

Introduction	Motivation	$p$ -Values and BFs	Multiple Testing	Methodology for PG	PG Example	Keyword Prior	Conclusions	References
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## 2014 SISG MODULE 4: Bayesian Statistics for Genetics

### Lecture 6: Bayesian and Frequentist Multiple Testing

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## Outline

Introduction and Motivating Examples

Motivation: Homocysteine Example

Connection Between  $p$ -Values and Bayes Factors

Review of  $p$ -Values

A Bayesian Perspective

Multiple Testing

Review of Non-Bayesian Procedures

An Example

Bayesian Approach

Methodological Framework

The Model

Homocysteine Example

Substantive Prior Information

Conclusions

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## Introduction

- With the advent of high-throughput technologies in genomics there is now the possibility of carrying out millions of tests, and so the implications of such **multiple testing** must be carefully considered.
- In this lecture we will review the rationale for  $p$ -values.
- We then explore the connection between  **$p$ -values and Bayes factors**.
- **Multiple testing** will be reviewed and a Bayesian perspective presented.
- An example in the context of a pharmacogenomics GWAS will be presented.
- The use of substantive prior information will also be demonstrated.

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## Motivating Data Description

- The Vitamin Intervention for Stroke Prevention (**VISP**) trial is an NIH-funded, multi-center, double-blind, randomized, controlled clinical trial.
- More detail in Wakefield *et al.* (2014).
- The aim is to determine whether a daily intake of high dose folic acid and vitamins B6 and B12 was associated with cardiovascular endpoints.
- We examine data on  $n = 1670$  individuals, with 837 randomized to the high dose and 833 to the low dose.
- The outcome is the intermediary variable **homocysteine** level: high levels in blood are associated with cardiovascular disease.
- In the VISP trial, levels were measured longitudinally but for simplicity we take as outcome the difference between the baseline and the first post-baseline measurements:  $Y$  will represent this difference.
- The change was  $-0.37 \mu\text{mol/L}$  in the low dose group versus  $-2.36 \mu\text{mol/L}$  in the high dose group, i.e., a **difference** of  $-1.99 \mu\text{mol/L}$  ( $p < 2 \times 10^{-16}$ ).

## Marker-Specific Treatment Effects

- An increasingly important venture is examining treatment effects by marker (e.g. **SNP**): a particular type of gene-environment interaction.
- Historically, candidate gene studies were popular, but now genome-wide scans are also being performed, see Daly (2010) for a review.
- **Pharmacogenomics-related traits**: Drug response, susceptibility to adverse drug reactions,...
- **Key Statistical Point**: The estimated interactions are based on subgroups of varying sizes, so that the **power** varies substantially across tests.
- In the **VISP** trial, there are  $J = 803,122$  SNPs and suppose we define subgroups as having **at least one copy of the minor allele**.
- The number in this subgroup ranges between **21** and **1564** across SNPs.

## Beyond single-marker analyses: mining whole genome scans for insights into treatment responses in severe sepsis

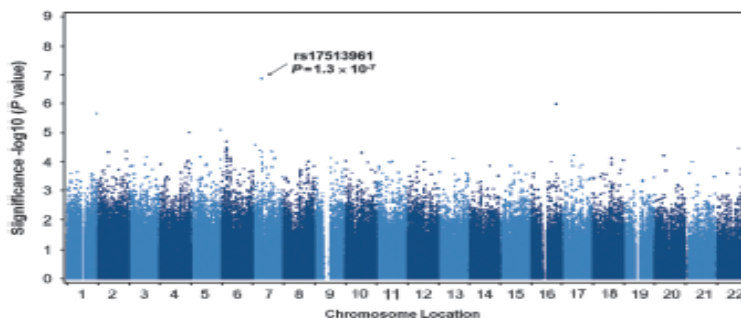
M Man<sup>1</sup>, SL Close<sup>2</sup>, AD Shaw<sup>3</sup>,  
GR Bernard<sup>4</sup>, IS Douglas<sup>5</sup>,  
RJ Kaner<sup>6</sup>, D Payen<sup>7</sup>, J-L Vincent<sup>8</sup>,  
S Fosceco<sup>1</sup>, JM Jones<sup>1</sup>,  
AG Leishman<sup>1</sup>, L O'Brien<sup>1</sup>,  
MD Williams<sup>9</sup> and JGN Garcia<sup>10</sup>

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Management of severe sepsis, an acute illness with high morbidity and mortality, suffers from the lack of effective biomarkers and largely empirical predictions of disease progression and therapeutic responses. We conducted a genome-wide association study using a large randomized clinical trial cohort to discover genetic biomarkers of response to therapy and prognosis utilizing novel approaches, including combination markers, to overcome limitations of single-marker analyses. Sepsis prognostic models were dominated by clinical variables with genetic markers less informative. In contrast, evidence for gene–gene interactions were identified for sepsis treatment responses with genetic biomarkers dominating models for predicting therapeutic responses, yielding candidates for replication in other cohorts. *The Pharmacogenomics Journal* advance online publication, 7 February 2012; doi:10.1038/tj.2012.1

**Keywords:** drotrecogin alfa (activated); epistasis; genetic markers; genome-wide association study; polymorphism; severe sepsis

- Aim: To identify marker-defined populations with improved response to DAA (for treatment of severe sepsis).



**Figure 1** Representative Manhattan plot from GWAS in the entire cohort for genotype BB vs not BB. All possible combinations of genotypes representing a dominant, heterozygous and recessive inheritance were evaluated. Three pairs of comparisons AA vs not AA, AB (heterozygous) vs not AB and BB vs not BB were completed. This figure represents the plot of chromosome position and *P*-values for homozygous genotype (BB) vs heterozygous (AB) or homozygous for the other allele (AA), or BB vs not BB. (Manhattan plots of GWAS results for genotype AA vs not AA and AB vs not AB are shown in Supplementary Figure 1). GWAS = genome-wide association study.

## The Statistical Set-Up

- We begin with a very simple situation in which we have a single parameter of interest  $\theta$ .
- Assume the null of interest is

$$H_0 : \theta = 0$$

with  $\theta$ , for example, a treatment difference, or a log odds ratio, or a log hazard ratio.

- We assume an analysis yields a statistic  $T$  for which large values indicate departures from the null.
- For example, the squared **Wald statistic**,  $T = \hat{\theta}^2 / V$ , with  $V$  the asymptotic variance of the MLE<sup>1</sup>.
- An alternative is the **likelihood ratio statistic**.

<sup>1</sup>  $T = Z^2$  where  $Z$  is the **Z-score**

## Types of Testing

- The observed  $p$ -value is given by:

$$p = \Pr(T > t_{\text{obs}} | H_0)$$

where  $t_{\text{obs}}$  is a number that is evaluated for the data at hand.

- To report  $p$  only, gives a **pure significance test**.
- A small  $p$ -value can arise because:
  - $H_0$  is true but we were “unlucky”.
  - $H_0$  is not true.
- to decide which explanation is responsible depends crucially on the **prior** belief on whether  $H_0$  is true or not.

**Key question:** How small is small?

## Types of Testing

- A **test of significance** sets a cut-off value (e.g.  $\alpha = 0.05$ ) and rejects  $H_0$  if  $p < \alpha$ .

**Again:** How to pick  $\alpha$ ?

- A type I error is to reject  $H_0$  when it is true, and a test of significance controls the type I error (whereas a pure significance test does not).
- A type II error occurs when  $H_1$  is true but  $H_0$  is not rejected.
- A **hypothesis test** goes one step further and specifies an alternative hypothesis.
- A decision is then taken as to which of  $H_0$  and  $H_1$  is chosen.
- The celebrated **Neyman-Pearson lemma** shows that for fixed  $\alpha$ -level the likelihood ratio statistic maximizes the power.
- Wouldn't it be more reasonable to **balance** type I and type II errors?



## The Dangers of Fixed Significance Levels

- **Example:** Sample,  $Y_1, \dots, Y_n$  of size  $n$  from  $\text{normal}(\theta, 1)$ ,

$$H_0 : \theta = 0, \quad H_1 : \theta = 1.$$

Obvious that we should reject  $H_0$  for  $\bar{Y} > k$ , a constant.

- The table below illustrates the problems of choosing a fixed  $\alpha$ , regardless of sample size — **imbalance** in  $\alpha$  and  $\beta$  as a function of  $n$ .

