Association testing II

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What You’ll Learn Today

- **Modeling association with population structure:**
  - When and how to use linear mixed models to handle relatedness and population structure.
- **Discoveries at scale:**
  - How to control error rates across millions of tests (Bonferroni genome-wide threshold, FDR).
- **Combine evidence across studies:**
  - GWAS meta-analysis (combine p-values or effect sizes; fixed vs. random effects)
  - how to interpret heterogeneity.

Regression Approach

- Generalized linear models (GLMs)

$$g(E(Y \mid X)) = X\beta + C\alpha + \varepsilon,$$

- g is the link function.
- X : the coded genotype
- C : covariates (age, gender, PCs)
- Test for genetic effect

$$H_0 : \beta = 0$$

- Can use likelihood ratio tests or score tests to test H_0
- Main advantage: it allows incorporation of covariates

Mixed Effect models

- Handle population structure, family relatedness, and cryptic correlations
- Particularly useful when measurements are made on clusters or related individuals (family).
- Model phenotypes using a mixture of fixed effects (SNPs, covariates) and random effects (family structure).

Linear Mixed Models (LMM)

- Basic linear model: $Y = X\beta + C\alpha + \varepsilon$
- Mixed model extension: $Y = X\beta + C\alpha + u + \varepsilon$
  - u : genetic random effects (heritability component)
  - ε : residual, non-heritable variation
- Assumptions on random effects: $E(u) = 0, \quad \mathrm{Var}(u) = \sigma_g^2 K.$
- Genetic covariance matrix: $K = \frac{GG^T}{M}$
  - $G : N \times M$ genotype matrix; N : number of individuals; M : number of SNPs.
- In GWAS, both N and M are very large $\rightarrow$ requires efficient methods

Linear Mixed Models (LMM)

- K captures genetic relatedness, including population structure, family relationships, and hidden relatedness
- σ_g^2:genetic variance parameter we aim to estimate
- Estimation methods: REML (Restricted Maximum Likelihood) or AI-REML (Average Information REML)
- LMMs also allow us to estimate the individual random effects(μ)

Association testing in the LMM framework

Two-step fitting procedure for LMM:

- STEP 1: fit the null model.

$$Y = C\alpha + u + \varepsilon$$

- We can regress out the effects of covariates:

$$\tilde{Y} = u + \varepsilon$$

- $E(u) = 0, \mathrm{Var}(u) = \sigma_g^2 K, \mathrm{Var}(\varepsilon) = \sigma_e^2 I$
- Using REML/AI-REML we can estimate $\widehat{\sigma_g^2}$ and $\widehat{\sigma_e^2}$.
- We can also get BLUP (best linear unbiased predictors)

$$\widehat{u} = \widehat{\mathrm{K}}\widehat{\sigma_g^2} \times \Sigma^{-1}\left(\mathrm{I} - \mathrm{C}\left(C^T\Sigma^{-1}\mathrm{C}\right)^{-1}C^T\Sigma^{-1}\right)\mathrm{Y}, \Sigma = \widehat{\sigma_g^2}\,\mathrm{K} + \widehat{\sigma_e^2}\mathrm{I}.$$

Association testing in the LMM framework

- STEP 2: test for association with each SNP $H_0 : \beta = 0$.

$$\widehat{\boldsymbol{Y}}^{\star}_{\text{resid}} = \widetilde{\boldsymbol{Y}} - \widehat{u}.$$

- Test for association in a linear (non-mixed) regression model

$$\widehat{\boldsymbol{Y}}^{\star}_{\text{resid}} = X\beta + \varepsilon$$

- STEP 1 is computationally demanding (large matrix operations like inversions), but it only needs to be done once under the null
- STEP 2 is then repeated for millions of SNPs

Efficient LMM Methods for GWAS

- BOLT-LMM (Nature genetics, 2015)
  - Software: https://alkesgroup.broadinstitute.org/BOLT-LMM/BOLTLMMmanual.html
- fastGWA (Nature genetics, 2019) - assumes a sparse GRM
  - Software: https://yanglab.westlake.edu.cn/software/gcta/\#Overview
- SAIGE (Nature genetics, 2018)
  - Software: https://github.com/weizhouUMICH/SAIGE
- Regenie (Nature genetics, 2021)
  - Software: https://rgcgithub.github.io/regenie/

