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# From cross-phenotype associations to pleiotropy in human genetic studies

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## Pleiotropy

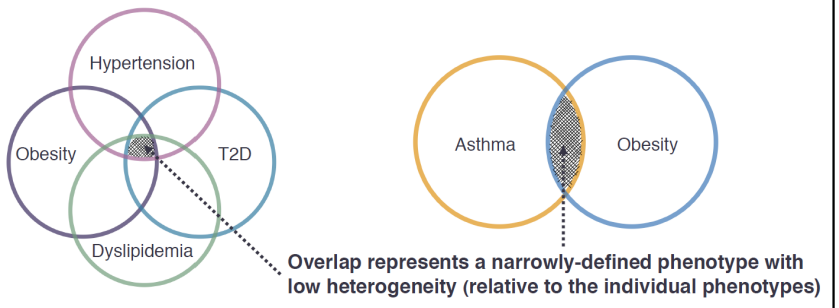
- Phenomenon in which a genetic locus affects more than one trait or disease
- Molecular level
  - Single gene with multiple physiological functions
  - Two domains of a single gene product with different functions and affecting multiple phenotypes
  - Gene product with a single function that affects multiple phenotypes be acting in multiple tissues
- Statistical level
  - A locus displaying cross-phenotype associations is often considered pleiotropic



## Pleiotropy and disease comorbidity

- Examples of correlated (comorbid) disease
  - Obesity, hypertension, dyslipidemia, type 2 diabetes (metabolic disorder)
  - Depression, anxiety, personality disorders (psychiatric disorder)
  - Asthma, obesity (pro-inflammatory conditions)
- Why do certain disease occur together
  - Causality
  - Shared environmental risk factors
  - Shared genetic risk factors

## Pleiotropy and disease comorbidity



# Pleiotropy and disease comorbidity

- Pleiotropy-informed analyses consider multiple phenotypes together and take into account the correlation between the phenotypes
  - Analyzing multiple correlated phenotype (e.g. comorbid diseases) is equivalent to analyzing a single narrowly-defined phenotype with low heterogeneity



## Pleiotropy and disease comorbidity

- Detecting shared genetics and/or molecular pathways between comorbid diseases can help us understand exactly how the etiology of the diseases overlap
- Etiologic overlaps:
  - provide opportunities for novel interventions that prevent or treat the comorbidity, rather than preventing/treating each disease separately
  - facilitate drug repurposing (that is, known drugs targeting a pleiotropic locus may be repurposed to treat other diseases controlled by that locus, precluding the need for the development and testing of a brand-new drug)

## Expression of autism spectrum and schizophrenia in patients with a 22q11.2 deletion

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Schizophrenia Research 143 (2013) 55–59

**Table 1**
Autistic symptoms and probable ASD during childhood, assessed in 77 adults with 22q11.2DS, comparing those with and without schizophrenia.

| | Total (n=77) | 22q11.2DS-SZ (n=36) | 22q11.2DS-Co (n=41) | p | | | | |
|---|---|---|---|---|---|---|---|---|
| Mean SRS T-score (95% CI) | 73.1 (69.7–76.6) | 72.4 (67.3–78.0) | 73.7 (68.9–78.6) | 0.36[a] | | | | |
| Subjects categorized as probable ASD | n (%) | n (%) | n (%) | p | Uncorrected OR (95% CI) | p | Corrected OR (95% CI) | p |
| SRS T score cut-off 60 | 59 (76.6%) | 27 (75.0%) | 32 (78.0%) | 0.75[b] | 0.84 (0.29–2.43) | 0.75 | 0.36 (0.085–1.52) | 0.17[c] |
| SCQ cut-off 15 | 13 (16.9%) | 3 (8.3%) | 10 (24.4%) | 0.06[b] | 0.28 (0.07–1.12) | 0.07 | 0.20 (0.03–1.46) | 0.11[d] |
| SCQ cut-off 12 | 27 (35.1%) | 13 (36.1%) | 14 (34.1%) | 0.86[b] | 1.10 (0.44–2.78) | 0.86 | 1.53 (0.49–5.27) | 0.49[d] |

CI= confidence interval.
[a] Mann–Whitney–Wilcoxon.
[b] Chi-square.
[c] Corrected for age and IQ.
[d] Corrected for gender, age, IQ.

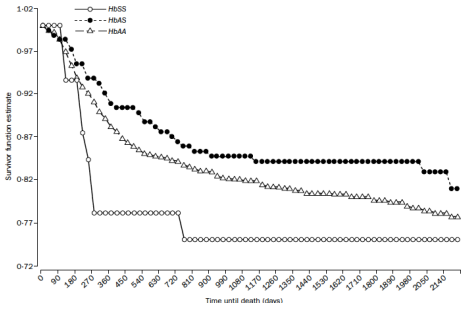
A B S T R A C T

*Background:* Copy number variants (CNVs) associated with neuropsychiatric disorders are increasingly being identified. While the initial reports were relatively specific, i.e. implicating vulnerability for a particular neuropsychiatric disorder, subsequent studies suggested that most of these CNVs can increase the risk for more than one neuropsychiatric disorder. Possibly, the different neuropsychiatric phenotypes associated with a single genetic variant are really distinct phenomena, indicating pleiotropy. Alternatively, seemingly different disorders could represent the same phenotype observed at different developmental stages or the same underlying pathogenesis with different phenotypic expressions.
*Aims:* To examine the relation between autism and schizophrenia in patients sharing the same CNV.
*Method:* We interviewed parents of 78 adult patients with the 22q11.2 deletion (22q11.2DS) to examine if autistic symptoms during childhood were associated with psychosis in adulthood. We used Chi-square, T-tests and logistic regression while entering cognitive level, gender and age as covariates.
*Results:* The subgroup of 22q11.2DS patients with probable ASD during childhood did not show an increased risk for psychosis in adulthood. The average SRS scores were highly similar between those with and those without schizophrenia.
*Conclusions:* ASD and schizophrenia associated with 22q11.2DS should be regarded as two unrelated, distinct phenotypic manifestations, consistent with true neuropsychiatric pleiotropy. 22q11.2DS can serve as a model to examine the mechanisms associated with neuropsychiatric pleiotropy associated with other CNVs.

## Protective effects of the sickle cell gene against malaria morbidity and mortality

*Michael Aidoo, Dianne J Terlouw, Margarette S Kolczak, Peter D McElroy, Feiko O ter Kuile, Simon Kariuki, Bernard L Nahlen, Altaf A Lal, Venkatachalam Udhayakumar*

*Lancet* 2002; **359:** 1311–12




| | Crude incidence/1000 person-months | | | Adjusted relative risk (95% CI) | | | |
|---|---|---|---|---|---|---|---|
| | *HbAA* | *HbAS* | *HbSS* | *HbAS vs HbAA* | p | *HbSS vs HbAA* | p |
| Severe malaria anaemia episodes | 4·0 | 2·0 | 1·5 | 0·40 (0·30–0·60) | 0·0001 | 0·29 (0·1–0·9) | 0·04 |
| All severe anaemia episodes | 8·8 | 6·8 | 7·5 | 0·61 (0·46–0·80) | 0·0006 | 0·63 (0·35–1·2) | 0·15 |
| (Hb <6 g/dL plus any parasitaemia) | | | | | | | |
| High density parasitaemia episodes | 20 | 17·3 | 15·8 | 0·73 (0·65–0·84) | 0·0001 | 0·52 (0·36–0·74) | 0·0002 |

Hb=haemoglobin. *Hb*SS was associated with lower parasite incidence than HbAA haemoglobin levels and parasitaemia were determined using routine monthly finger-prick blood samples and samples collected any time the children were reported ill. All data points collected monthly for the entire time children participated in the study were used in data analyses unless indicated otherwise. Only birthweight among the various covariates considered (same as for survival analysis) was controlled for in the final model.