$n$	$\alpha$	$\beta$	$k$
1	0.01	0.91	6.21
25	0.01	0.0038	0.42
100	0.01	$8 \times 10^{-15}$	$2.5 \times 10^{-12}$

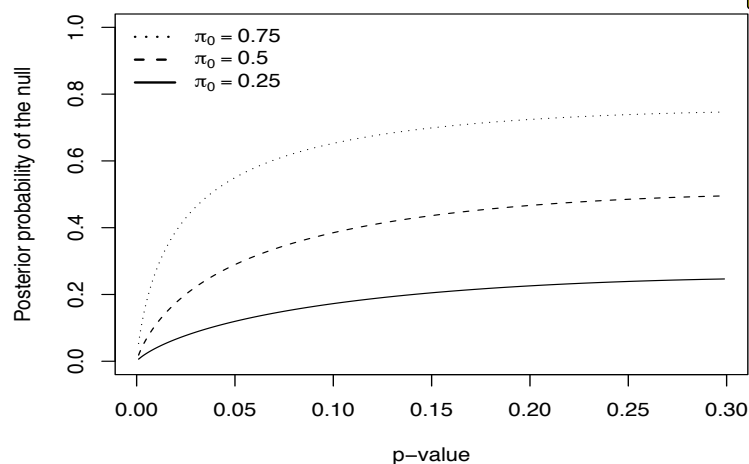
- **Also:** Statistical versus practical significance.
- For both  $p$ -values and  $\alpha$  levels we need thresholds that **decrease** as a function of the sample size  $n$ . Pearson (1953, p. 68), "...the quite legitimate device of reducing  $\alpha$  as  $n$  increases".

## Calibrating $p$ -values

- With  $\pi_0 = \Pr(H_0)$ , Sellke, Bayarri and Berger (2001) show that:

$$\Pr(H_0 | \text{data}) \geq \left\{ 1 - \frac{1}{2.72} \frac{1}{p \log p} \times \frac{1 - \pi_0}{\pi_0} \right\}^{-1} \quad (1)$$

- A small  $p$ -value doesn't translate to a small probability that the null is not true.




## Why does anyone use $p$ -values?

- Historically, it was usual to carry out well-powered (single) experiments, and the prior on the alternative was not small.
- With respect to (1) and with  $\pi_0 = 0.5$ :
  - $p\text{-value} = 0.05$  gives  $\Pr(H_0 | \text{data}) > 0.29$ .
  - $p\text{-value} = 0.01$  gives  $\Pr(H_0 | \text{data}) > 0.11$ .
- Scientists well-calibrated in their own discipline?
- Perhaps, but if you're going to be subjective, why not be formal about it?
- **Aside:** Reason for lack of replication in observational epidemiology? Along with confounding, data dredging, measurement error,...

## Calibrating $\alpha$ -Levels

- We want  $\Pr(H_0 | \text{data})$ , where “data” corresponds to the event  $T > t_{\text{fix}}$ , but to obtain this we must specify alternatives – consider a simple alternative, say  $H_1 : \theta = \theta_1$ .
- Then,

$$\begin{aligned}
 \text{Posterior Odds of } H_0 &= \frac{\Pr(T > t_{\text{fix}} | H_0)}{\Pr(T > t_{\text{fix}} | H_1)} \times \frac{\Pr(H_0)}{\Pr(H_1)} \\
 &= \frac{\alpha}{1 - \beta} \times \text{Prior Odds of } H_0
 \end{aligned}$$

- For **ranking** associations (which does not involve the prior odds if constant across tests): must consider the **power**,  $\Pr(\text{data} | H_1)$ . 
- For **calibration**: must consider the **prior odds** of  $H_0$ .

## A Sanity Check via a Simple Example

- The model:

$$Y_i|\theta \sim_{iid} \text{normal}(\theta, \sigma^2), \quad \sigma^2 \text{ known},$$

$$i = 1, \dots, n.$$

- The distribution of the **MLE** is:

$$\hat{\theta} = \bar{Y} \sim \text{normal}(\theta, V)$$

$$\text{with } V = \sigma^2/n,$$

$$T = \frac{n\bar{Y}^2}{\sigma^2}.$$

- Null and alternative hypotheses are

$$H_0 : \theta = 0, \quad H_1 : \theta \neq 0,$$

## A Sanity Check via a Simple Example

- Under  $H_1$  assume the prior  $\theta \sim \text{normal}(0, W)$ .
- Recall from Lectures 3 and 4, that the evidence in the data for a pair of hypotheses is summarized in the **Bayes factor**.
- The Bayes factor is

$$\text{BF} = \frac{p(\mathbf{y}|H_0)}{p(\mathbf{y}|H_1)} = \frac{\prod_{i=1}^n \text{dnorm}(y_i|0, \sigma^2)}{\int_{\theta} \prod_{i=1}^n \text{dnorm}(y_i|\theta, \sigma^2) \times \text{dnorm}(\theta|0, W) d\theta}$$

where **dnorm** is shorthand for the density of a normal random variable.

- We take  $W = \sigma^2$ , which corresponds to the “unit information prior” of Kass and Wasserman (1995) (this choice not so important).
- With a prior odds, PO, and ratio of costs  $R$  this gives the decision rule to reject  $H_0$ :

$$\text{BF} \times \text{PO} = \sqrt{1+n} \times \exp\left(-\frac{T}{2} \frac{n}{1+n}\right) \times \text{PO} < R$$


- Notice how this depends on **T** and **n**.



## A Bayesian Test Statistics Threshold

- Rearrangement gives a threshold for rejection of:

$$T > \frac{2(1+n)}{n} \log \left( \frac{PO}{R} \sqrt{1+n} \right) \quad \text{🗨️}$$


- For relatively large prior odds on the null **PO**: require **T** to be larger (more evidence).
- For relatively large cost of Type II errors **R**  that we are averse to type II error, i.e. missing signals): require **T** to be smaller (less evidence).
- Not such a simply summarization for **n** but, beyond a certain point, as **n** gets larger, we require larger **T** (more evidence).
- The above should be contrasted with the usual frequentist approach of


$$T > \text{const}$$

with the constant usually chosen to control the type I error.

## A Bayesian Test Statistic Threshold

- The table below evaluates the probability of rejection given  $H_0$ . We assume  $R = 1$ .
- For  $\pi_0 = 0.5$  and  $n = 20, 50, 100$  the thresholds give  $\approx 0.05$  — the situation in which this infamous threshold was first derived?



	$\pi_0 = 0.25$	$\pi_0 = 0.50$	$\pi_0 = 0.95$
$n = 10$	0.64	0.10	0.0025
$n = 20$	0.35	0.074	0.0022
$n = 50$	0.18	0.04 	0.0016
$n = 100$	0.12	0.031	0.0011
$n = 1000$	0.030	0.0085	0.00034

## Calibration with $p$ -values

**Interesting question:** When do Bayes and frequentist  $p$ -value inference coincide?

- Consider an approximate Bayes approach. We have parameter of interest  $\theta$  with
  - Data:** MLE  $\hat{\theta}$  and standard error  $\sqrt{V}$  to give likelihood  $\hat{\theta}|\theta \sim \text{normal}(\theta, V)$ ,
  - Prior:**  $\theta \sim \text{normal}(0, W)$ .
- The null and alternative hypotheses of interest are

$$H_0 : \theta = 0 \quad H_1 : \theta \neq 0.$$

- This leads to the approximate Bayes factor (ABF)

$$\begin{aligned} \text{ABF} &= \frac{1}{\sqrt{1-r}} \exp\left(-\frac{Z^2}{2}r\right) \\ &= \sqrt{\frac{V+W}{V}} \exp\left(-\frac{Z^2}{2} \frac{W}{V+W}\right) \end{aligned}$$

where  $r = W/(V + W)$  and  $Z = \hat{\theta}/\sqrt{V}$ .

## Calibration with $p$ -values

- $\text{ABF} = \sqrt{\frac{V+W}{V}} \exp\left(-\frac{Z^2}{2} \frac{W}{V+W}\right)$ , which depends on  $n$ , because  $V$  does.
- Recall we reject if  $\text{ABF} \times \text{PO} > \text{threshold } R$ .
- We are trying to find a Bayes factor that does not depend on  $n$ , to correspond with a  $p$ -value rule.
- We can reverse engineer a version of ABF that does not depend on  $n$  by taking the prior variance  $W = K \times V$ , where  $K$  is a constant.
- Then we have approximate Bayes factor

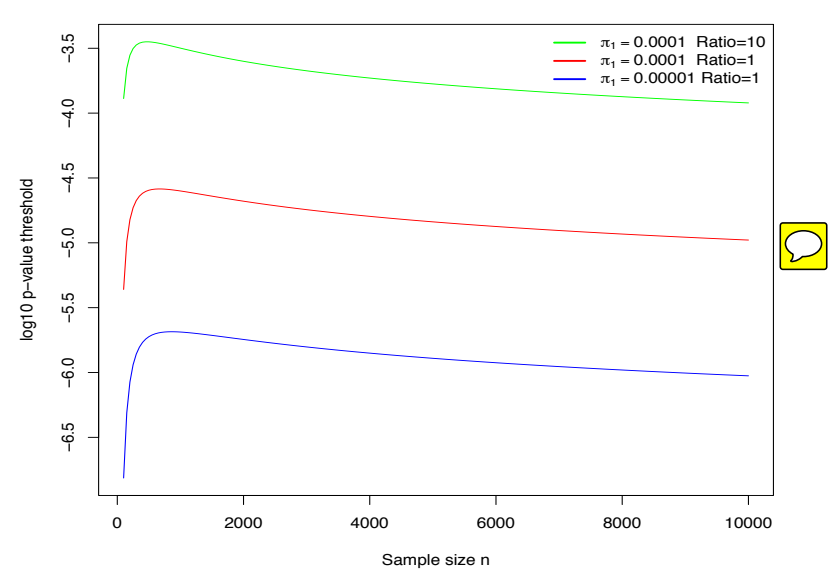
$$\text{ABF}^p = \sqrt{1+K} \exp\left(-\frac{Z^2}{2} \frac{K}{1+K}\right).$$

- Important point:** No dependence on  $n$ , i.e. it depends on  $Z$  only, and therefore on the  $p$ -value only.
- If we use the above prior and Bayes factor in multiple tests, then the **rankings** of  $p$ -values and  $\text{ABF}^p$  will be **identical**. The problem is that this prior makes no sense.