Heritability Estimation from GWAS

- Variance decomposition: $Var(Y) = Var(G) + Var(\varepsilon)$
- **Heritability:**$h^2 = \frac{Var(G)}{Var(Y)}$
- Earlier: used **trait covariance among relatives** (no genotypes)
- Now: estimate heritability directly from **GWAS data**
- Focus on **SNP heritability** → proportion of variance explained by common SNPs

Heritability Estimation from GWAS

$$Y = \mu + \sum_{m=1}^{M} a_m X_m + \epsilon$$

- If causal variants (QTLs) were known, genetic variance could be computed as:

$$\sigma_g^2 = \mathrm{Var}(G) = \sum_{m=1}^{M} \mathrm{Var}\left(a_m X_m\right) = \sum_{m=1}^{M} a_m^2 2 p_m \left(1 - p_m\right).$$

- Problem: **causal variants are unknown**
- Workaround: use significant SNPs as proxies for causal variants
- Pitfalls: may include too few (if the selection is too strict) or too many (If the selection is too lenient).

Heritability Estimation from GWAS

- $\text{cov}\left(y_j, y_k\right) = \text{cov}\left(\sum_{m=1}^{M} a_m X_{jm}, \sum_{m=1}^{M} a_m X_{km}\right) = \sigma_g^2 K_{\text{causal}}\left[\text{j}, \text{k}\right]$
- K_{causal} : relatedness matrix from causal SNPs
  - Cannot compute directly (causal SNPs unknown)
- Instead, approximate with: $K = GG^T / M$ based on all SNPs.
- Using REML/AI-REML to estimate $\widehat{\sigma_g^2}$
- $h^2 = \frac{\widehat{\sigma_g^2}}{\sigma_Y^2}$

Family-based Designs

- Family-based studies have a long tradition in genetics (association and linkage)
- Example: compare the genotypes of affected individuals with their unaffected siblings
- Using siblings as controls removes confounding from **population stratification**

Family-based designs

- Family-based designs are **robust to confounding** caused by population structure
- Rejecting H_0 (no association) means more than simple correlation:
    - The tested marker is likely **linked** to the true disease locus
- In contrast, population-based designs may give false positives due to structure

Question: why is this robustness lost in population-based studies?

Indirect association

- Genetic association studies typically test **markers**, not causal mutations
- A marker may be correlated with the true causal variant → this is **indirect association**
- **Linkage disequilibrium (LD)** (or correlation) between a marker and a causal locus (DSL) creates an apparent association with the phenotype
- Key idea: the marker is not causal, but **tags the causal variant** through LD

The trio design and Transmission Disequilibrium Test (TDT)

- Setup: **affected offspring** and their **biological parents**
- From parental genotypes, Mendel’s laws of segregation predict the expected offspring genotype distribution
- **TDT test:** compares observed offspring genotypes with expectations from parental genotypes
- Advantage: immune to bias from **population stratification**
- If there is a genotype–phenotype association:
    - Expect **over- or under-transmission** of certain alleles from parents to offspring

The trio design and Transmission Disequilibrium Test (TDT)

- Each parent has a transmitted allele and an untransmitted allele.
- w= #homozygous AA parents and z=#homozygous aa parents
- w and z are not informative
- x=#heterozygous parents Aa that transmit A allele.
- $y = \#$ heterozygous parents Aa that transmit a allele.
- If no association, we have $\mathrm{E}[\mathrm{x}] = \mathrm{E}[\mathrm{y}]$.
- Hence, conditioning on $\mathrm{x} + \mathrm{y}$, the count x is $\mathrm{Bin}(\mathrm{x} + \mathrm{y}, 0.5)$.