# Pleiotropy in gene mapping

- Mapping a single genotype to multiple phenotypes has the potential to uncover novel links between traits or diseases
- It can also offer insights into the mechanistic underpinnings of known comorbidities
- It can increase power to detect novel associations with one or more phenotypes



# A practitioners' guide for studying pleiotropy in genetic epi studies

Am J Epidemiol. 2017 Aug 11. doi: 10.1093/aje/kwx296. [Epub ahead of print]

**Statistical Analysis of Multiple Phenotypes in Genetic Epidemiological Studies:From Cross-Phenotype Associations to Pleiotropy.**

Salinas YD, Wang Z, DeWan AT.

**Abstract**

In the context of genetics, pleiotropy refers to the phenomenon in which a single genetic locus affects more than one trait or disease. Genetic epidemiological studies have identified loci associated with multiple phenotypes, and these cross-phenotype associations are often incorrectly interpreted as examples of pleiotropy. Pleiotropy is only one possible explanation for cross-phenotype associations. Cross-phenotype associations may also arise due to issues related to study design, confounder bias, or non-genetic causal links between the phenotypes under analysis. Therefore, it is necessary to dissect cross-phenotype associations carefully to uncover true pleiotropic loci. In this review, we describe statistical methods that can be used to identify robust statistical evidence of pleiotropy. First, we provide an overview of univariate and multivariate methods for discovery of cross-phenotype associations and highlight important considerations for choosing among available methods. Then, we describe how to dissect cross-phenotype associations by using mediation analysis. Pleiotropic loci provide insights into the mechanistic underpinnings of disease comorbidity, and may serve as novel targets for interventions that simultaneously treat multiple diseases. Discerning between different types of cross-phenotype associations is necessary to realize the public health potential of pleiotropic loci.



**KEYWORDS:** genetic epidemiology; mediation analysis; pleiotropy



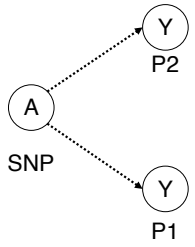
# Guidelines for generating robust statistical evidence of pleiotropy

**Discover** CP associations ⇨ **Dissect** CP associations ⇨ **Classify** them as examples of biological, mediated, or spurious pleiotropy



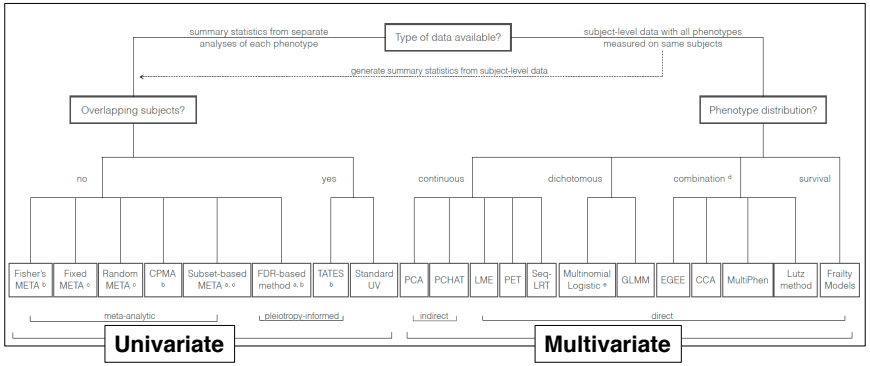
# Cross-phenotype (CP) associations

Statistical associations between a **single genetic locus** – a single gene or a single variant within a gene – and **multiple phenotypes**




Note that the dashed lines denote uncertainty about whether the SNP has a direct effect on the phenotypes.

# Analytic options for discovery of CP associations

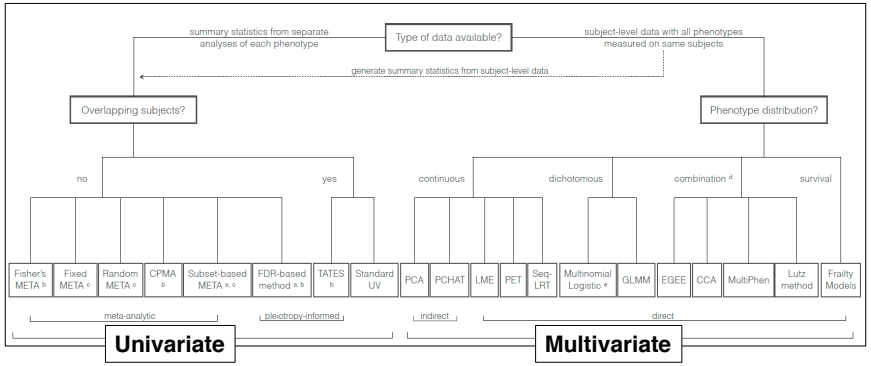



Key distinction:

- Univariate methods examine the association between a given SNP and each trait *separately*
- Multivariate methods examine the association between a given SNP and each trait by modeling the traits *jointly*



# Analytic options for discovery of CP associations




Choice between univariate and multivariate approaches depends on:

- Types of data available on our phenotypes of interest
  - Summary statistics vs. individual-level data?
  - Are the phenotypes measured on the same subjects?
- Distribution of the phenotypes (e.g., quantitative or disease trait)

# Univariate methods are by far the most commonly used to detect CP associations

- Univariate methods include (but are not limited to) the methods you've discussed in class so far:
  - allelic Chi-Square test
  - genotypic Chi-Square test
  - regression-based methods
- The overall approach is to:
  - obtain univariate association p-values for each phenotype
  - declare CP associations at genetic loci that are statistically significantly associated with each phenotype

# Hypothetical example: Discovery of CP associations for hypertension and heart disease by using logistic regression

**Step 1. Fit two univariate regression models within PLINK**

$$E[hypertension] = \beta_0 + \boldsymbol{\beta_1} * SNP$$

$$E[heart\ disease] = \beta_0 + \boldsymbol{\beta_1} * SNP$$

**Word of caution:** The univariate tests of association should be marginal tests (conducted irrespectively of the second phenotype) NOT conditional tests (conducted on a subset defined based on absence/presence of the second phenotype). In this example, what that means is that the regression for hypertension should be fit on all subjects *irrespectively* of their heart disease status; and the regression for heart disease should be fit on all subjects *irrespectively* of their hypertension status. More on this later!

evidence to declare a CP association at this SNP.

## Hypothetical example: Discovery of CP associations for hypertension and heart disease by using logistic regression

**Step 1. Fit two univariate regression models within PLINK**

$$E[hypertension] = \beta_0 + \boldsymbol{\beta_1} * SNP$$

$$E[heart\ disease] = \beta_0 + \boldsymbol{\beta_1} * SNP$$

**Step 2. For a given SNP, examine p-values for $\boldsymbol{\beta_1}$ from <u>each</u> model.**

- P-value for $\boldsymbol{\beta_1}$ in hypertension model = $1.03 \times 10^{-12}$
- P-value for $\boldsymbol{\beta_1}$ in heart disease model = $6.02 \times 10^{-9}$

**Step 3. Declare CP associations at a given SNP, if the p-values for $\boldsymbol{\beta_1}$ in <u>each</u> model surpass the study significance threshold.**

- Assuming the standard GWAS significance threshold (alpha=$5 \times 10^{-8}$), there is a statistically significant association with both hypertension and heart disease at this particular SNP. Therefore, we have sufficient statistical evidence to declare a CP association at this SNP.