## Calibration with $p$ -values

- The ABF with  $W$  not depending on  $n$  is **consistent** (you get the right answer with a lot of data), whereas the “ $p$ -value” Bayes factor is not.
- The original ABF can be inverted to give a rule for  $Z^2$  that depends on  $PO$ ,  $R$  and  $n$  (as with the simple example presented previously).
- For more details, see Wakefield (2009).
- Figure 1 shows the behavior of this rule as a function of the sample size  $n$ , and for different choices of the prior on the alternative  $\pi_1$  and the ratio of costs of type II to type I errors.
- Larger values on the  $y$  axis correspond to less extreme test statistics.
- The curves have the expected ordering and, as  $n$  gets large, a greater and greater level of evidence is required.
- This is as we would expect because **as the sample size increases we want both Type I and Type II errors to go to zero.**

## A Bayes Factor Threshold



**Figure 1 :** Threshold for rejection, on the  $-\log_{10}(p)$ -value scale, versus sample size. Notice how the threshold is decreasing with increasing sample size.

## Multiple Testing

**The problem:**  $m$  tests being carried out, often (in a GWAS context, for example) with a tiny probability of any association being non-null.

We describe:

- **Family-wise error:** Bonferroni and Sidak.
- Control of **expected number of false discoveries.**
- Control of **false discovery rate.**
- A **Bayesian perspective.**

## Framework for Multiple Testing



Possibilities with  $m$  tests and when  $K$  are flagged as requiring further attention:

	Non-Flagged	Flagged	
$H_0$	$A$	$B$	$m_0$
$H_1$	$C$	$D$	$m_1$
	$m - K$	$K$	$m$

- $m_0$  is the number of true nulls.
- $B$  is the number of type I errors.
- $C$  is the number of type II errors.

**Problem:** To select a rule that will determine  $K$ .

We discriminate between:

- A sensible **criterion.**
- How the criterion should **depend on sample size.**

## The Family-Wise Error Rate

	Non-Flagged	Flagged	
$H_0$	$A$	$B$	$m_0$
$H_1$	$C$	$D$	$m_1$
	$m - K$	$K$	$m$

- The **family-wise error rate** (FWER) is the probability of making at least one Type I error, i.e.

$$\Pr(B \geq 1 | \text{all } H_0 \text{ true}).$$

- Let  $B_i$  be the event that the  $i$ -th null is incorrectly rejected, so that  $B = \cup_{i=1}^m B_i$  is the total number of incorrectly rejected nulls.

- The **FWER** is given by:

$$\begin{aligned} \text{FWER} = \Pr(B \geq 1 | \text{all } H_0 \text{ true}) &= \Pr(\cup_{i=1}^m B_i | \text{all } H_0 \text{ true}) \\ &\leq \sum_{i=1}^m \Pr(B_i | \text{all } H_0 \text{ true}) \\ &= m\alpha^* \end{aligned}$$

where  $\alpha^*$  is the level for each test.

- This is true regardless of whether the tests are independent or not.
- Bonferroni takes  $\alpha^* = \alpha/m$  to give  $\text{FWER} \leq \alpha$ .
- Example:** For control at  $\alpha = 0.05$  with  $m = 500K$  tests take  $\alpha^* = 0.05/500,000 = 10^{-7}$ .
- Such stringent rules lead to a loss of power, but not ridiculous if you think there is a reasonable chance that all nulls could be true (but  $\alpha$  should depend on  $n$ , in particular should decrease as  $n$  gets larger and larger).

## Sidak Correction

- If all the tests are independent:

$$\begin{aligned}
 \Pr(B \geq 1) &= 1 - \Pr(B = 0) \\
 &= 1 - \Pr(\cap_{i=1}^m B'_i) \\
 &= 1 - \prod_{i=1}^m \Pr(B'_i) \\
 &= 1 - (1 - \alpha^*)^m
 \end{aligned}$$

- So to achieve  $\text{FWER} = \alpha$  take  $\alpha^* = 1 - (1 - \alpha)^{1/m}$  — the **Sidak correction** (Sidak, 1967).
- **Example:** with  $m = 500K$  tests take

$$\alpha^* = 1 - (1 - 0.05)^{1/500,000} = 1.03 \times 10^{-7}.$$

## Expected Number of False Discoveries

- We describe an alternative criterion.
- For  $i = 1, \dots, m$  tests let  $B_i$  be the 1/0 random variable representing whether the null was incorrectly rejected or not, so that  $B = \cup_{i=1}^m B_i$ .
- The **expected number of false discoveries** (EFD), with significance level  $\alpha$  for each test, is given by

$$\text{EFD} = E[B] = \sum_{i=1}^m E[B_i] = m\alpha$$

if all nulls are true.

## Expected False Discoveries

For  $m_0$  true nulls:  $E[B] = m_0\alpha$ , but  $m_0$  is unknown, so all we can say is

$$\text{EFD} = E[B] \leq m\alpha.$$

- In a GWAS context suppose  $m = 500K$  and  $\alpha = 0.05$ ; this gives  $\text{EFD} \leq 25,000$ , so conventional levels will clearly not work!
- We can easily put an upper bound on the EFD.
- For example, if we set  $\alpha = 1/m$  the expected number of false discoveries is **bounded** by 1.
- With  $\alpha = 5/m$  the expected number of false discoveries is **bounded** by 5.
- Compare to Bonferroni which controls the FWER via  $\alpha/m$ .

## False Discovery Rate

- A very popular criterion is the **false discovery rate (FDR)**.

	Non-Flagged	Flagged	
$H_0$	$A$	$B$	$m_0$
$H_1$	$C$	$D$	$m_1$
	$m - K$	$K$	$m$

- Define the false discovery proportion (FDP) as the **proportion of incorrect rejections**:

$$\text{FDP} = \begin{cases} \frac{B}{K} & \text{if } K > 0 \\ 0 & \text{if } K = 0 \end{cases}$$

- Then the **false discovery rate (FDR)**, the **expected proportion of rejected nulls that are actually true nulls**, is given by

$$\text{FDR} = E[\text{FDP}].$$

## False Discovery Rate

We describe an algorithm for **controlling the FDR**.

- Consider the following procedure for independent  $p$ -values:
  - Let  $P_{(1)} < \dots < P_{(m)}$  denote the ordered  $p$ -values.
  - Define  $I_i = i\alpha/m$  and  $R = \max\{i : P_{(i)} < I_i\}$  where  $\alpha$  is the value at which we would like FDR control.
  - Then define the  $p$ -value threshold as  $p_T = P_{(R)}$ .
  - Reject all  $H_{0i}$  for which  $P_i \leq p_T$ .
- Benjamini and Hochberg (1995) show that if this procedure is applied, then regardless of how many nulls are true ( $m_0$ ) and regardless of the distribution of the  $p$ -values when the null is false

$$\text{FDR} \leq \frac{m_0}{m} \alpha < \alpha.$$

## False Discovery Rate

- If all the nulls are true then  $B = K$  (all rejections are false) and

$$\text{FDR} = E \left[ \frac{B}{K} \right] = 1 \times \Pr(B \geq 1) = \text{FWER}.$$

- FDR in this form and with extensions, e.g. Storey and Tibshirani (2003) (description of the  **$q$ -value methodology**) have been successfully used in the microarrays field, where the number of non-null associations is not small.
- Unfortunately less successful in a GWAS, because the proportion of nulls is very close to 1.