The trio design and Transmission Disequilibrium Test (TDT)

Case-control design is more powerful but less robust than TDT

- Power of TDT is expected to be lower than for case - control design with the same number of cases because, e.g., homozygous parents do not contribute.
- Also, the trio design is more expensive: three genotypes compared to two in a case-control design.
- It can be difficult to obtain parental genotypes for late-onset diseases, e.g. Alzheimer's disease.
- Other family-based designs: discordant sibships, trios with multiple affected siblings, multi-generational pedigrees.

Family-based association test (FBAT)

- Originally, TDT limited to binary data and trio design
- Many extensions to handle other genetic models, missing data etc.
- FBAT is a unified family-based approach, an extension of TDT to:
- Missing parental genotypes, continuous phenotypes, time-to-onset, different genetic models.
- www.biostat.harvard.edu/~fbat/fbat.htm

FBAT

- FBAT score statistic:

$$U = \sum_{\substack{\text{family } i, \\ \text{offspring } j}} Y_{ij} \left(X_{ij} - E \left(X_{ij} \mid P_i \right) \right)$$

$$Z = \frac{U}{\sqrt{\text{Var}(U)}} \sim N(0, 1)$$

- The centering of the offspring genotype by its expected value conditional on parental genotypes helps maintain robustness to population stratification.
- If parental genotypes are missing one can use genotypes of other relatives for the conditioning above.

Exercises

1. What is the alternative hypothesis for a TDT test (or any FBAT test), and how does that compare with the alternative of a test of association from a case-control or cohort study? Why is this important from a practical perspective?
2. The TDT is a conditional test. What are the random variables used in computing the null distribution of the test, and what variables are being conditioned on?

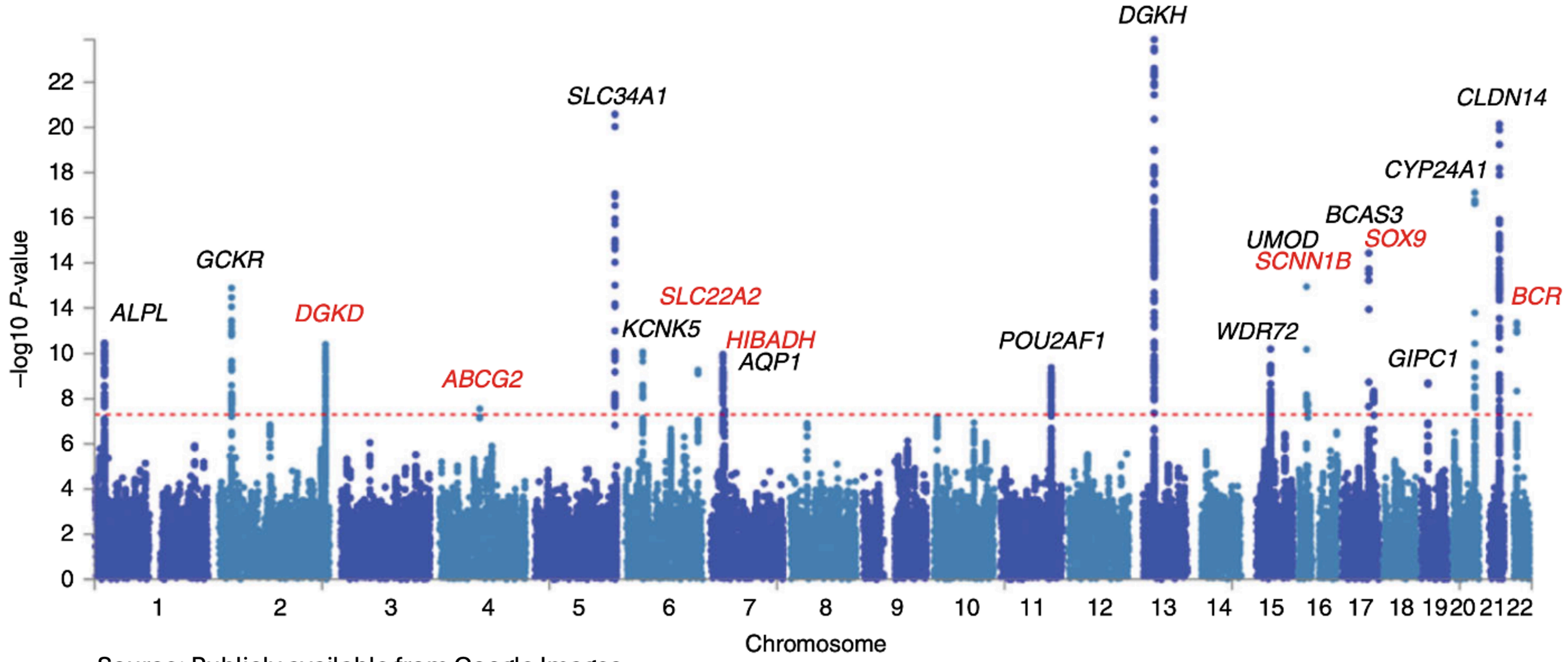
Complications when testing association with millions of markers in large GWAS studies