## Using multivariate methods to increase the power to detect cross-phenotype associations

## A comparison of univariate and multivariate GWAS methods for analysis of multiple dichotomous phenotypes

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[1] Department of Chronic Disease Epidemiology; [2] Department of Biostatistics, Yale School of Public Health, Yale University, 60 College St, New Haven, Connecticut, USA

Genet. Epidemiol. 41 (7), 689-689

## Statistical power of multi-trait methods

- For *quantitative* trait methods, it has been shown that:
  - Multivariate analyses achieve greater power than univariate analyses both in the presence **(Allison 1998)** and absence of cross-trait genetic correlation or pleiotropy **(Galesloot 2014)**
- Therefore, joint analysis of *quantitative* phenotypes has the potential to enhance the statistical power of genetic studies.

## Statistical power of multi-trait methods

- With this potential for greater statistical power, multivariate methods could contribute to the investigation of the 'missing heritability' of complex diseases.
- However, it is unknown whether the trends observed for *quantitative* traits also hold for methods that can analyze multiple *disease* (case-control) phenotypes.
- Understanding the performance of these methods is essential to their successful application to real data.

## Objective

- To evaluate the relative statistical power of methods for analysis of two disease (case/control) phenotypes in the presence and absence of pleiotropy using simulated genotype and phenotype data.

## Data Simulation

- Genotypes were simulated for a bi-allelic SNP with **MAF = 0.20** by sampling two alleles independently from a binomial distribution.
- Genotypes (coded as 0/1/2) are the sum of the two alleles.

## Simulation scenarios

| # traits associated | $h_i^2$ | $r_{Y1,Y2}$ | $P_j$ |
|---|---|---|---|
| 1 | $h_1^2$=0.1%,$h_2^2$=0% | [-0.9,0.9] | P1 = P2 = 10% |
| | | | P1 = P2 = 20% |
| | | | P1 = 10%, P2 = 20% |
| | | | P1 = 20%, P2 = 10% |
| 2 | $h_1^2 = h_2^2$= 0.1% | [-0.9,0.9] | P1 = P2 = 10% |
| | | | P1 = P2 = 20% |
| | | | P1 = 10%, P2 = 20% |
| | | | P1 = 20%, P2 = 10% |
| 2 | $h_1^2$ = 0.1%,$h_2^2$ = 0.05% | [-0.9,0.9] | P1 = P2 = 10% |
| | | | P1 = P2 = 20% |
| | | | P1 = 10%, P2 = 20% |
| | | | P1 = 20%, P2 = 10% |

# Methods evaluated

1. **Standard univariate approach**
   - **models fitted**
     $\text{logit}[E(Y_{i1})] = \beta_0 + \beta_1 X_i$
     $\text{logit}[E(Y_{i2})] = \beta_0 + \beta_1 X_i$
   - **p-value extracted**
     - the minimum of the two univariate p-values
2. **Reversed ordinal logistic regression (MultiPhen)**
   - **model fitted**
     $\text{logit}[E(X_i \leq c)] = \alpha_c + \beta_1 Y_{i1} + \beta_2 Y_{i2}$, for c = 1, 2, or 3 genotype categories
   - **p-value extracted**
     - the p-value for a Likelihood Ratio Test for model fit, evaluating the null hypothesis that $\beta_1 = \beta_2 = 0$
3. **Generalized estimating equations (GEEs)**
   - **model fitted**
     $\text{logit}[E(Y_{ij})] = \beta_0 + \beta_1 j_i + \beta_2 X_i + \beta_{12} X_i j_i$
   - **p-value extracted**
     - the p-value for the test of the hypothesis that $\beta_2 = \beta_{12} = 0$
4. **Generalized linear mixed models (GLMMs)**
   - **model fitted**
     $\text{logit}[E(Y_{ij})] = \beta_0 + \beta_1 j_i + \beta_2 X_i + \beta_{12} X_i j_i + b_{ij}$
   - **p-value extracted**
     - the p-value for the test of the hypothesis that $\beta_2 = \beta_{12} = 0$

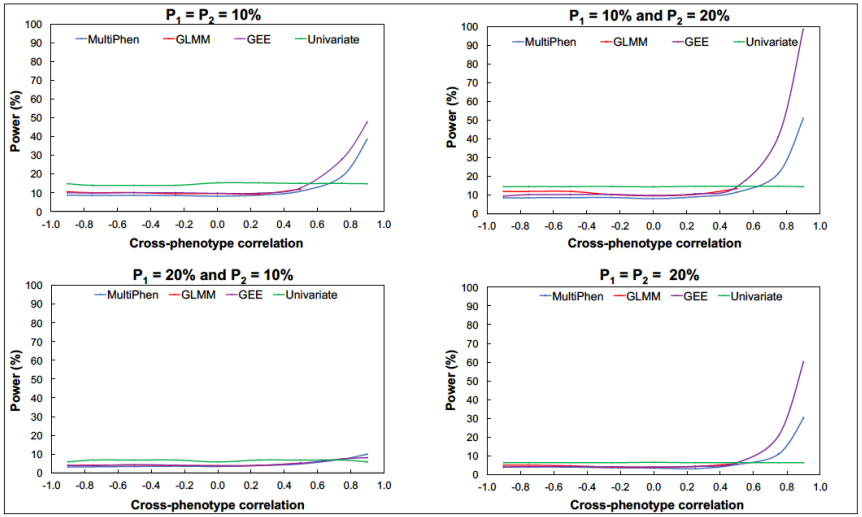
* $Y_{ij}$ represent the case/control status of the $i^{th}$ subject, measured for phenotypes j = 1 or 2; $X_i$ are the individual genotypes; $b_{ij}$ are the random effects correlated within the $i^{th}$ subject; and $j_i$ is an indicator variable for the phenotypes (coded as 0/1).

# Power

- We defined power as the percentage of the 10,000 replicates for which the extracted p-value for a given scenario was smaller than a genome-wide significance level of $5 \times 10^{-8}$ .

**PLEIOTROPY ABSENT**

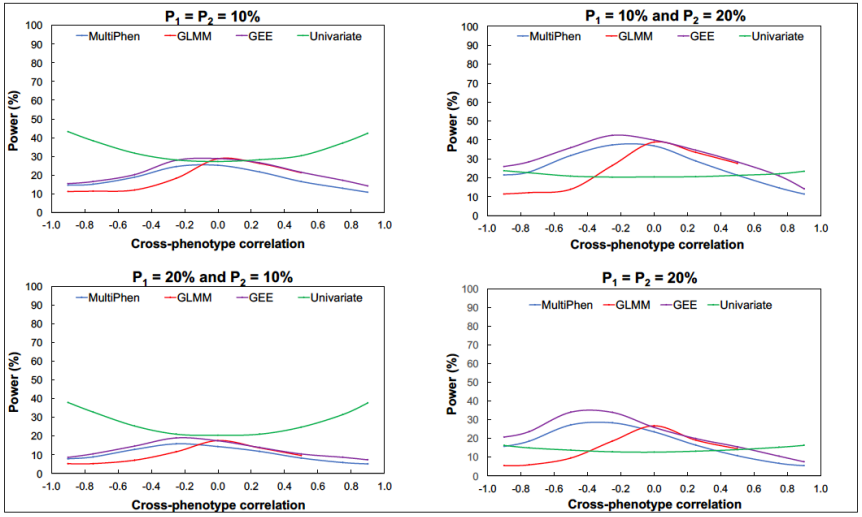
**Figure 1. Power when one phenotype ($Y_1$) is associated with the SNP ($h_1^2 = 0.1\%$; $h_2^2 = 0\%$) [a]**




[a] Results for GLMMs are shown for $r_{Y1,Y2} \leq 0.5$ only, since the models experienced convergence issues for $r_{Y1,Y2} > 0.5$.

