT multi-locus tests)

PBAT screening for mDSL

- Little has been done in the context of family-based screening for epistasis
- First assess how a method is capable of detecting multiple DSL
- Simulation strategy (10,000 replicates):
 - Genetic data from Affymetrix SNPChip 10K array on 467 subjects from 167 families
 - Select 5 regions; 1 DSL in each region
 - Generate traits according to normal distribution, including up to 5 genetic contributions
 - For each replicate: generate heritability according to uniform distribution with mean $h = 0.03$ for all loci considered (or $h = 0.05$ for all loci)

(Van Steen et al 2005)

General theory on FBAT testing

- Test statistic:

- works for any phenotype, genetic model
- use covariance between offspring trait and genotype

$$U = \sum (Y - \mu)(X - E(X|P))$$

- Test Distribution:

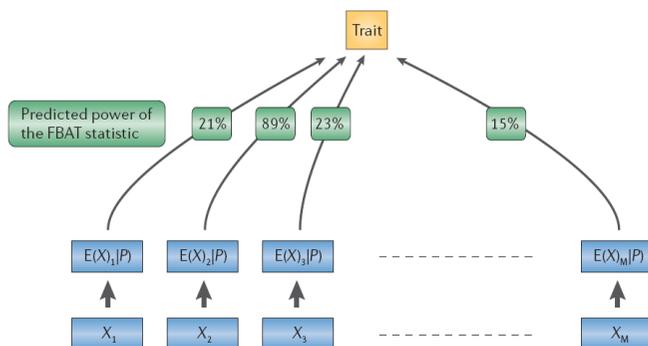
- computed assuming H_0 true; random variable is offspring genotype
- condition on parental genotypes when available, extend to family configurations (avoid specification of allele distribution)
- condition on offspring phenotypes (avoid specification of trait distribution)

(Horvath et al 1998, 2001; Laird et al 2000)

Screen

- Use ‘between-family’ information $[f(S, Y)]$
- Calculate conditional power (a_b, Y, S)
- Select *top N* SNPs on the basis of power

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$



Test

- Use ‘within-family’ information $[f(X/S)]$ while computing the FBAT statistic
- This step is independent from the screening step
- Adjust for *N* tests (not 500K!)

$$E[Y] = \mu + a_w(X - E[X|P]) + a_b(E[X|P])$$

(↑ Van Steen et al 2005)

(← Lange and Laird 2006)

Power to detect genes with multiple DSL

DSLs	Identified genes ($h = 0.03$)					DSLs	Identified genes ($h = 0.05$)				
	1	2	3	4	5		1	2	3	4	5
2	0.646	0.000	–	–	–	2	0.969	0.000	–	–	–
	0.665	0.000					0.984	0.000			
3	0.776	0.079	0.000	–	–	3	0.984	0.390	0.000	–	–
	0.823	0.063	0.000				0.996	0.287	0.000		
4	0.846	0.247	0.010	0.000	–	4	0.972	0.643	0.116	0.003	–
	0.914	0.255	0.025	0.000			0.997	0.754	0.246	0.015	
5	0.730	0.205	0.005	0.000	0.000	5	0.947	0.534	0.051	0.000	0.000
	0.822	0.222	0.025	0.000	0.000		0.987	0.696	0.185	0.005	0.000

top : top 5 SNPs in the ranking

bottom: top 10 SNPs in the ranking

(Van Steen et al 2005)

Power to detect genes with multiple DSL

DSLs	Identified genes ($h = 0.03$)					DSLs	Identified genes ($h = 0.05$)				
	1	2	3	4	5		1	2	3	4	5
2	0.059	0.000	–	–	–	2	0.413	0.000	–	–	–
	0.201	0.000					0.587	0.000			
3	0.138	0.000	0.000	–	–	3	0.630	0.004	0.000	–	–
	0.303	0.000	0.000				0.799	0.004	0.000		
4	0.258	0.000	0.000	0.000	–	4	0.770	0.018	0.000	0.000	–
	0.485	0.010	0.000	0.000			0.909	0.071	0.000	0.000	
5	0.368	0.000	0.000	0.000	0.000	5	0.833	0.003	0.000	0.000	0.000
	0.563	0.008	0.000	0.000	0.000		0.937	0.033	0.003	0.000	0.000

top : Benjamini-Yekutieli FDR control at 5% (general dependencies)

bottom: Benjamini-Hochberg FDR control at 5%

(Van Steen et al 2005)