- So far, we have discussed one test/one genetic marker at a time.
- **Multiple testing in GWAS** - millions of tests at once
- **Meta-analysis of multiple datasets** - combine data/results from multiple studies

Multiple Testing

GWAS of Kidney Stone Disease

- In a typical GWAS we perform millions of tests. How do we account for that? What Significance level should we use?



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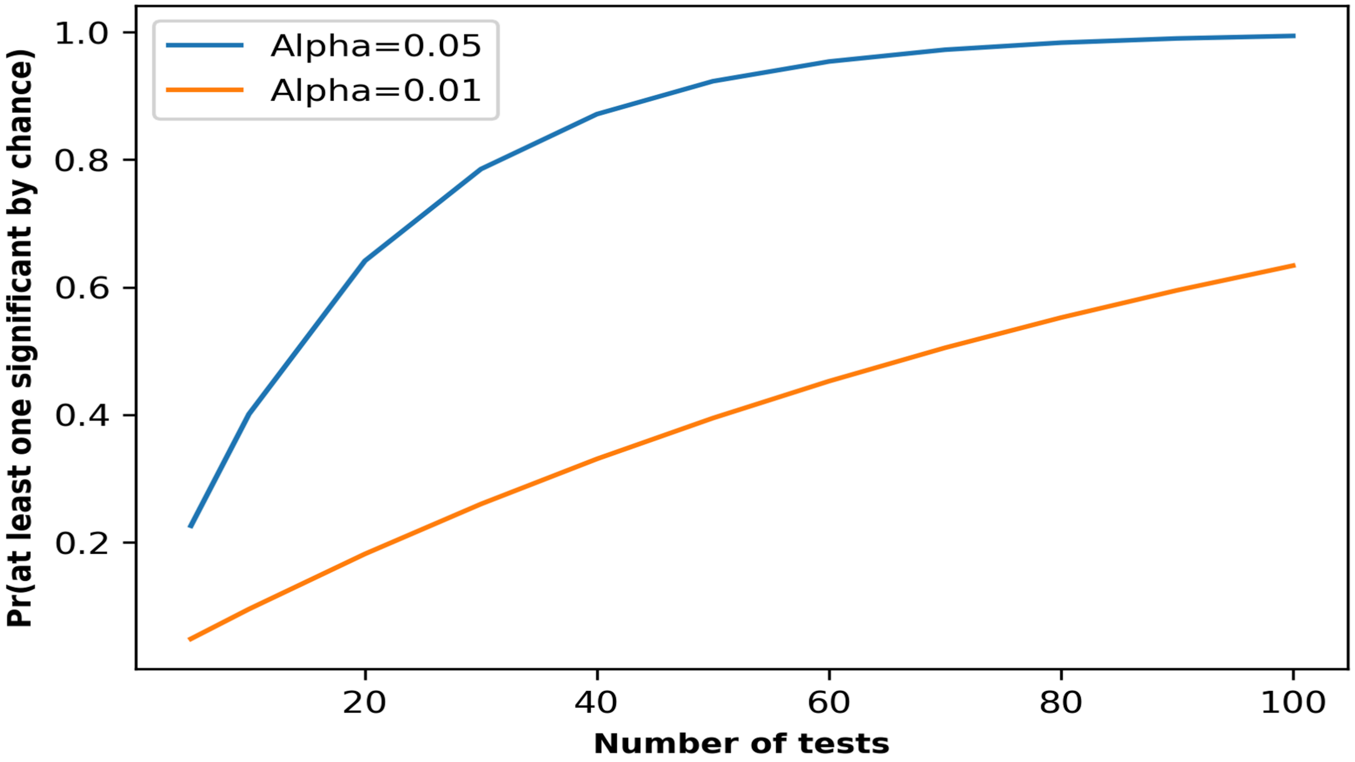
Multiple Testing