**PLEIOTROPY PRESENT**
**equal effect sizes**

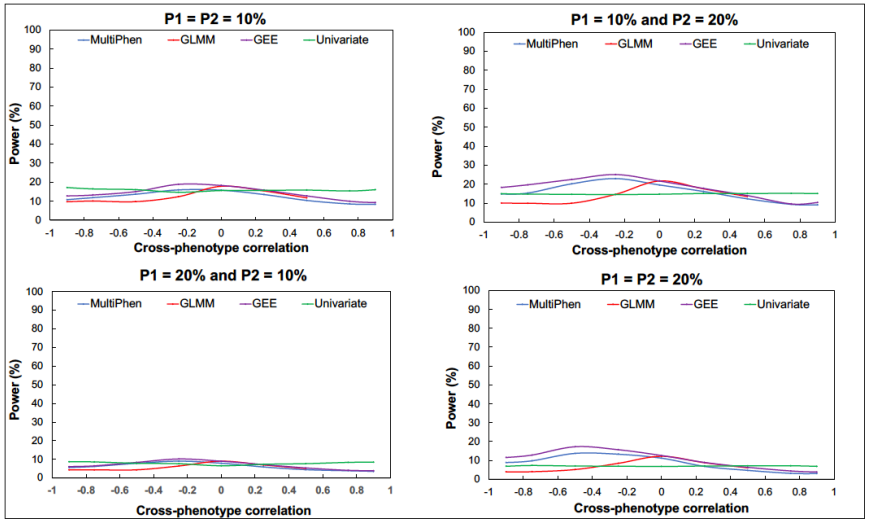
**Figure 2. Power when both phenotypes are associated with the SNP ($h_1^2 = h_2^2 = 0.1\%$) [a]**




[a] Results for GLMMs are shown for $r_{Y1,Y2} \leq 0.5$ only, since the models experienced convergence issues for $r_{Y1,Y2} > 0.5$.

PLEIOTROPY PRESENT
unequal effect sizes

**Figure 3. Power when both phenotypes are associated with the SNP ($h_1^2$ = 0.1%, $h_2^2$=0.05%) [a]**




[a] Results for GLMMs are shown for $r_{Y1,Y2} \leq 0.5$ only, since the models experienced convergence issues for $r_{Y1,Y2} > 0.5$.

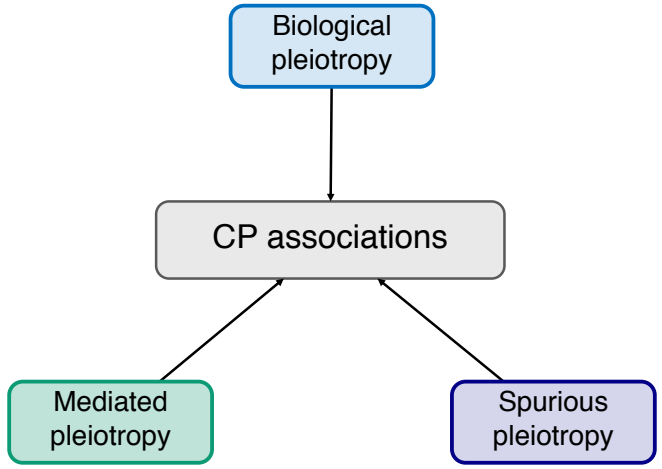
# Conclusions

- The performance of the univariate approach appeared to complement that of multivariate methods, with notable patterns:
  - **in the absence of pleiotropy**, multivariate methods had better performance **for $r_{Y1,Y2} > 0.5$** while univariate methods had better performance **for $r_{Y1,Y2} < 0.5$**
  - **in the presence of pleiotropy (positive genetic correlation)**, the multivariate approach lost power **for $r_{Y1,Y2} > 0$**, while the univariate approach gained power across this range of values
- Thus, to improve GWAS discovery, it may be beneficial to use univariate and multivariate approaches in parallel.



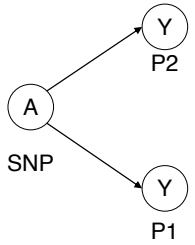
## Problem: CP associations need not be indicative of pleiotropy





# Biological pleiotropy

Independent associations between a genetic locus (A) and multiple phenotypic outcomes (Y)

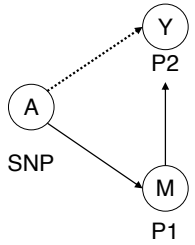



The SNP has a direct effect on each phenotype. (Note that direct or causal effects are depicted with solid lines).



# Mediated pleiotropy

Association between a genetic locus (A) and an intermediate phenotype (M) that causes a second phenotypic outcome (Y)

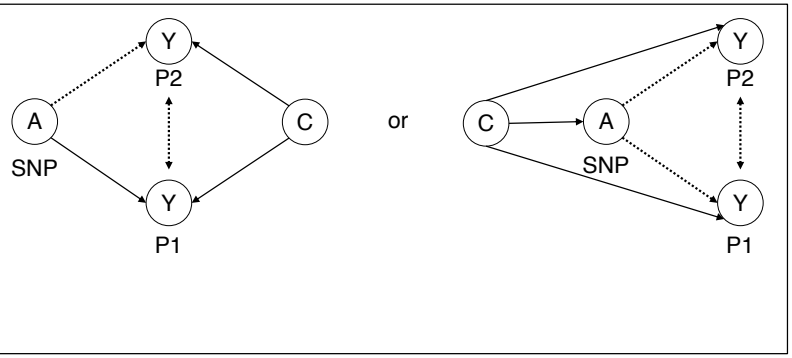



A non-genetic causal link between M and Y induces an association between A and Y, even in the absence of a direct effect of A on Y.



# Spurious pleiotropy

Artifactual associations with multiple phenotypes due to issues related to study design, confounding, or associations with markers in strong linkage disequilibrium* with multiple causal variants in different genes




*Linkage disequilibrium is the non-random co-segregation of alleles.



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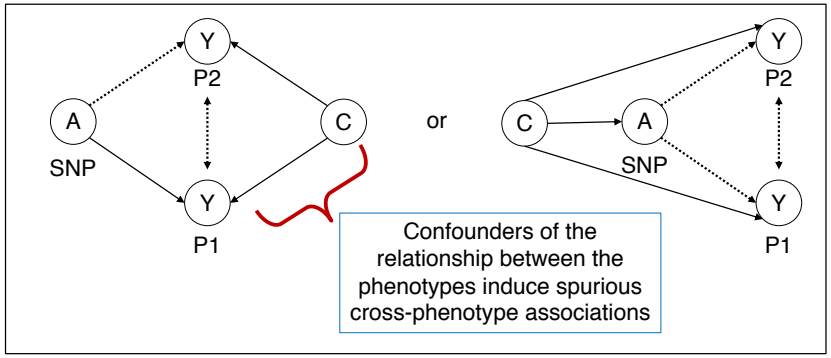


Confounders of the relationship between the phenotypes induce spurious cross-phenotype associations

*Linkage disequilibrium is the non-random co-segregation of alleles.