FBAT multi-locus tests

Human
Heredit

Original Paper

Hum Hered 2008;66:122–126
DOI: 10.1159/000119111

Published online: March 31, 2008

FBAT-SNP-PC: An Approach for Multiple Markers and Single Trait in Family-Based Association Tests

Cyril S. Rakovski^a Scott T. Weiss^b Nan M. Laird^a Christoph Lange^{a, b}

^aDepartment of Biostatistics, Harvard School of Public Health, and ^bChanning Laboratory, Harvard Medical School, Boston, Mass., USA

(Rakovski et al 2008)

- The new test has an overall performance very similar to that of FBAT-LC

- FBAT-SNP-PC attains higher power in candidate genes with lower average pair-wise correlations and moderate to high allele frequencies with large gains (up to 80%).

An Efficient Family-Based Association Test Using Multiple Markers

Xin Xu,^{1*} Cyril Rakovski,² Xiping Xu,³ and Nan Laird²

¹Program for Population Genetics, Harvard School of Public Health, Boston, Massachusetts

²Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts

³Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, Chicago, Illinois

In genetic association studies, multiple markers are usually employed to cover a genomic region of interest for localizing a trait locus. In this report, we propose a novel multi-marker family-based association test (T_{LC}) that linearly combines the single-marker test statistics using data-driven weights. We examine the type-I error rate in a numerical study and compare its power to identify a common trait locus using tag single nucleotide polymorphisms (SNPs) within the same haplotype block that the trait locus resides with three competing tests including a global haplotype test (T_H), a multi-marker test similar to the Hotelling- T^2 test for the population-based data (T_{MM}), and a single-marker test with Bonferroni's correction for multiple testing (T_B). The type-I error rate of T_{LC} is well maintained in our numeric study. In all the scenarios we examined, T_{LC} is the most powerful, followed by T_B , T_{MM} and T_H are the poorest. T_H and T_{MM} have essentially the same power when parents are available. However, when both parents are missing, T_{MM} is substantially more powerful than T_H . We also apply this new test on a data set from a previous association study on nicotine dependence. *Genet. Epidemiol.* 30:620–626, 2006. © 2006 Wiley-Liss, Inc.

Key words: multi-marker family-based association; FBAT

(FBAT-LC : Xin et al 2008)