- Multiple testing issues arise when many markers are tested as in GWAS.
- Major statistical problem as it leads to loss of power, and increased false positive rates if not accounted for.
- Idea: Test each marker separately and adjust the significance level of each test.

Why do we need to adjust for multiple testing?



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Methods based on P-value Adjustment

- Test each SNP separately and adjust the significance level of each test in order to preserve the overall error rate.
- Two different error rates:

   i. Family-wise error rate (FWER)

   ii. False discovery rate (FDR)

- If M is the total number of tested SNPs, then for each $m = 1, \ldots, M$, we define the null hypothesis:
  $H_0(m)$: no association between the m-th SNP and the phenotype.

Bonferroni method

- **FWER (family-wise error rate)** or experiment-wise error rate:

$$FWER = P\,(\text{ reject at least one } H_0(m) \mid H_0(m) \text{ is true for all } m)$$

- **Bonferroni**: fix $FWER = \alpha$ and set individual significance levels at $\frac{\alpha}{M}$.
- This ensures that the FWER is less than the desired level α (e.g. 0.05).
- If markers are not independent (due to linkage disequilibrium) the Bonferroni adjustment is conservative.
- E.g. extreme case: only one independent marker among M and the true FWER is $\frac{\alpha}{M}$.

Bonferroni threshold for GWAS

- The effective number of independent tests in a dense GWAS study is 1 million, so the Bonferroni adjustment corresponds to a significance level of 5×10^{-8}.
- This corresponds to a finding by chance 1 in 20 GWAS studies.
- Large sample sizes are needed for such a stringent threshold.

False Discovery Rate (FDR)

- Rather than control the Type-1 error, FDR limits the expected number of null-hypotheses that are rejected incorrectly.
- FDR=5% means that on average 5% of the SNPs we rejected are in fact false positives.
- FDR is less conservative than FWER $\longrightarrow$ higher power.
- FDR is less accepted in the GWAS setting, but useful for GWAS where results are followed up.

False Discovery Rate (FDR)

	Declared non-significant	Declared significant	
True null hypotheses	U	V	M_0
False null hypotheses	T	S	$M - M_0$
	$M - R$	R	M

- Assume there are M independent markers.
- The false discovery rate is $\mathrm{E}\left(\frac{V}{R}\right)$ (expectation of false discovery proportion).
- Goal: keep the FDR below a specific threshold, e.g. 0.05 or 0.10.

Benjamini–Hochberg (BH) procedure

- Benjamini and Hochberg (1995) procedure can be used to control the FDR.
- Rank the M p-values from smallest to largest:

$$p_{(1)}, \dots, p_{(M)}$$

- For a specified FDR level (e.g. 0.05), compare

$$p_{(i)} \leq \frac{i}{m} FDR$$

- Find largest i for which this inequality holds, and then reject tests that correspond to $1, \dots, i$.
- For dependent tests, extensions are available (e.g. Benjamini-Yekutieli 2001).

Example

i	1	2	3	4	5	6	7	8	9	10
$p_{(i)}$	0.002	0.005	0.006	0.008	0.009	0.009	0.017	0.025	0.105	0.54
$10\frac{p_{(i)}}{i}$	0.02	0.022	0.02	0.02	0.017	0.015	0.025	0.031	0.11	0.54

FDR = 5% reject hypotheses 1-8 –> more than Bonferroni that rejects only two.

Meta-analysis

Meta-analysis

- Meta-analysis is essential in GWAS.
- GWAS studies are extremely large $\rightarrow$ require combining many smaller cohorts.
    - Example: the largest GWAS on height (2022) analyzed 5.4 million individuals across diverse ancestries and identified 12,111 independent SNPs.
- Purpose: to combine information from multiple independent studies.