## Simulated Example

- We illustrate control by the family-wide error rate (**FWER**), the expected number of false discoveries (**EFD**) and the false discovery rate (**FDR**).
- We simulate data for  $m = 100$  tests, with  $m_1 = 5$  being non-null.
- True table:

	Non-Flagged	Flagged	
$H_0$	$A$	$B$	95
$H_1$	$C$	$D$	5
	$m - K$	$K$	100

## Simulated Example

- We begin by plotting, in Figure 2 the observed  $p$ -values versus those expected under the null, i.e.  $i/(m+1)$  for  $i = 1, \dots, m$ .
- Hard to interpret, so we truncate the scales in Figure 3.
- Finally we stretch the scale in Figure 4 to show  $-\log_{10}$  the observed  $p$ -values versus expected  $p$ -values.
- On this scale, a value of 2 corresponds to a  $p$ -value of 0.01, and a value of 3 corresponds to a  $p$ -value of 0.001.
- We see that the **FWER** is very conservative ( $p = 0.05/100 = 5 \times 10^{-4}$ , or  $-\log_{10}(p) = 3.30$ ) and only flags one test as being significant.
- The **EFD**=1 gives a  $p$ -value threshold of 0.01, or  $-\log_{10} p = 2$  and picks up all 5 signals.
- The **FDR** control at 5% gives the green diagonal line and rejects 3 tests.

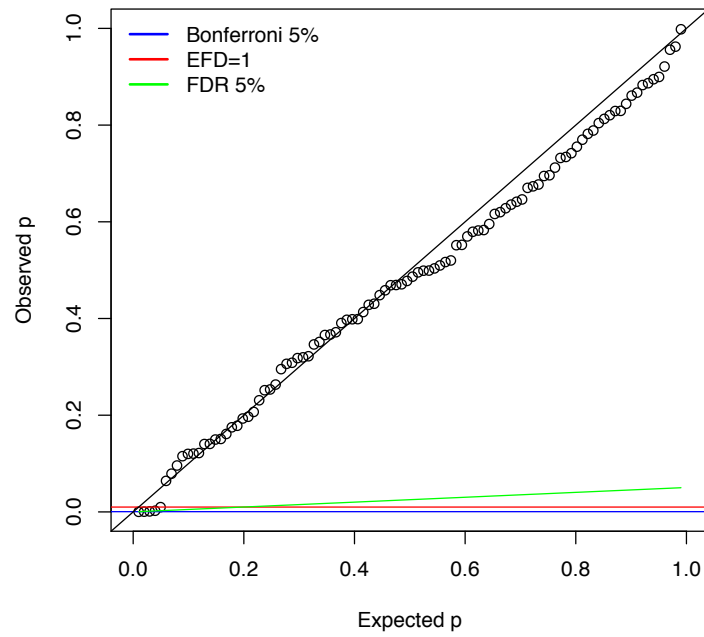


Figure 2 : Observed versus expected  $p$ -values.

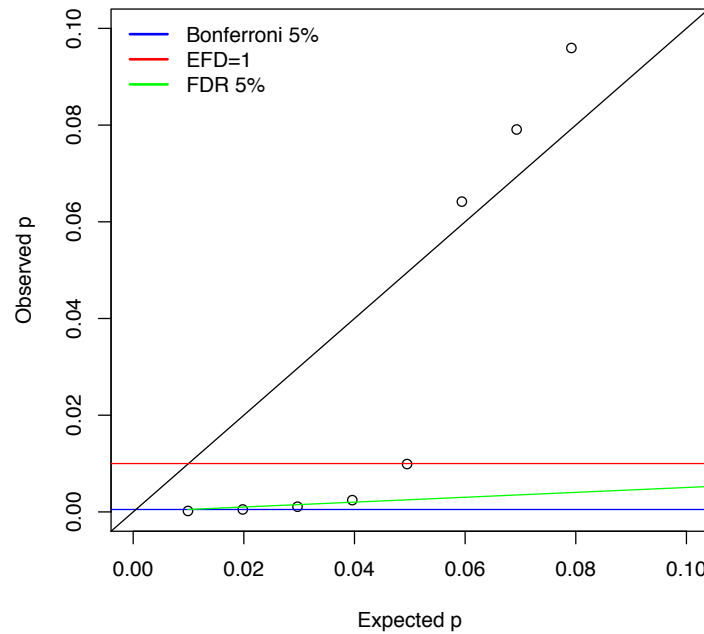


Figure 3 : Observed versus expected  $p$ -values with truncated scale.

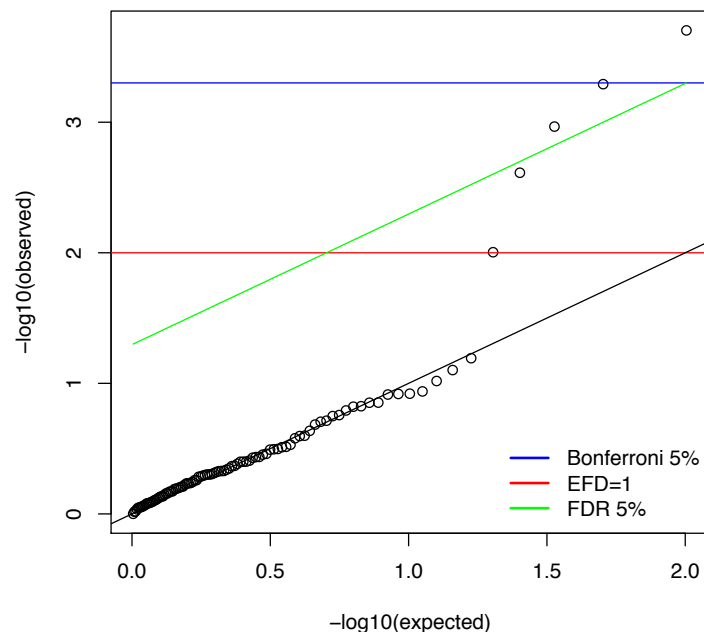


Figure 4 : Observed versus expected  $p$ -values, on  $-\log_{10}$  scale.

## Bayesian False Discoveries/Non-Discoveries

- In a Bayesian approach, based on Bayes factors we have a rule to flag a single association as **noteworthy** if:

$$\text{Posterior Odds} = \text{Bayes Factor} \times \text{Prior Odds} > R$$

where  $R$  is the ratio of costs of type II to type I errors.

- In a multiple testing situation in which  $m$  associations are being examined nothing, in principle, changes.
- We simply apply the same rule  $m$  times, perhaps changing the priors if we have different priors for different associations.
- The choice of threshold,  $R$ , and hence the procedure, does not depend on: **the number of tests being carried out**<sup>2</sup>.

<sup>2</sup>unless the prior on the null, or the ratio of costs of errors depends on the number of tests

## Bayesian False Discoveries/Non-Discoveries

- As we have seen, the Bayes factor depends, crucially, on the **sample size**.
- In contrast, multiple testing based on  $p$ -values (e.g. Bonferroni/Sidak) does not depend on the sample size but, crucially, on the **number of tests  $m$** .
- We have already noted that  $p$ -value calibration is very difficult, and we would like a procedure by which  $p$ -value thresholds **decrease to zero** with increasing sample size.
- The same would also be required of EFD or FDR based procedures.
- To summarize in the case of normal test statistics:

**The Bayesian decision is based on the  $Z$  score and on the sample size,  $n$ , but not on the number of tests,  $m$ .**

- In contrast:

**The Bonferroni decision is based on the  $Z$  score and on the number of tests,  $m$ , but not on the sample size,  $n$ .**

## Bayesian Multiple Testing

In a Bayesian context, for a single test:

- If we call a hypothesis **noteworthy** then  $\Pr(H_0 | \text{data})$  is the probability of a **false discovery**.
- If we call a hypothesis **not rejected** then  $\Pr(H_1 | \text{data})$  is the probability of a **false non-discovery**.
- In a multiple-hypothesis testing situation (and assuming ordered so the first  $K$  are rejected), we have

$$\text{Expected number of false discoveries} = \sum_{i=1}^K \Pr(H_{0i} | \text{data}_i)$$

$$\text{Expected number of false non-discoveries} = \sum_{i=K+1}^m \Pr(H_{1i} | \text{data}_i).$$

**A Key Point:** A Bayesian analysis of a single SNP alone, or the same SNP from multiple SNPs will produce the same decision (assuming the prior is the same).

## Overall Treatment Effect

- We now describe the methodology for the VISIP trial.
- Suppose we have two treatments  $T = 0/1$  (e.g. low dose/high dose), a **continuous response**  $Y$  and  $n/2$  subjects in each treatment group, where  $n$  is the number of trial participants.
- Let  $Y_i$  be the response for the  $i$ -th individual and  $T_i$  the treatment indicator.
- To estimate the overall treatment effect we fit the model

$$Y_i = \alpha + \beta T_i + \epsilon_i$$

with  $\text{var}(\epsilon_i) = \sigma^2$ , so that  $\beta$  is the parameter of interest.

- $H_0 : \beta = 0$  is the null of interest, i.e. no **treatment effect**?
- Test statistic:

$$Z = \frac{\hat{\beta}}{\text{s.e.}(\hat{\beta})} \sim \text{normal}(0, 1) \text{ under } H_0$$

where  $\hat{\beta} = \bar{Y}_{HI} - \bar{Y}_{LO}$  and  $\text{s.e.}(\hat{\beta}) = \hat{\sigma}/\sqrt{n}$ .