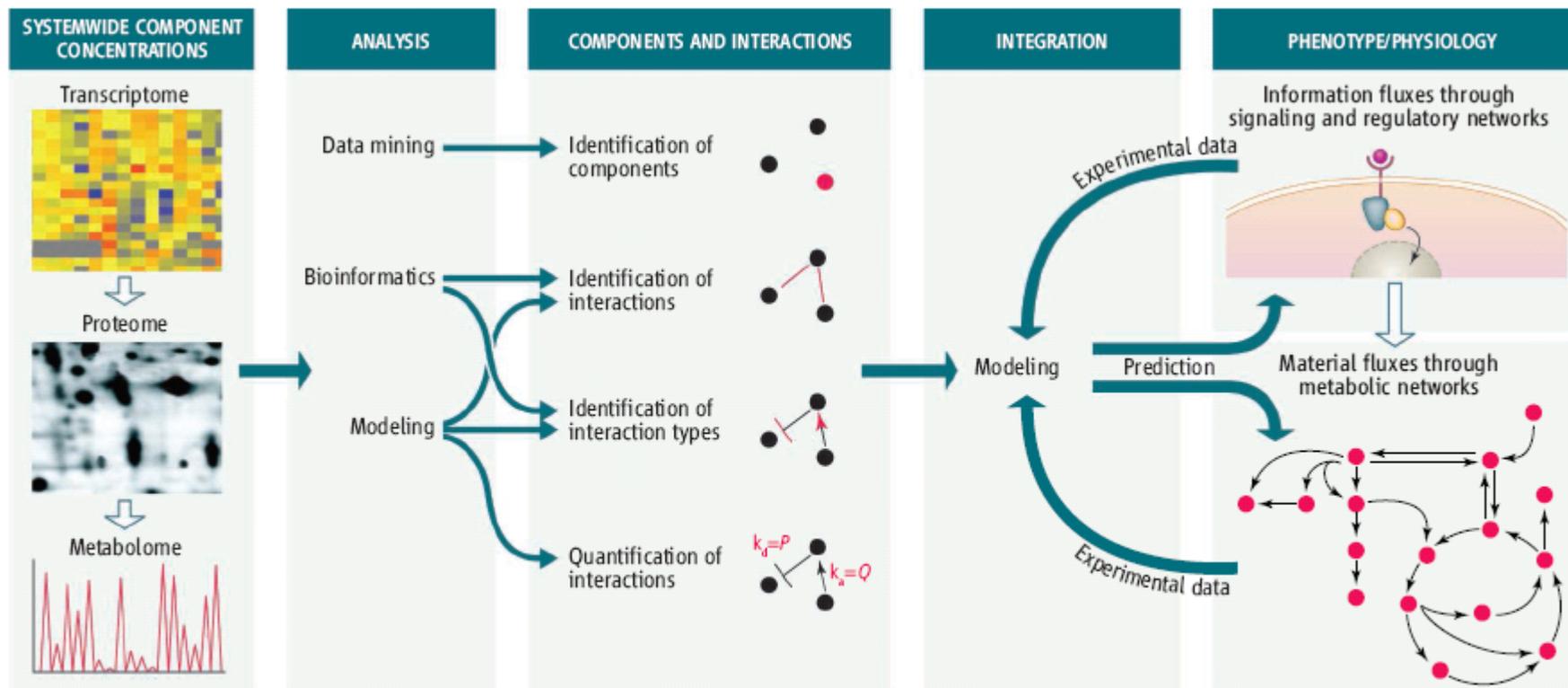
In contrast: popular multi-locus approaches for unrelateds

- Parametric methods:
 - Regression
 - Logistic or (Bagged) logic regression
- Non-parametric methods:
 - Combinatorial Partitioning Method (CPM)
 - quantitative phenotypes; interactions
 - Multifactor-Dimensionality Reduction (MDR)
 - qualitative phenotypes; interactions
 - Machine learning and data mining

- The multiple testing problem becomes “unmanageable” when looking at (genetic) interaction effects? More about this in the future!

7 Future challenges

Integration of –omics data in GWAs



A systems roadmap. The comprehensive component concentrations reported by Ishii *et al.* provide input data for inferring component interactions using computational methods. The challenge for computational modeling methods yet to be developed is to predict the functional network state from the concentrations and to infer the information processing network that controls the functional state.

Integrations of –omics data in GWAs

PERSPECTIVE

GENOMEWIDE ASSOCIATION STUDIES — ILLUMINATING BIOLOGIC PATHWAYS

Genomewide Association Studies — Illuminating Biologic Pathways

Joel N. Hirschhorn, M.D., Ph.D.

Human geneticists seek to understand the inherited basis of human biology and disease, aiming either to gain insights that could eventually improve treatment or to produce useful diagnostic or predictive tests. As recently as 2004, few genetic variants were known to reproducibly influence common polygenic diseases (including cancer, coronary artery disease, and diabetes) or quantitative phenotypes (including lipid levels and blood pressure). This relative ignorance limited potential insights into the pathophysiology of common diseases.

Gelehrter predicted that no more than three new common variants would be reproducibly associated with common diseases by the time the American Society of Human Genetics (ASHG) held its meeting in the autumn of 2008.

During the past 2 years, however, genomewide association studies have identified more than 250 genetic loci in which common genetic variants occur that are reproducibly associated with polygenic traits.¹⁻⁴ This explosion represents one of the most prolific periods of discovery in human genetics, with most new loci

be evenly distributed across the genome, he concludes that every gene in the genome could theoretically be implicated, a scenario that would prohibit useful biologic insights.