Meta-analysis

- Combines information from multiple independent studies.
- Increases statistical power by boosting sample size.
- Helps assess consistency of findings across datasets.
- Methods:
    - Combine p-values or Z-scores (e.g., Fisher's method).
    - Combine effect sizes.

Combine p-values or Z-scores

- Combine p -values p_{mk} for variant m and stage k using **Fisher's method**:

$$X^2_{2K} = -2 \sum_{k=1}^{K} \ln\left(p_{mk}\right) \sim \chi^2_{2K}.$$

- This approach does not take into account sample size differences between studies.
- We would like to give more weight to the larger studies, we can combine Z-scores:

$$Z_m = \left(\frac{1}{\sqrt{\sum_k n_k}} \sum_k \sqrt{n_k} Z_{mk} \right) \sim N(0,1).$$

Fixed-effects meta-analysis

- Constant effect size across studies.
- Observed effect size in each study varies due to random sampling error.
- **Combined effect estimates the fixed effect size (the same underlying parameter across studies)**.
- Might be realistic if, for example, the studies have all been conducted in the same population, consistently measured phenotypes, same inclusion criteria etc.
- In practice, that may not be true.

Random-effects meta analysis

Random-effects meta-analysis.

- True effect size may vary from study to study (so there is a study-specific true effect).
- Observed effect size in each study varies due to both random error and differences in true effect sizes across studies.
- **Combined effect estimates the mean of the distribution of true effects**.

Fixed-effect model

- We assume we have K studies.
- Let $\hat{\beta}_1, \ldots, \hat{\beta}_K$ be the effect-size estimates (e.g. $\log(OR)$ or regression coefficients).
- Let $\hat{\sigma}_i = SE\left(\hat{\beta}_i\right)^2$ (within study variance) and $w_i = \hat{\sigma}_i{}^{-1}$.
- The overall inverse-variance-weighted effect-size is:

$$\hat{\beta} = \frac{\sum_{i=1}^{K} w_i \widehat{\beta}_i}{\sum_{i=1}^{K} w_i}.$$

Fixed-effect model

- $\hat{\beta}$ follows a normal distribution, with $\mathrm{SE}(\hat{\beta}) = 1/\sqrt{\sum_{i=1}^{K} w_i}$.

$$\hat{\beta} \sim \mathrm{N}\left(\beta, \left(\sum_{i=1}^{K} w_i\right)^{-1}\right)$$

- Larger studies are given higher weight compared with smaller studies. So if one very large study and other small studies, the large study will dominate.

Random-effect model

- In the RE model, we assume that the true effects $\beta_i = \beta + \xi_i, \xi_i \sim N\left(0, \tau^2\right)$.
- The overall effect-size is estimated as:

$$\hat{\beta} = \frac{\sum_{i=1}^{K} w_i^* \hat{\beta}_i}{\sum_{i=1}^{K} w_i^*}$$

- $w_i^* = \left(\hat{\sigma}_i + \hat{\tau}^2\right)^{-1}$.
- The weight here depends both on the within study variance and also on the between-study heterogeneity in true effects.
- $$\hat{\beta} \sim \mathrm{N}\left(\beta, \left(\sum_{i=1}^{K} w_i^*\right)^{-1}\right).$$