## Treatment-by-Marker Interactions

- Now consider the situation in which we wish to examine the treatment effect by marker.
- To be concrete, define the subgroups relative to a **recessive** model so that at a generic SNP we have  $S = 0$ :

*No Copies of the Minor Allele*

and  $S = 1$ :

*One or Two Copies of the Minor Allele.*

- There are therefore  $m$  comparisons of interest, with summary data at marker  $j$ , as below:

	Marker		Sample Size
	$S = 0$	$S = 1$	
$T = 0$	$\bar{Y}_{00}$	$\bar{Y}_{01}$	$n/2$
$T = 1$	$\bar{Y}_{10}$	$\bar{Y}_{11}$	$n/2$
	$n - n_s$	$n_s$	$n$

**Table 1 :** Summary data at a generic marker, under two treatments  $T = 0/1$ ; there are  $n$  individuals in total, of which  $n_s$  possess the marker of interest.

## Treatment-by-Marker Interactions

- Let  $S_i = 0/1$  be a marker indicator for individual  $i$  and a generic SNP.
- For the treatment effect and at each marker we fit the model

$$Y_i = \alpha + \beta T_i + \gamma S_i + \underbrace{\Delta}_{\text{Interaction}} T_i \times S_i + \epsilon_i$$

with  $\text{var}(\epsilon_i) = \sigma^2$ .

- $H_0 : \Delta = \Delta_0$  is the null of interest, i.e. is there a differential treatment effect of a certain size at the SNP, e.g.  $\Delta_0 = 0$ , to compare to the marginal treatment effect.
- Test statistic

$$Z = \frac{\hat{\Delta} - \Delta_0}{\text{s.e.}(\hat{\Delta})} \sim \text{normal}(0, 1) \text{ under } H_0.$$

- To emphasize, the same 833/837 responses are used in each of the  $m$  comparisons, but they are distributed into the four treatment  $\times$  marker cells differently.
- **Key Observation:** The standard error will vary considerably across SNPs.

## VISP Example

- After data cleaning, there were  $m = 803,122$  SNPs on which data were available, with at least 5 individuals in each treatment  $\times$  marker subgroup.
- Suppose we are interested in detecting marker subgroups for which there is an **enhanced** effect, i.e. an increased reduction over the marginal treatment effect.
- Figure 5 shows the standard errors in the VISP trial – large variability and so the power ranges considerably also.
- Now refresh memory on the Bayesian approach to testing.

## Computation of Bayes Factors

- Recall that

$$\begin{aligned}\hat{\Delta}|\Delta &\sim N(\Delta, V) \\ \Delta &\sim N(\Delta_0, W).\end{aligned}$$

where  $\sqrt{V}$  is the standard error of the estimator leads to a simple form for the Bayes factor:

$$BF = \sqrt{\frac{V+W}{V}} \exp\left(-\frac{Z^2}{2} \frac{W}{V+W}\right)$$

where

$$Z = \frac{\hat{\Delta} - \Delta_0}{\sqrt{V}}.$$

## Bayesian Boundaries

- We again use the Bayes factors as a mechanism by which  $Z$ -score boundaries can be calculated, as a function of the standard error  $\sqrt{V}$ .
- The Bayesian  $Z^2$  score threshold is:

$$Z^2 > z_B^2 = \left(\frac{V+W}{W}\right) \left\{ \log\left(\frac{V+W}{V}\right) + 2 \log\left(\frac{PO}{R}\right) \right\}$$

to give a threshold which is an explicit function of  $V$ ,  $R$  and  $PO$ .

- If the prior odds  $PO$  on the null increases, threshold increases: require **more evidence**.
- If cost of Type II to Type I errors  $R$  increase, threshold decreases: require **less evidence**.

## Bayesian Boundaries

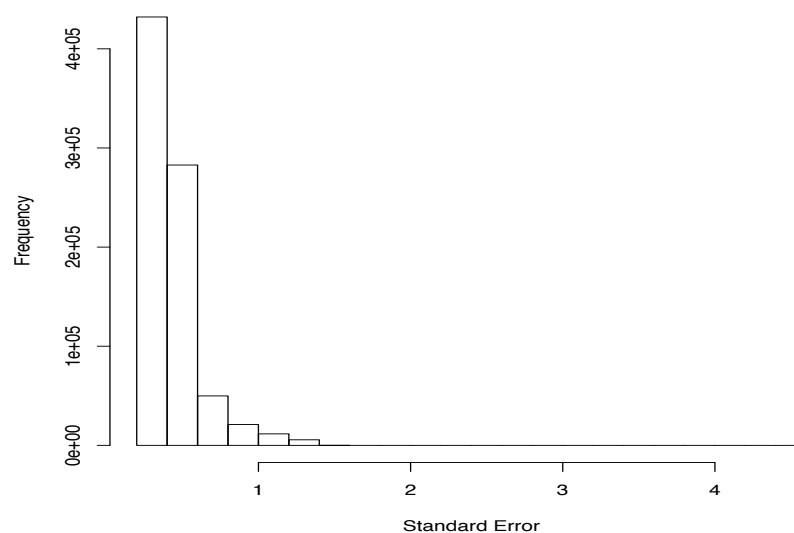
- The Bayesian boundary:

$$Z^2 > z_B^2 = \left( \frac{V+W}{W} \right) \left\{ \log \left( \frac{V+W}{V} \right) + 2 \log \left( \frac{PO}{R} \right) \right\}.$$

- Beyond a certain point, as  $V$  decreases the Type I error decreases to zero.
- Specifically, let  $n$  denote an appropriate measure of sample size and  $V = \sigma^2/n$ . Then, as  $n \rightarrow \infty$ ,

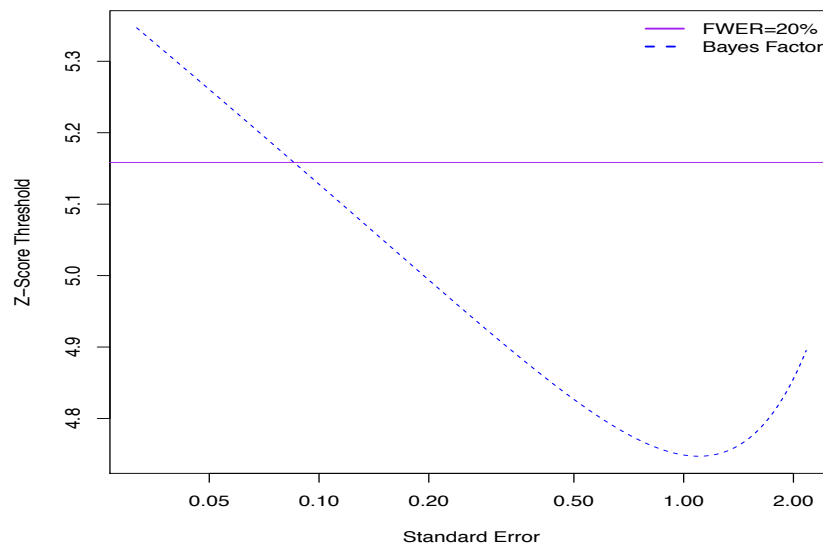
$$z_B^2 \rightarrow \underbrace{\log \left( 1 + \frac{nW}{\sigma^2} \right)}_{\rightarrow \infty} + 2 \log \left( \frac{PO}{R} \right).$$

- Relative to a fixed boundary:
  - For **small  $n$ /large standard error** the Bayesian approach requires more evidence because of the **low power**.
  - For **large  $n$ /small standard error** the Bayesian approach requires more evidence because of the **high power** and the **comparison** with the distribution under  $H_A$ .



**Figure 5 :** Histogram of standard errors of the interaction parameter estimates  $\hat{\Delta}$  in the VISP study.





**Figure 6 :** Bayesian Z-score threshold as a function of the standard error. The Bayesian threshold is based on a prior on the alternative of 0.0001,  $R = 1$  and a prior standard deviation on the interaction effect size of  $\sqrt{W} = 5.1$ ; this prior gives a 95% interval on  $\Delta$  of  $(-10,10)$ .

## A Priori Operating Characteristics

- Ranking is straightforward with Bayes factors, since the only choice is the prior on the effect parameter ( $W$ ), and inference is relatively insensitive to this value.
- There is much greater sensitivity to the ratio of costs  $R$  and the prior odds  $PO$ .
- Deciding upon values for  $R$  and  $PO$  is not straightforward, but only the ratio  $PO/R$  is needed.
- We assume  $R = 1$  (equal costs of type I and type II errors) and  $\pi_1 = 0.001, 0.0001, 0.0001$ .
- For  $m = 803,122$  SNPs this corresponds to expecting 803, 80 and 8 non-null interactions, respectively.
- These signals will not reflect 803, 80, 8 different causal variants since typically multiple SNPs will tag each causal variant.
- Figure 7 plots various useful operating characteristics.