I believe that the skeptics' arguments either misconstrue the primary goal of genomewide association studies or are contradicted by their findings. The main goal of these studies is not prediction of individual risk but rather discovery of biologic pathways underlying polygenic diseases and traits. It is already clear that the genes being identified expose rel-

(Hirschhorn 2009)

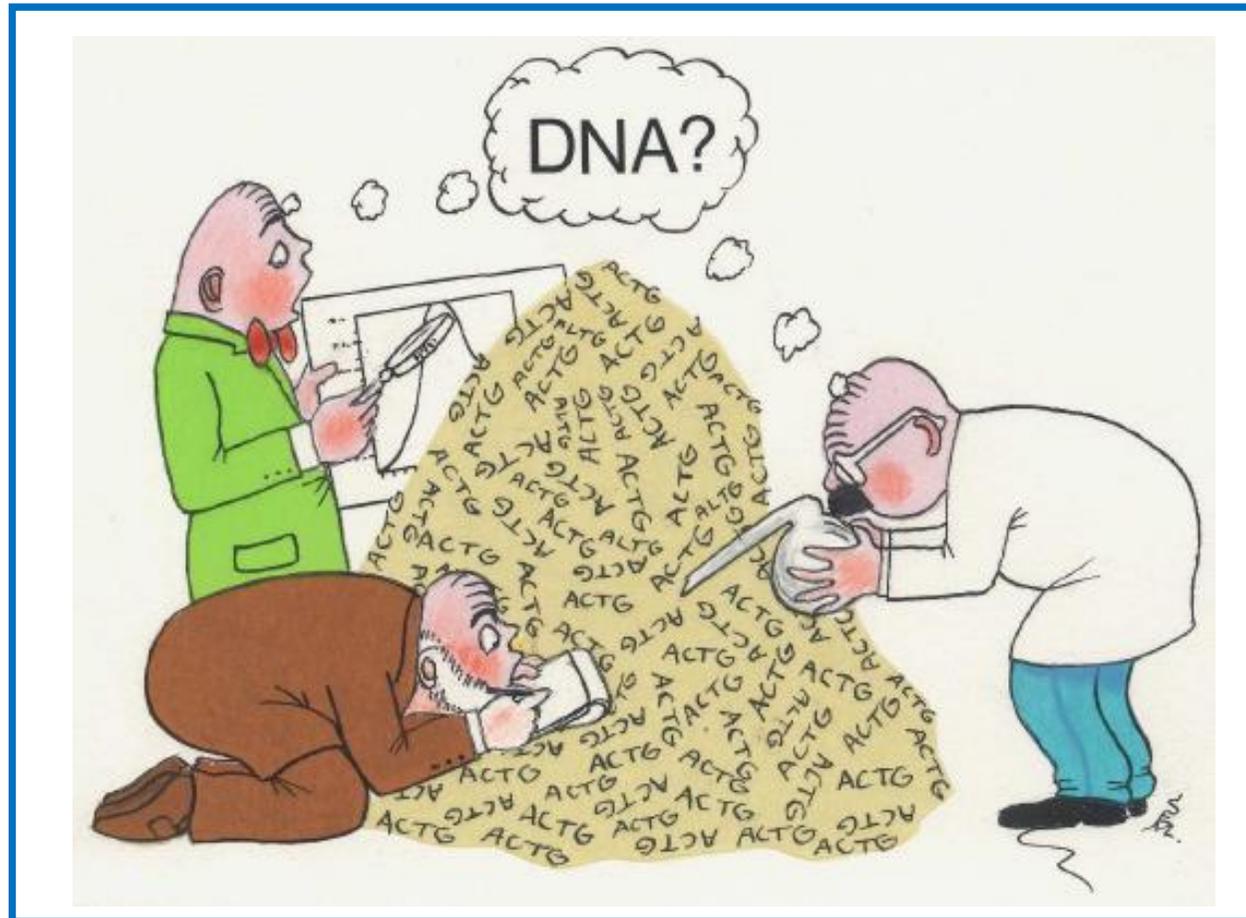
Integration of –omics data in GWAs

A few “straightforward” examples, just using “gene expressions”:

- Post-analysis
 - As validation tool in main effects GWAs
 - Links to gene-expression networks?
 - Implication factor for GxG model via biological data bases large enough?
- During the analysis:
 - Epistasis screening (FAM-MDR)
 - Use expression values to prioritize multi-locus combinations

		Locus 3		
		AA	Aa	aa
Locus 4	BB	3.00	0.42	1.14
	Bb	0.75	4.00	2.66
	bb		1.27	0.54

Extensive boundary crossing collaborations



Statistical Genetics Research Club (www.statgen.be)

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