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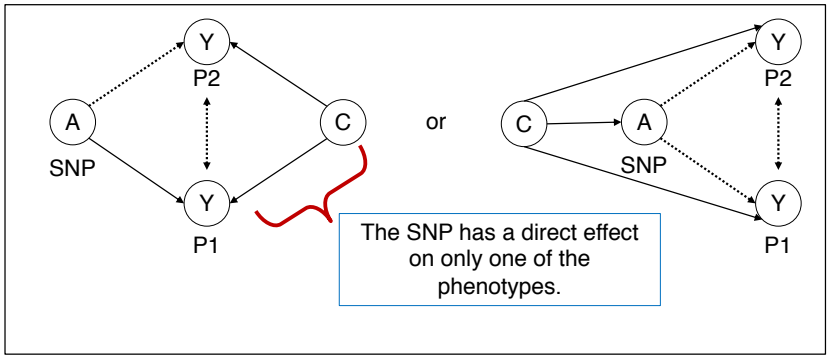


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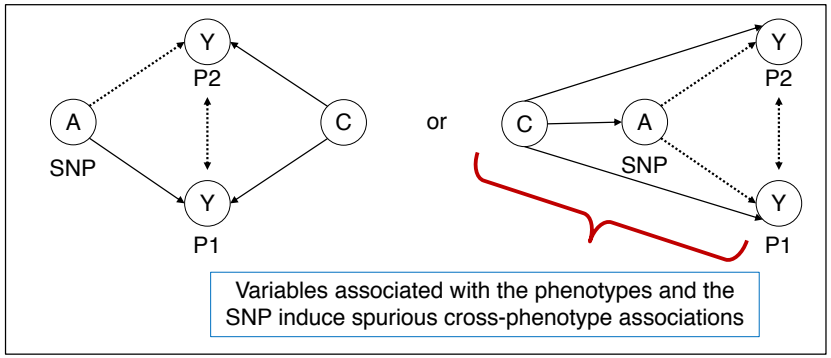


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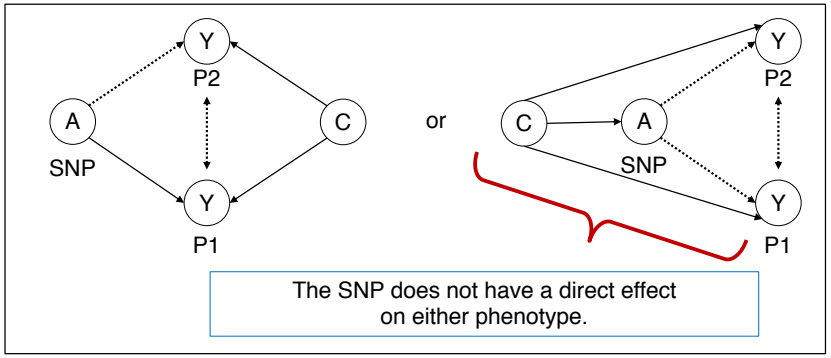


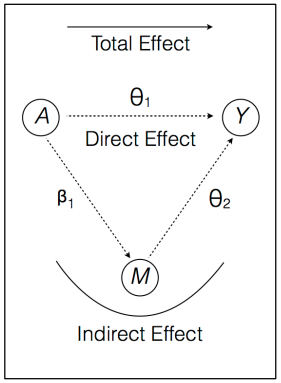

*Linkage disequilibrium is the non-random co-segregation of alleles.



# Guidelines for generating robust statistical evidence of pleiotropy

# Mediation analysis provides a tool for dissecting CP associations

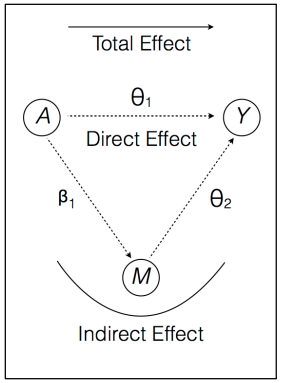
- Mediation analysis decomposes the **total effect** of the SNP (*A)* on a phenotypic outcome (*Y* ) into:
  - **Direct effect:** effect of *A* on *Y* that occurs independently of an intermediate phenotype (*M)*
  - **Indirect effect:** effect of *A* on *Y* that occurs through the intermediate phenotype *M*






# Mediation analysis: Data requirements

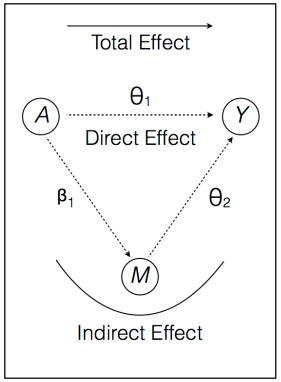
- All phenotypes must be measured on the same subjects
- Temporality must be ascertained
  - The occurrence of the intermediate variable *M* must precede that of the phenotypic outcome variable *Y*






# Mediation analysis: Assumptions

- There must be no unmeasured:
  - confounders of the total effect
  - confounders of the relationship between SNP *A* and the mediator *M*
  - confounders of the relationship between mediator *M* and phenotypic outcome *Y*

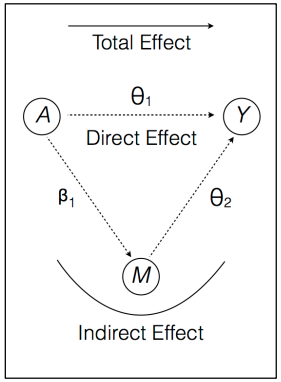





# Mediation analysis: Assumptions

Typically met in genetic epi studies!

- There must be no unmeasured:
  - confounders of the total effect
  - confounders of the relationship between SNP *A* and the mediator *M*
  - confounders of the relationship between mediator *M* and phenotypic outcome *Y*



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  - confounders of the relationship between SNP *A* and the mediator *M*
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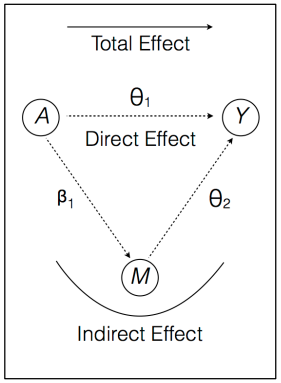


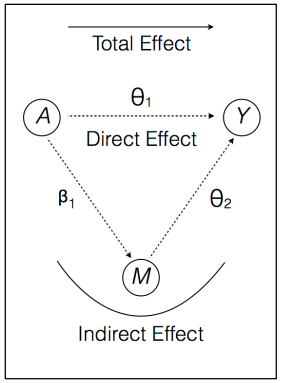

Requires adjustment for known confounders to prevent bias
(Note: this effectively restricts the use of mediation analyses to datasets in which data on such variables have been collected)



# Mediation analysis: Regression-based approach

- Requires fitting two regression models, one for mediator *M* and one for phenotypic outcome *Y:*
  - $E[M\,|a,c] = \beta_0 + \boldsymbol{\beta_1} a + \beta_2' c$
  - $E[Y\,|a,m,c\,] = \theta_0 + \boldsymbol{\theta_1} a + \boldsymbol{\theta_2} m + \theta_4' c$

Assesses the effect of *A* on *M*, while controlling for measured confounders *(C)*



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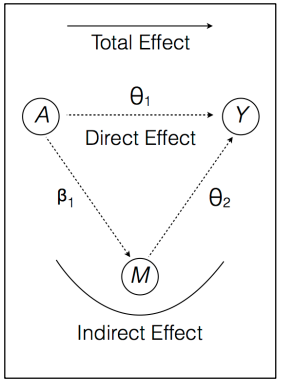
Assesses the effect of *A* on *Y*, while controlling for both *M* and *C*



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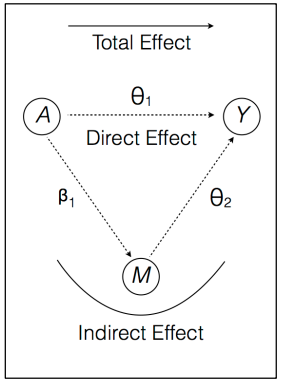
  - $E[Y\,|a,m,c\,] = \theta_0 + \boldsymbol{\theta_1} a + \boldsymbol{\theta_2} m + \theta_4' c$
- The parameter estimates from these models (**namely $\boldsymbol{\beta_1}$, $\boldsymbol{\theta_1}$, and $\boldsymbol{\theta_2}$**) are used to estimate the direct and indirect effects