## Operating Characteristics

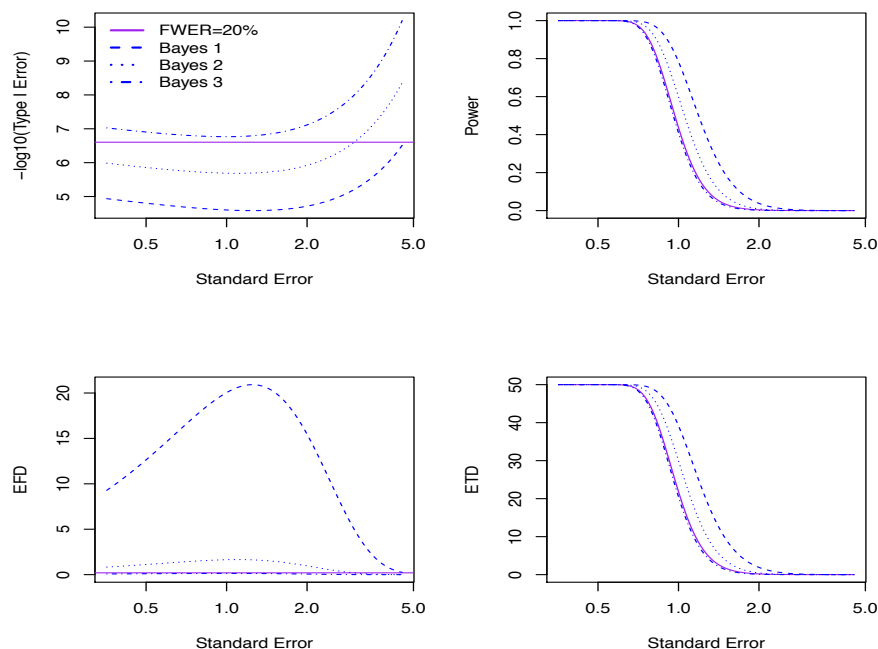
- To determine the EFD and ETD we require specification of the number of null and non-null signals, which we label as  $m_0$  and  $m_1$ , respectively (so that  $m = m_0 + m_1$ ).
- We take the true number of signals as  $m_1 = 50$  so that there are  $m_0 = 803,072$  null signals.
- Then

$$\text{EFD} = m_0 \times \alpha$$

$$\text{ETD} = m_1 \times (1 - \beta)$$

where  $\alpha$  and  $\beta$  are the type I and type II errors.

- We emphasize that in a GWAS in which the fraction of non-null associations is close to zero, the ETD is highly sensitive to the choice of  $m_1$  (in contrast to EFD, which is insensitive)



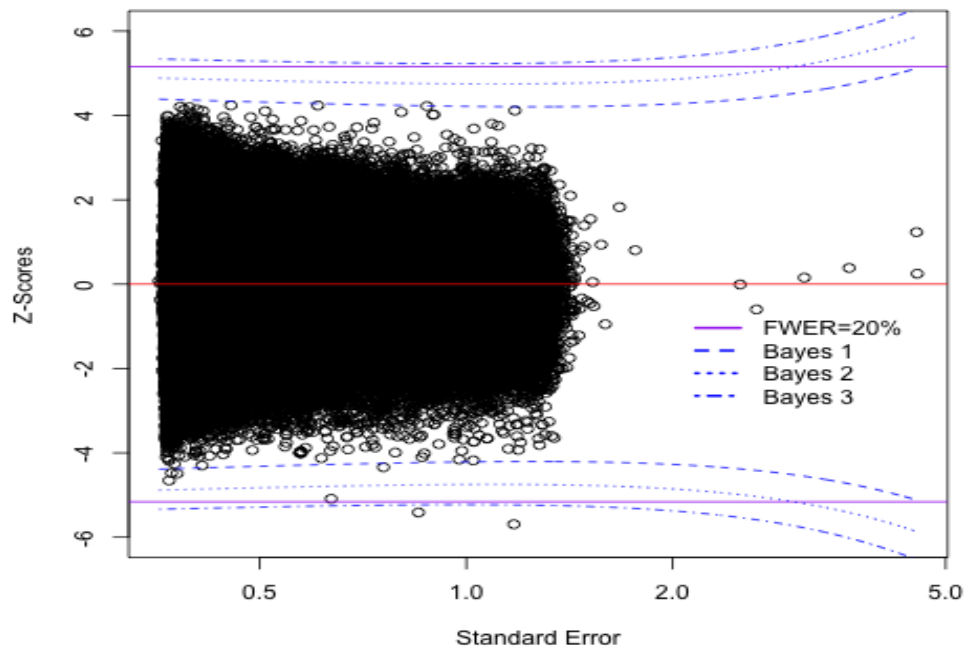
**Figure 7 :** Operating characteristics of Bayes/Bonferroni. For Bayes boundaries  $R = 1$  and “Bayes 1”, “Bayes 2”, “Bayes 3” correspond to priors of  $\pi_1 = 0.001, 0.0001, 0.0001$ . Power is to detect a drop of 5 units. For EFD/ETD we set  $m_1 = 50$ .

## Operating Characteristics

- The most liberal prior of  $\pi_1 = 0.001$  produces a large number of type I errors (around 20 for standard errors in the mid-range) and might be judged to give unacceptably poor performance.
- The most sceptical prior is more conservative than Bonferroni (with a FWER of 20%) and the prior with  $\pi_1 = 0.0001$  is a compromise for this choice of  $m_1$ .
- **For example:** For a standard error of 1, around 2 false discoveries would be expected (as in the lower left panel) but with around 10 more true signals being detected (as seen in the lower right panel), which seems a reasonable trade-off.
- Note, however, that if we think the number of true signals is smaller than  $m_1 = 50$  then the number of true signals will fall proportionally.
- **For example:** At a standard error of 1, if  $m_1 = 5$  then we would only expect to detect a single additional signal, when compared to the use of Bonferroni.
- Armed with this information we move to an analysis of the VISP data.

## Motivating Homocysteine Example

- We fitted the interaction model with adjustment for age and gender.
- The genetic subgroups are defined as having at least one copy of the minor allele as compared to two copies of the major allele.
- The number in the former subgroup ranges between 21 and 1,564 across SNPs.
- We choose  $W$  to give a 95% prior interval for the interactions  $\Delta$  of  $\pm 10$ .
- Figure 8 plots the Z-scores versus the standard error, along with boundary corresponding to a FWER of 20%.
- For both the most conservative prior and the Bonferroni approach (with a FWER of 20%, which gives a  $p$ -value threshold of  $2.5 \times 10^{-7}$ ) two SNPs are flagged.
- With a FWER of 5% the Bonferroni threshold is  $6.2 \times 10^{-8}$  and results in a single SNP being deemed significant.
- With the more optimistic prior of  $\pi_1 = 0.0001$ , a further signal is flagged (and these are not significant using Bonferroni).



**Figure 8 :** Z-score threshold as a function of the standard error for the VISP data, ratio of costs of type II to type I errors  $R = 1$  and varying priors on the alternative of  $\pi_1 = 0.001, 0.0001, 0.00001$  (to give Bayes 1, Bayes 2, Bayes 3 boundaries).

### Flagged Signals

SNP ID	Chrom	$\hat{\Delta}$	$\widehat{s.e.}(\hat{\Delta})$	<i>p</i> -value	Bayes Factor	Post Prob
rs3736238	17	-6.68	1.38	$1.5 \times 10^{-8}$	$9.3 \times 10^{-7}$	0.99
rs16893296	6	-4.61	0.85	$7.1 \times 10^{-8}$	$3.9 \times 10^{-6}$	0.96
rs1739317	6	-3.23	0.64	$4.0 \times 10^{-7}$	$2.3 \times 10^{-5}$	0.81
rs11819196	10	-1.72	0.37	$3.5 \times 10^{-6}$	$2.9 \times 10^{-4}$	0.26

**Table 2 :** The SNPs in the VISP study that had posterior probabilities on the alternative of greater than 0.25 ( $R = 3$ ), with a prior on the alternative of  $\pi_1 = 0.0001$  and under the equal variances recessive genetic model.

## VISP Results

- Figure 9 plots the posterior probabilities of the alternative hypothesis (with  $\pi_1 = 0.0001$ ) versus chromosomal position (this is similar to a Manhattan plot in which  $-\log_{10}$  p-values are plotted against position).
- The 3 SNPs that fall outside of the boundary in Figure 8 are highlighted.
- The strongest signal is for SNP rs3736238 on chromosome 17. For this SNP there are 42 individuals in the  $M = 1$  subgroup, of which 24 and 18 are in the low and high dose groups, respectively.
- The probability of this signal being a false discovery is 0.01 *under* our assumed prior.