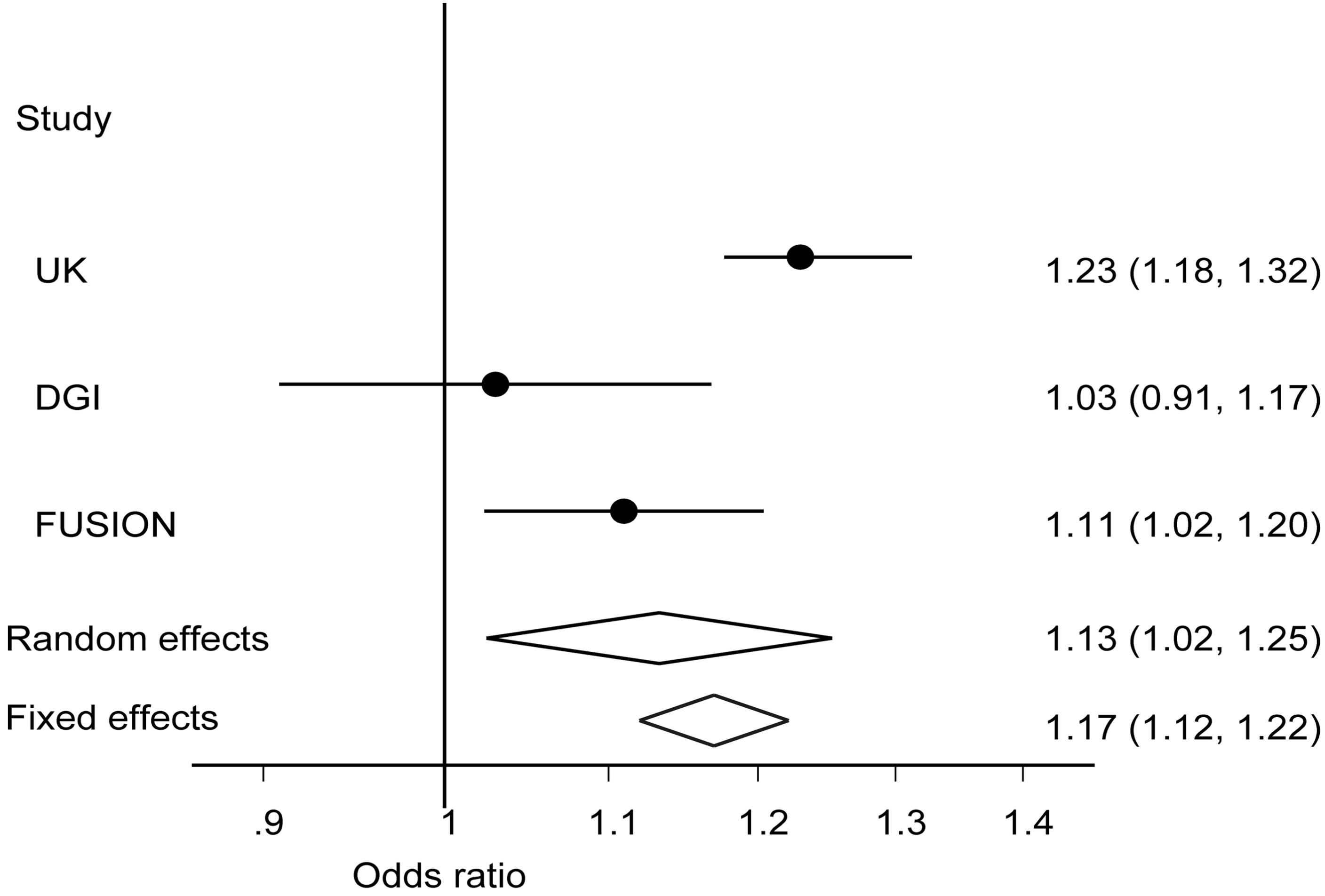
Random-effect model

- $w_i^* = \left(\hat{\sigma}_i + \hat{\tau}^2\right)^{-1}$ (in fixed effect $w_i = \hat{\sigma}_i{}^{-1}$)
- Smaller studies are given relatively more weight than in the fixed effect model
- The calculated standard error is smaller from a fixed effects meta-analysis than that from a random-effects meta-analysis.
- How to estimate the between-study variance $\hat{\tau}^2$?
- Many different methods, e.g. DerSimonian and Laird (DL)

Fixed vs. Random Effect model

- The decision needs to be done before the analysis based on knowledge about the individual studies
- **Fixed effect meta-analysis is typically used in genetics**, without regard to heterogeneity.
- Random effect meta-analysis is typically too conservative (less powerful).

Meta-analysis of three GWAS studies assessing the link between the FTO rs8050136 variant and type 2 diabetes



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What's Next

- Population Stratification
- Genotype Imputation
- Quality Control

What questions do you have about anything from today?