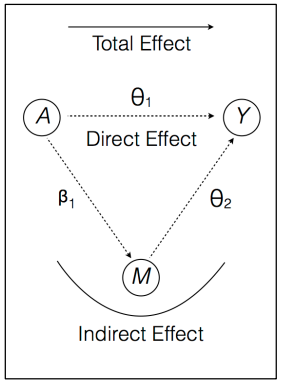
# Guidelines for generating robust statistical evidence of pleiotropy

**Discover** CP associations → **Dissect** CP associations → **Classify** them as examples of biological, mediated, or spurious pleiotropy



# Mediation analysis: Interpretation

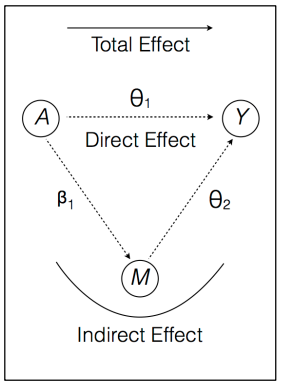
- **Biological pleiotropy:** SNP A is associated with mediator M, and the total effect of SNP A on phenotypic outcome Y is equal to its direct effect (i.e., the indirect effect is equal to 0)






# Mediation analysis: Interpretation

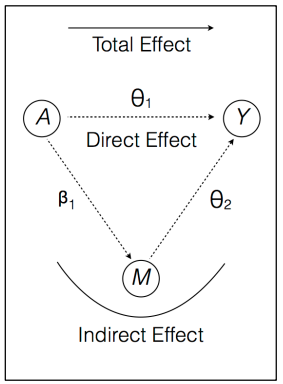
- **Mediated pleiotropy**
  - Complete mediation:
    - SNP *A* is associated with mediator *M* and the total effect of *A* on phenotypic outcome *Y* is equal to its indirect effect (i.e., the direct effect is equal to 0).
  - Incomplete mediation:
    - SNP *A* is associated with mediator *M* and *A* has both direct and indirect effects on phenotypic outcome *Y* (i.e., the total effect is equal to the sum of the direct and indirect effects)






# Mediation analysis: Interpretation

- **Spurious pleiotropy**
  - SNP A is not associated with mediator M after controlling for measured confounders



# mediation R package

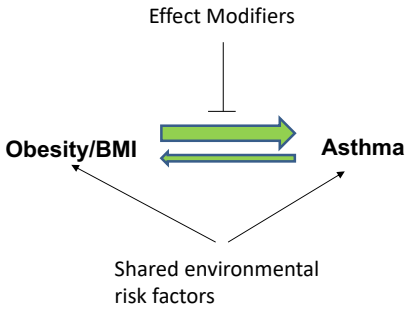
```
> med.fit<-glm(W1~rs1_2, data=combined, family=binomial("logit"))
> out.fit<-glm(W2~W1+rs1_2, data=combined, family=binomial("logit"))
> med.out<-mediate(med.fit,out.fit, treat="rs1_2", mediator="W1", boot=TRUE, boot.ci.type="bca", sims=1000)
> summary(med.out)

Causal Mediation Analysis

Nonparametric Bootstrap Confidence Intervals with the BCa Method
```

| | Estimate | 95% CI Lower | 95% CI Upper | p-value |
|---|---|---|---|---|
| ACME (control) | 0.02152 | 0.01823 | 0.03 | <2e-16 *** |
| ACME (treated) | 0.02199 | 0.01868 | 0.03 | <2e-16 *** |
| ADE (control) | 0.00723 | 0.00415 | 0.01 | <2e-16 *** |
| ADE (treated) | 0.00771 | 0.00443 | 0.01 | <2e-16 *** |
| Total Effect | 0.02922 | 0.02461 | 0.03 | <2e-16 *** |
| Prop. Mediated (control) | 0.73634 | 0.65429 | 0.84 | <2e-16 *** |
| Prop. Mediated (treated) | 0.75247 | 0.67272 | 0.85 | <2e-16 *** |
| ACME (average) | 0.02175 | 0.01847 | 0.03 | <2e-16 *** |
| ADE (average) | 0.00747 | 0.00426 | 0.01 | <2e-16 *** |
| Prop. Mediated (average) | 0.74441 | 0.66254 | 0.84 | <2e-16 *** |



## Empirical searches for pleiotropic loci for asthma and obesity

# Asthma-obesity comorbidity




Ford ES. The epidemiology of obesity and asthma. J Allergy Clin Immunol. 2005;115(5):897-909; quiz 10.
Stukus DR. Obesity and asthma: The chicken or the egg? J Allergy Clin Immunol. 2014.
Kim SH, Sutherland ER, Gelfand EW. Is there a link between obesity and asthma? Allergy Asthma Immunol Res. 2014;6(3):189-95.
Egan KB, Ettinger AS, DeWan AT, Holford TR, Holmen TL, Bracken MB. Longitudinal associations between asthma and general and abdominal weight status among Norwegian adolescents and young adults: the HUNT Study. Pediatric obesity. 2014.



Am J Hum Genet. 2009 Jul;85(1):87-96. doi: 10.1016/j.ajhg.2009.06.011. Epub 2009 Jul 2.

**PRKCA: a positional candidate gene for body mass index and asthma.**

Murphy A[1], Tantisira KG, Soto-Quirós ME, Avila L, Klanderman BJ, Lake S, Weiss ST, Celedón JC.

## Study design

- Two phases:
  - genome-wide linkage analysis of BMI
  - follow-up family-based candidate-gene association study of BMI and asthma
- Strategy for candidate-gene study:
  - Authors focused on a single gene (*PRKCA*) within the BMI linkage peak because:
    - animal models suggest role of PRKCA in obesity; and
    - published association studies of other genes within the linkage peak had found no association with BMI.

# Study population

- Costa Rica study
  - N = 415 asthmatic children + parents
- Childhood Asthma Management Program
  - N = 493 non-Hispanic White asthmatic children + parents

Note that ALL children in both study populations are asthmatic



# Phenotype definitions

- Body mass index (BMI)
  - calculated from objective measures of height and weight
- Asthma
  - physician-diagnosed asthma + one of the following:
    - 2 respiratory symptoms or asthma attacks in prior year
    - increased airway responsiveness or bronchodilator response



# Statistical methods

- Univariate family-based association tests (FBATs) were used to test *PRKCA* SNPs for association with BMI and asthma *separately*
  - Note: The FBAT statistic takes into account the phenotype of the **offspring only**
- Significance threshold used by study authors: $\alpha = 9.5 \times 10^{-5}$



# Results for BMI

**Table 3. Evidence for Association of *PRKCA* with BMI in Costa Rica and CAMP**

| Marker | Location (BP)[a] | Minor Allele | Allele Frequency CR | Allele Frequency CAMP | Number of Informative Families[b] (number of offspring with 0/1 recoded genotype) CR | Number of Informative Families[b] (number of offspring with 0/1 recoded genotype) CAMP | Effect Size[c] CR | Effect Size[c] CAMP | CR p Value[d,e] | CAMP Replication p Value[d,e] (two-sided) | Joint p Value[f] (CR, CAMP two-sided) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| rs228883 | 61874457 | T | 0.27 | 0.33 | 91 (67/24) | 110 (80/39) | 2.45 | 1.60 | +0.0011 | +0.0038 (+0.0076) | $5.6 \times 10^{-5}$** ($1.0 \times 10^{-4}$) |
| rs1005651 | 61868473 | C | 0.26 | 0.33 | 83 (60/23) | 113 (83/39) | 2.27 | 1.60 | +0.0019 | +0.0039 (+0.0077) | $9.5 \times 10^{-5}$** ($1.8 \times 10^{-4}$) |
| rs228875 | 61924337 | A | 0.29 | 0.35 | 101 (70/31) | 129 (92/46) | 1.71 | 1.22 | +0.0109 | +0.0182 (+0.0364) | 0.0019 (0.0035) |
| rs2244497 | 61931405 | C | 0.31 | 0.36 | 120 (86/34) | 136 (98/47) | 1.69 | 1.21 | +0.0160 | +0.0171 (+0.0341) | 0.0025 (0.0046) |