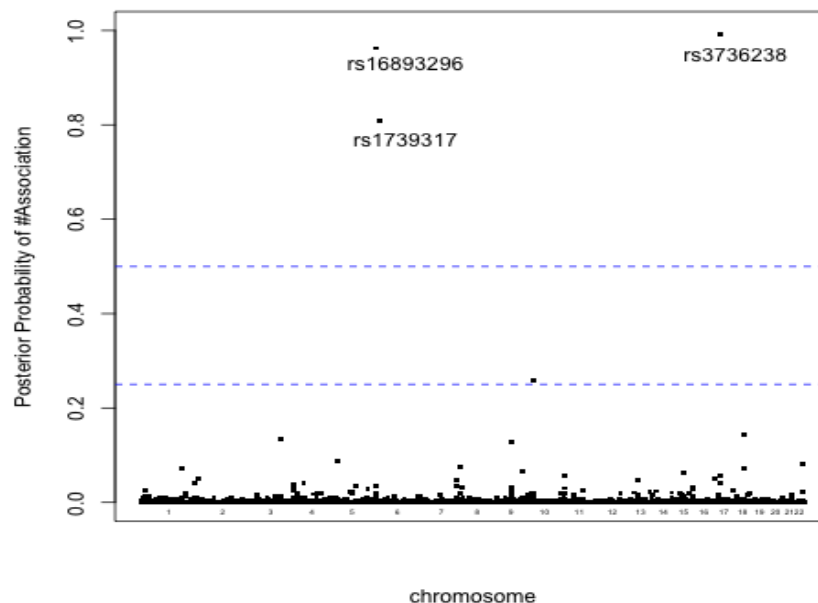



Figure 9 : Posterior probability on the alternative plotted versus genomic position for the VISP data. The prior on the alternative is  $\pi_1 = 0.0001$ . 

## VISP Example

- Figure 10 shows that the  $p$ -values and Bayes factors differ in their rankings due to the differing sample sizes/standard errors.
- The points are color-coded by the size of the standard error and we see that the points with larger standard errors are consistently ranked as giving greater evidence for the alternative under the Bayesian approach.
- This behavior occurs here because of the association between the  $Z^2$  boundary and the standard error for these priors, as shown in Figure 6.
- Specifically, the majority of the signals occur in that portion of the latter curve in which the Bayes boundary lies below the FWER boundary.
- Figure 11 shows an example in which distinctly different behavior occurs.

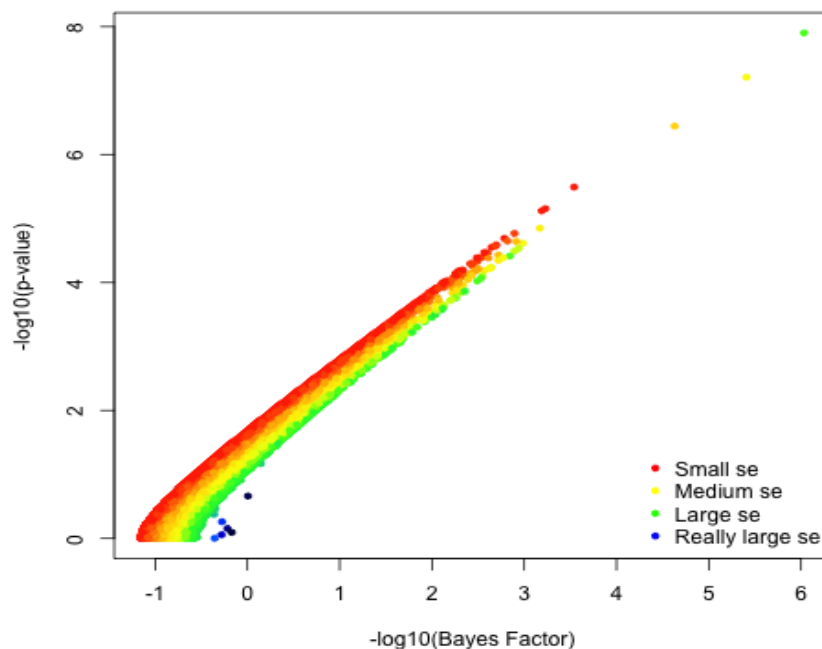


Figure 10 :  $-\log_{10}\text{BFs}$  vs  $-\log_{10} p$ -values, color-coded by standard error with  $W = 10$ .

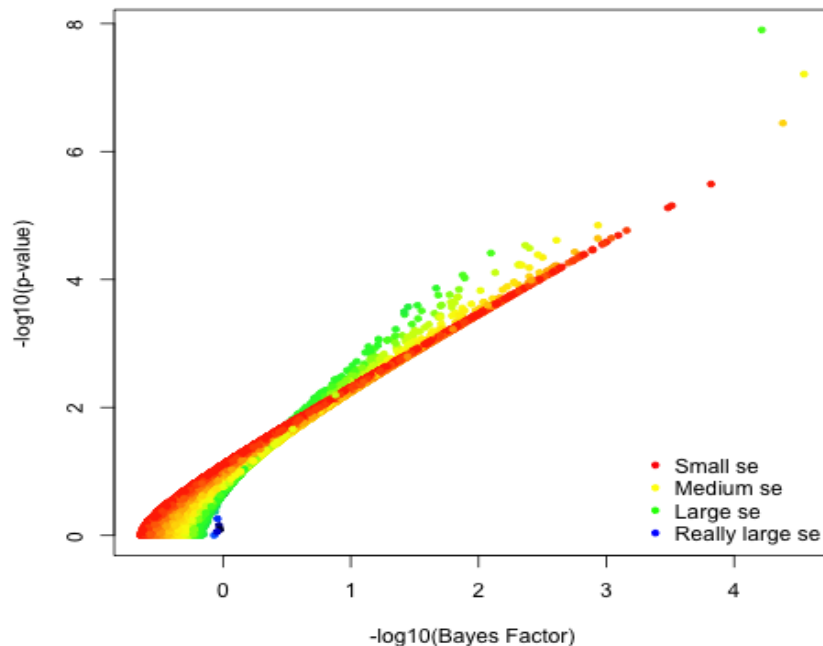


Figure 11 :  $-\log_{10} \text{BFs}$  vs  $-\log_{10} p$ -values, color-coded by standard error with  $W = 3$ .

## VISP Example

- A related interesting exercise is to simulate the distribution of observed effect sizes under our assumed priors (on both the proportion of non-null signals and the effect sizes), using the observed distribution of standard errors.
- The distribution of effect sizes is  $N(\Delta, V + W)$  for the non-null signals and  $\text{normal}(0, V)$  for the null signals.
- We can then evaluate the power, and hence determine the number of signals we would expect to detect given our prior assumptions.
- For the VISP data, with a proportion of non-null signals  $\pi_1 = 0.0001$ ,  $R = 1$  and 95% range for the effect sizes of  $\pm 10$ , we would expect to see 52 true positives and one false positive.
- Given we only observed three non-null signals, this implies that either the range of effect sizes (as defined through  $W$ ) was too wide or, more probably, that our estimate of  $\pi_1$  was optimistic.
- Repeating this exercise with  $\pi_1 = 0.00001$  gives 5 true positives and close to 0 false positives, which is more consistent with that which was observed.
- Figure 12 gives the posterior probabilities for this prior.

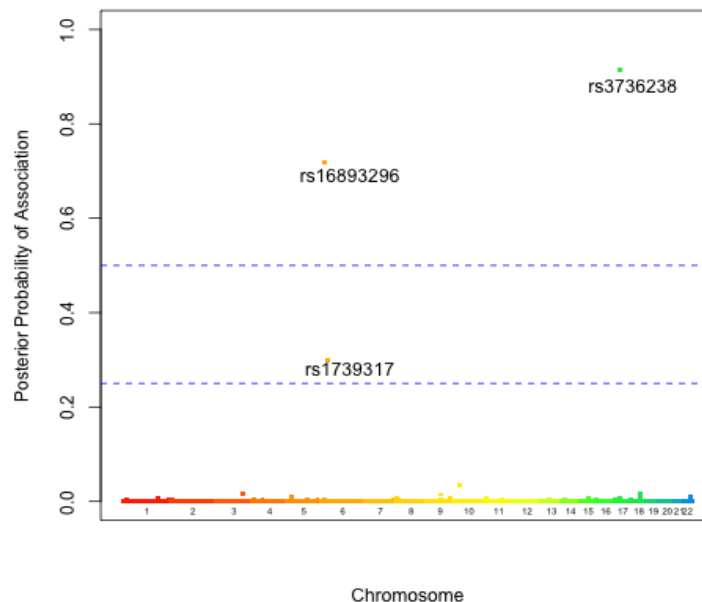


Figure 12 : Posterior probability on the alternative plotted versus genomic region for the VISP data. The prior on the alternative is the more conservative choice of  $\pi_1 = 0.00001$ . ).

## VISP Discussion

- We chose the value  $\pi_1 = 0.0001$  by examining frequentist summaries before the real data analysis was performed.
- We define  $\pi_1$  as the proportion of SNPs that would be associated with the disease, if the power were 1.
- After the data are analyzed we can, for those SNPs declared as null (i.e. all but 3 SNPs in the VISP trial), sum up the posterior probabilities of being non-null, and this gives the **expected number of false non-discoveries** .
- For the VISP data, **this expected number is 24.6** so that we are missing a large number of signals, with lack of power being a major issue.
- For the three significant signals, at the 0.5 threshold, the probabilities of the null being true are 0.01, 0.04 and 0.19, so that **the expected number of false discoveries is 0.24**.
- Taking the threshold of significance as 0.25 gives an additional SNP as being declared significant.
- The sum of the posterior probabilities of the null is 0.98 in this case and so, under this prior, we would expect one of the reports signals to be a false discovery.



## Sensitivity to $\pi_1$

- The posterior probability of the alternative is highly dependent on the choice of prior on the null  $\pi_0$ , and a sensitivity analysis is always warranted.
- Ideally, rather than fix  $\pi_0$  as we have done, one would estimate of  $\pi_0$  from the totality of data (i.e. over all  $m$  SNPs), but this is difficult because in a GWAS the proportion of *detectable* null signals is typically very close to 1; there may be many thousands of small but non-zero effects, but the power to detect these signals is low, with the usual sample sizes.
- In other contexts, such as the analysis of gene expression data (Storey and Tibshirani, 2003), the data can be used to estimate  $\pi_0$  more reliably.
- If the same prior on the null is used for all the tests, the rankings based on the Bayes factor will remain the same as the ranking based on posterior probabilities.
- However, **calibrating** the Bayes factors to the probability scale requires prior probabilities.
- Within a sensitivity exercise one may include an analysis in which any available information on particular SNPs may be included.

## An Alternative Approach to Significance

- The posterior probability (and the Z-score threshold) is equally sensitive to R as to  $\pi_1$ .
- The form of the latter suggests that all we need to do is to fix PO/R.
- As mentioned above, in the VISIP analysis we selected  $\pi_1$  by examining the frequentist operating characteristics.
- An alternative method (Wakefield, 2012) for obtaining PO/R is to specify a value for the  $Z^2$  boundary,  $z_B^2$ , at a particular  $V$  (for example, at a MAF and sample size that one is familiar with) and then solve for  $U = \log(\text{PO/R})$  via

$$\hat{U} = \frac{z_B^2 \times W}{2(V + W)} - \frac{1}{2} \log \left( \frac{V + W}{V} \right).$$

- With this value of  $\hat{U} = \text{PO/R}$  one can then proceed to use

$$Z^2 > z_B^2 = \left( \frac{V + W}{W} \right) \left\{ \log \left( \frac{V + W}{V} \right) + 2 \log(\hat{U}) \right\}$$

across the observed range of standard errors.

## Incorporating Prior Information

- We now briefly describe a method for assigning priors to SNPs based on substantive information.
- In collaboration with scientists at IARC and at the Department of Computer Science at Sheffield University a method had been developed that searches through PubMed abstracts for pre-assigned keywords and key concepts.
- More details in Johansson *et al.* (2012).
- This information is used to assign prior probabilities of association with the phenotype for each SNP of interest.
- Three prior groups were assigned, depending on the number of hits.
- The priors can subsequently be incorporated with the association results of GWAS using the previously described Bayesian framework.
- The method has acronym: **Adjusting Association Priors with Text (AdAPT)**.
- Details of the method can be found in Johansson *et al.* (2012).
- The **AdAPT** software is available here:  
<http://services.gate.ac.uk/lld/gwas/service/config>

## Incorporating Prior Information in a GWAS

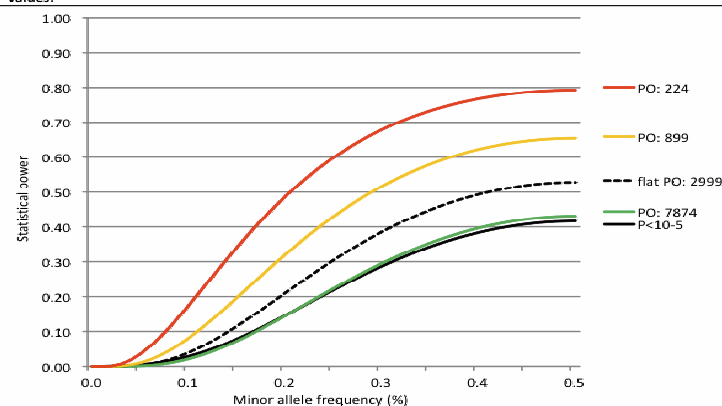
- SNPs are assigned to a group, based on the number of keywords that were found to be associated with this SNP.
- For the priors, keywords were ranked by priority: In the:
  - **1st group**  $G_1$ : were words specific to lung cancer (eg, smoking, lung carcinoma).
  - **2nd group**  $G_2$ : were more general words specifically relevant to lung cancer (smoking, nicotine, non-small cell carcinoma),
  - **3rd group**  $G_3$ : were more general words (carcinogen, DNA damage).
- Each SNP was then placed in one of three prior categories:
  1.  $C_1 = \{\text{not } G_1, \text{not } G_2, \text{not } G_3\}$ .
  2.  $C_2 = \{\text{at least one of } G_1, G_2, G_3 \text{ but not all } \}$ .
  3.  $C_3 = \{G_1, G_2, G_3\}$ .
- We then assigned prior odds (PO) to  $\Pr(H_0|C_j)/\Pr(H_1|C_j)$ . Specifically for the three categories, the PO was set to 7874 ( $C_1$ ), 899 ( $C_2$ ) and 224 ( $C_3$ ).
- These were used in the analysis to obtain the posterior odds on  $H_0$ :

$$\frac{\Pr(H_0|y, C_j)}{\Pr(H_1|y, C_j)} = \frac{\Pr(y|H_0)}{\Pr(y|H_1)} \times \frac{\Pr(H_0|C_j)}{\Pr(H_1|C_j)}.$$

## Incorporating Prior Information

- First, the power was evaluated for the three categories, see Figure 13.
- The method was tested by comparing rankings of known **susceptibility alleles** in a previous lung cancer GWAS of 1989 cases and 2625 controls in 6 central European countries.
- The rankings of **6 SNPs** that have been independently replicated in multiple studies were calculated.

**Figure 1 | Comparison of the statistical power when evaluating the noteworthiness of SNPs by BFDP and p-values.**



These power calculations assume an evaluation of 300,000 SNPs of which 100 are truly associated with the outcome and distributed evenly across three prior categories, respectively. The overall distribution of SNPs across the three prior categories is assumed to be [87.5%; 10%; 2.5%]. Flat PO assumes one single prior category.

**Figure 13 :** Power as a function of MAF, for three prior categories, for a single prior, and for a  $p$ -value approach.

## Incorporating Prior Information: Proof of Principle Results

- The results below show that known susceptibility SNPs were ranked more highly by AdAPT BFDPs than by p-values.
- Rankings based on initial data with informative priors for the Bayes rankings:

SNP	p-value ranking	Bayes ranking
rs8034191	1	1
rs1051730	2	2
rs4324798	4	5
rs401681	73	30
rs2736100	76	32
rs3117582	121	34

## Incorporating Prior Information: New Study Results

- Subsequently, the method was applied on a novel two phase GWAS of oral cancer, with 791 cases and 7,012 controls included in the discovery phase.
- A **Bayes threshold on the null** of 0.8 was assigned and 6 SNPs passed this test.
- One of these was already replicated, the replication was carried out for the remaining 5 AdAPT ranked SNPs in 1,046 cases and 2,131 controls from 4 case-control studies.
- rs991316**, located in the ADH gene region of 4q23, displayed a statistically significant association with oral cancer risk in the replication phase (per-rare-allele log additive  $p$ -value =  $2.5 \times 10^{-3}$ ).
- This SNP was ranked 76th in the  $p$ -value list and so would not have been selected to carry forward, but was ranked 4th in the BFDP list.
- The combined **odds ratio** associated with having one additional rare allele was 0.84 (95% CI: 0.75–0.94).

## Conclusions

- Bayesian analysis is attractive in a multiple testing context, but the results are very **sensitive to the prior** on the proportion of nulls,  $\pi_0$ .
- Fast methods are required for large  $m$  (e.g. in a GWAS context) of tests, which is still a drawback for many Bayesian approaches.
- As we have seen, **priors** can be made a function of characteristics of the SNP (e.g. non-synonymous, previously implicated,...).
- Such priors can have a major impact on rankings and posterior probabilities.
- In genetics, journals are sympathetic to Bayes analyses (not true in all disciplines).
- A huge GWAS enterprise used  $p$ -values and Bayes factors to assess significance (Wellcome Trust Case Control Consortium, 2007).
- Stephens and Balding (2009) provide a review of Bayesian approaches in GWAS.

## References

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