Two BMI-associated variants

# Results for asthma

**Table 4. Evidence for Association of *PRKCA* with Asthma in Costa Rica and CAMP**

| Marker | Location (BP)[a] | Minor Allele | Allele Frequency CR | Allele Frequency CAMP | Number of Informative Families[b] (number of offspring with 0/1 recoded genotype) CR | Number of Informative Families[b] (number of offspring with 0/1 recoded genotype) CAMP | Costa Rica p Value[c,d] | CAMP Replication p Value[c,d] (two-sided) | Joint p Value[e] (CR, CAMP two-sided) |
|---|---|---|---|---|---|---|---|---|---|
| rs732191 | 61779673 | G | 0.46 | 0.35 | 168 (117/51) | 141 113/43 | −0.0194 | −0.0214 (−0.0428) | 0.0036 (0.0067) |
| rs9895580 | 61789701 | C | 0.47 | 0.35 | 168 (117/51) | 141 114/43 | −0.0171 | −0.0160 (−0.0320) | 0.0025 (0.0047) |
| rs4411531 | 61793662 | A | 0.29 | 0.12 | 88 (70/18) | 25 (24/1) | −0.0058 | −0.0058 (−0.0117) | 0.0004 (0.0007) |
| rs8080771 | 61824330 | G | 0.46 | 0.35 | 164 (116/48) | 108 (90/29) | −0.0161 | −0.0070 (−0.0140) | 0.0011 (0.0021) |
| rs11652956 | 61839798 | G | 0.29 | 0.12 | 83 (65/18) | 23 (22/1) | −0.0101 | −0.0111 (−0.0222) | 0.0011 (0.0021) |
| rs7221968 | 61848731 | C | 0.27 | 0.11 | 79 (63/16) | 18 (17/1) | −0.0122 | −0.0216 (−0.0432) | 0.0024 (0.0045) |
| rs7405806 | 61862056 | A | 0.49 | 0.31 | 164 (109/55) | 90 (77/20) | −0.0309 | −0.0009 (−0.0018) | 0.0003 (0.0006) |
| rs11079657 | 61862528 | A | 0.38 | 0.23 | 129 (94/35) | 60 (56/8) | −0.0092 | −0.0002 (−0.0004) | $2.6 \times 10^{-5}$** ($5.0 \times 10^{-5}$**) |

One asthma-associated variant



# Conclusions

- **Authors' conclusion: *PRKCA* displays pleiotropy for asthma and BMI (pleiotropy at gene level)**
  - Two variants (rs228883 and rs1005651) displayed statistically significant associations with body mass index
  - A different variant (rs11079657) displayed a statistically significant association with asthma.



# Conclusions

- **Our conclusion: *PRKCA* is associated with asthma and with BMI among asthmatics (no true CP association!)**
  - There is insufficient evidence to declare a CP association at *PRKCA* because the test of association with BMI was not a marginal test
    - FBAT test for BMI only took into account the phenotype of the offspring – which were ALL asthmatic
  - Thus, it remains to be seen whether the association with BMI is also present among non-asthmatics subjects
  - Without that information, we would not be able to assess whether asthma is a **mediator** or a **moderator** of the relationship between *PRKCA* and BMI.



# A GWAS study: Salinas et al. (In Press)

**Discovery and mediation analysis of cross-phenotype associations with asthma and body mass index in 12q13.2**

Salinas YD, Wang Z, and DeWan AT

# Study design

- Two parts:
  - Genome-wide search for cross-phenotype associations with asthma and body mass index
  - Follow-up mediation analysis to dissect genome-wide significant CP associations



# Study population

- N = 305,945 White, British subjects from the UK Biobank (a population-based prospective cohort study of > 500,000 subjects, aged 40-69 years at baseline)

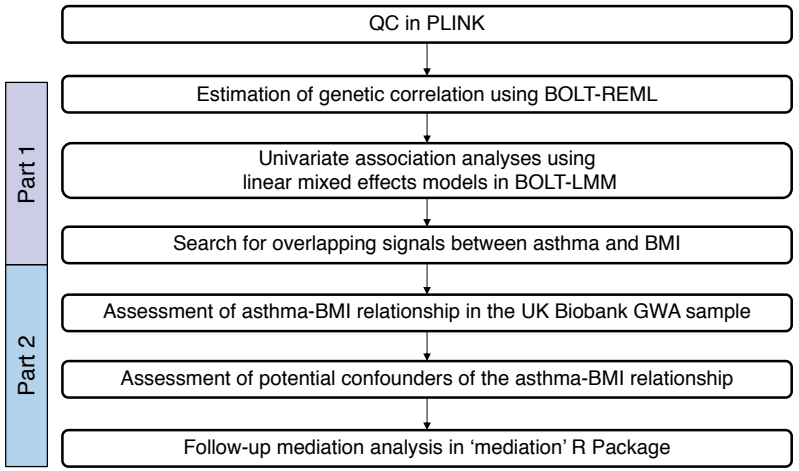


# Phenotype definitions

- BMI at baseline (kg/m$^2$):
  - calculated based on height and weight measurements collected by trained UK Biobank staff at the recruitment sites
- Asthma diagnosed prior to baseline (yes/no):
  - ascertained via the question “Has a doctor ever told you that you had asthma?”
  - Note: In mediation analyses, two subgroups were created based on age-at-diagnosis



# Statistical Methods



# Overlap in GWA signals

Association with BMI among the 1,457 SNPs with genome-wide significant p-values for asthma

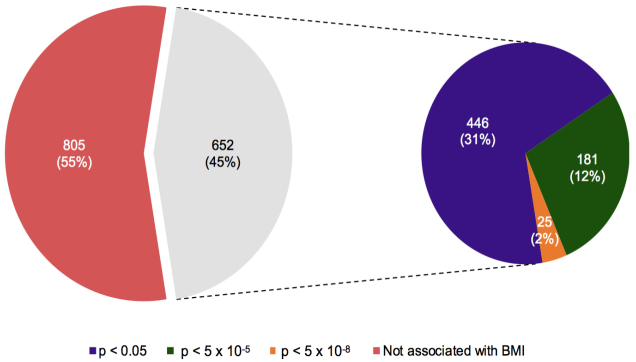
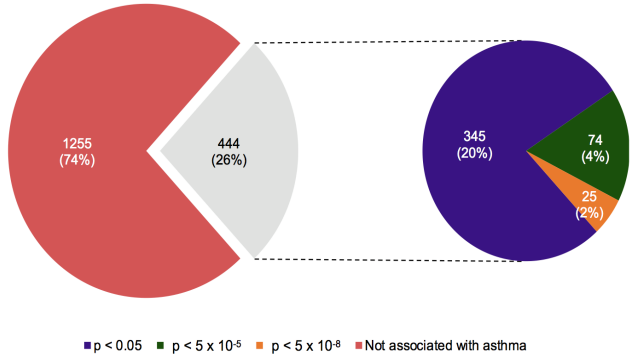



**Figure 1. Overlap in GWA signals between asthma and BMI.** Results for asthma are for the analysis of all asthmatic subjects (35,373 asthmatics vs. 270,572 non-asthmatics). Results for BMI are for the quantitative BMI analysis (n=305,945). Both analyses are sex- and age-adjusted. The threshold for genome-wide significance was alpha=5x10$^{-8}$.



# Overlap in GWA signals

Association with asthma among the 1,699 SNPs with genome-wide significant p-values for BMI

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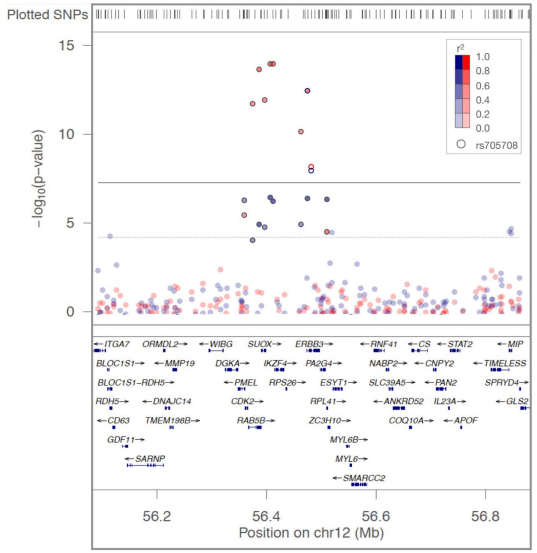
# Regional plot around rs705708 for BMI (blue) and asthma (red)








# Cross-phenotype associations in 12q13.2

Table 2. Cross-phenotype associations in 12q13.2 [a]

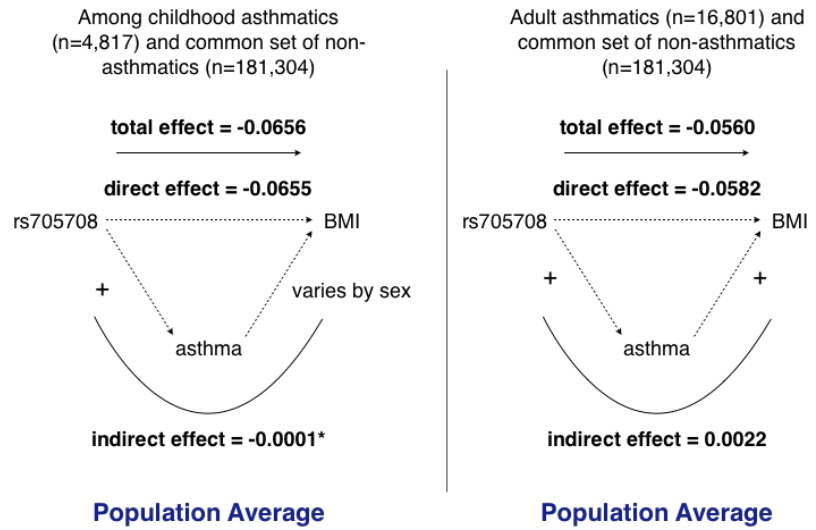
| | | | | | Asthma | | BMI[c] | |
|---|---|---|---|---|---|---|---|---|
| SNP | Gene | BP | Effect/reference allele | EAF | OR (95% CI) | P[c] | beta (95% CI) | P[d] |
| rs2069408 | *CDK2* | 56,364,321 | G/A | 0.3388 | 1.04 (1.02, 1.06) | $3.30\times10^{-6}$ | -0.06 (-0.08, -0.04) | $5.40\times10^{-7}$ |
| rs1873914 | *RAB5* | 56,379,427 | C/G | 0.4237 | 1.06 (1.04, 1.08) | $2.40\times10^{-12}$ | -0.05 (-0.07, -0.02) | $7.90\times10^{-5}$ |
| rs705702 [b] | *SUOX* | 56,390,636 | G/A | 0.3376 | 1.07 (1.05, 1.09) | $3.10\times10^{-14}$ | -0.05 (-0.08, -0.03) | $1.10\times10^{-5}$ |
| rs10876864 [b] | *SUOX* | 56,401,085 | G/A | 0.4279 | 1.06 (1.04, 1.08) | $1.50\times10^{-12}$ | -0.05 (-0.07, -0.03) | $1.60\times10^{-5}$ |
| rs1701704 | *IKZF4* | 56,412,487 | G/T | 0.3433 | 1.07 (1.05, 1.09) | $1.50\times10^{-14}$ | -0.06 (-0.09, -0.04) | $3.70\times10^{-7}$ |
| rs2456973 | *IKZF4* | 56,416,928 | C/A | 0.3432 | 1.07 (1.05, 1.09) | $1.50\times10^{-14}$ | -0.06 (-0.08, -0.04) | $6.00\times10^{-7}$ |
| rs11171739 [b] | *ERBB3* | 56,470,625 | C/T | 0.4337 | 1.06 (1.04, 1.07) | $8.80\times10^{-11}$ | -0.05 (-0.07, -0.03) | $1.10\times10^{-5}$ |
| rs2292239 | *ERBB3* | 56,482,180 | T/G | 0.3470 | 1.07 (1.05, 1.08) | $4.50\times10^{-13}$ | -0.06 (-0.08, -0.04) | $4.20\times10^{-7}$ |
| rs705708 | *ERBB3* | 56,488,913 | A/G | 0.4712 | 1.05 (1.03, 1.07) | $7.20\times10^{-9}$ | -0.06 (-0.09, -0.04) | $1.30\times10^{-8}$ |
| rs11171747 [b] | *ESYT1* | 56518408 | T/G | 0.6180 | 1.04 (1.02, 1.05) | $2.90\times10^{-5}$ | -0.06 (-0.08, -0.04) | $4.50\times10^{-7}$ |

Abbreviations: BP = base-pair ; BMI = body mass index; CI = confidence interval; EAF = effect allele frequency; OR = odds ratio; SNP = single-nucleotide polymorphism

a. Results shown for SNPs with p < 5x10$^{-8}$ for asthma and p < 0.05 for BMI.
b. For intergenic SNPs, the nearest gene is listed, with priority given to genes directly downstream of variant.
c. P-value from BOLT-LMM, derived using the standard "infinitesimal" mixed model.
d. P-value from BOLT-LMM, derived using the Gaussian mixture model.

# Decomposing the effect of rs705708 on BMI via mediation analysis






**Note**: Effect estimates shown are adjusted for common determinants of asthma and BMI: age, sex, breast-feeding status, exposure to maternal smoking, and smoking status at asthma diagnosis (adult analyses only). Unless otherwise noted by an asterisk(*), all paths are significant at the 0.05 level.



## Conclusions

- rs705708 has a positive direct effect on asthma
  - Stronger in magnitude for childhood asthma
- rs705708 has a negative direct effect on BMI
  - Consistent in magnitude and direction in analyses including childhood vs. adult asthmatics
- This suggests that locus 12q13.2, tagged by rs705708, has pleiotropic effects on asthma and BMI.



## Conclusions

- 12q13.2 is multigenic and our CP associations span genes *CDK2, RAB5*, *SUOX*, *IZK4*, *RPS26*, *ERBB3*, and *ESYT1*.
  - rs705708 is the top regional BMI signal and resides in *ERBB3*.
  - The top regional asthma signal, rs2456973, resides in *IZKF4*.
  - While rs705708 and rs2456973 could be in LD with the same causative variant in either *ERBB3* or *IKZF4* or another gene in 12q13.2, it is also possible that each variant could tag a distinct, trait-specific causative variant in different genes.
- Therefore, locus 12q13.2 displays pleiotropic effects on asthma and BMI, but this may not be an example of pleiotropy at the gene level (biological pleiotropy).