Expression QTLs mapping and analysis: a Bayesian perspective

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Abstract

The aim of expression Quantitative Trait Locus (eQTL) mapping is the identification of DNA sequence variants that explain variation in gene expression. Given the recent yield of trait-associated genetic variants identified by large-scale genome wide association analyses (GWAS), eQTL mapping has become a useful tool to understand the functional context where these variants operate and eventually narrow down functional gene targets for disease. Despite its extensive application to complex (polygenic) traits and disease, the majority of eQTL studies still rely on univariate data modeling strategies, i.e., testing for association of all transcript-marker pairs. However these "one at-a-time" strategies are (*i*) unable to control the number of false positives when an intricate Linkage Disequilibrium structure is present and (*ii*) are often underpowered to detect the full spectrum of *trans*-acting regulatory effects. Here we present our viewpoint on the most recent advances on eQTL mapping approaches, with a focus on Bayesian methodology. We review the advantages of the Bayesian approach over frequentist methods and provide a simple empirical example of polygenic eQTL mapping to illustrate the different properties of frequentist and Bayesian methods. Finally, we discuss how multivariate eQTL mapping approaches have distinctive features with respect to detection of polygenic effects, accuracy and interpretability of the results.

Key words: Expression QTL (eQTL), polygenic eQTL, *trans*-eQTLs, LASSO, penalized-regression, Bayesian Variable Selection

1. Introduction

Genetics shape the landscape of phenotypic variation between humans through changes in the mechanisms regulating gene transcription and, consequently, gene expression. Detecting genetic drivers of gene expression can help understand the functional effects of DNA sequence variations at the cellular level. In particular, with the growing number of genetic variants associated with complex traits and diseases by Genome Wide Association Studies (GWAS), understanding how these variants act though changes to the transcriptome might help elucidating their cellular context and prioritize functional gene targets [1].

Expression Quantitative Trait Loci (eQTLs) are genetic loci that control variation in the expression level of a gene (or transcript) in a given tissue or cell-type. In the literature eQTLs are distinguished by their relative position to the gene they regulate, as *cis*- (or proximal) and *trans*-acting. This distinction is important, as it can be informative on the mechanisms underlying variation in gene expression. For example, *cis*-eQTLs can be located within the promoter or enhancer region of the gene, and thus indicate interactions with the gene's own regulatory elements. Typically, *cis*-eQTLs are more easily detected and are of large genetic effect, whereas *trans*-eQTLs have relatively smaller effects and can reveal secondary regulatory mechanisms of gene expression. While it has been reported that a substantial fraction of observed *trans*-eQTL associations can be explained by *cis*-mediation [2], the identification of large clusters of *trans*-eQTLs can be informative of coordinated genetic regulation of gene expression and regulatory networks underlying complex traits [3–6].

The classical set-up of an eQTL mapping study involves quantifying the expression levels of selected genes or of the whole transcriptome using microarrays or RNA-sequencing analysis, and then treating each expression level as a quantitative trait to be mapped against a set of genetic markers. The goal is to estimate the number, effect size and kind (i.e., *cis*- or *trans*-acting) of eQTLs in a given tissue or cell-type. eQTLs can be detected using linkage or association mapping, much the same as in GWAS for quantitative traits. Linkage mapping is typically used to detect genetic linkages in pedigrees of related individuals for highly penetrant phenotypes with a few major effect genes (or under monogenic control), while association is more powerful when working with traits determined by many small-effect variants (i.e., polygenic) and in populations of unrelated individuals. There is vast literature on linkage-based eQTL mapping in inbred populations, families as well as in experimental model systems; however, in this review we restrict our attention to association mapping, as it is more relevant to the interpretation of GWAS signals in common disease.

Due to the complex genetic architecture of expression traits, statistical power is key when choosing an eQTL-mapping strategy. Contemporary eQTL studies are characterized by the "large p, small n paradigm", as the number of predictors (genetic markers) is orders of magnitude larger than the number of genotyped samples. Typically, the contribution of most predictors to the expression trait is negligible, so most experiments aim to discover the few SNPs with substantial effects and use separate analyses to detect *cis*- and

trans-effects. In this, *cis*-eQTLs are usually investigated by analyzing only the SNPs located nearby the gene, therefore reducing the need for multiple testing adjustments. However, frequentist models, which estimate individual SNP's contribution to the gene expression, are less capable of identifying the full spectrum of (*cis*- and *trans*-acting) eQTLs in the genome, giving way to multivariate selection approaches.

A wide range of genetic mapping programs, tailored for eQTL analysis, is currently available, using either frequentist or Bayesian inference [7–20]. These methods vary greatly in terms of statistical power to detect associations, interpretability of results and computational efficiency and the choice between different approaches is usually influenced by the trade-off between these three factors. Frequentist univariate models, for example, are fast and usually come with straightforward conversions to false-positive rates and false discovery rates (FDR), but have limited ability to detect small-effect *trans*-eQTLs and polygenic contributions to gene expression. Multivariate selection models (using penalization on the regression coefficients or sparsity prior on their number) are substantially more powerful than univariate approaches since they are able to decrease the uncertainty of the results by selecting (non-collinear) independent predictor variables avoiding at the same time over-fitting. However these advantages do come at a price: these methods are computationally more demanding and less efficient to deal with genome-wide eQTL-mapping experiments.

Another problem of frequentist univariate models relates to their ability to distinguish a tissue-specific eQTL (i.e., a genetic marker linked to gene expression in a specific tissue or cell-type) from an eQTL that is conserved across tissues. In contrast, the simultaneous and multivariate eQTL mapping of expression levels across tissues has been shown to increase power to detect common *trans*-eQTLs [20–22] in comparison with a naïve intersection of eQTLs mapped separately within individual tissues.

Here we review statistical methodologies that are most commonly used for the discovery of eQTLs. In this, after introducing eQTL mapping that use the frequentist approach, we focus on Bayesian approaches and appraise their advantages and distinctive features. For illustrative purposes, we report an example of eQTL mapping of simultaneous *cis*- and *trans*-effects (i.e., polygenic control of gene expression) as well as the extension to multiple tissues, to illustrate features specific to each eQTL mapping method.

2. Frequentist eQTL mapping

In classical statistics, the observed data are considered an instance of infinitely many possible independent samples, while the tested hypothesis h, and any model parameters, are fixed and unknown. Hypothesis testing aims at deciding to accept or reject the null hypothesis with a high probability, which amounts to estimating the likelihood of observing the current instance of the data (or any function of it) under the null hypothesis. The *p*-value, a measure of the probability of observing under the same experimental conditions future samples equal or more extreme than the observed data, is

used to decide on a hypothesis, based on whether it is smaller than an arbitrary significance level (typically <5% when a single hypothesis is tested).

(a) *Simple parametric models*

Early attempts of eQTL mapping were predominantly frequentist, and utilized mapping strategies that were used in ordinary linkage of GWAS analysis settings. Most these methods test the association of the expression level of each transcript to each marker independently, partitioning the samples in groups based on their genotype – e.g., in isogenic populations this is essentially differential expression analysis using the allele as grouping variable [23, 24] while in multi-allelic data an ANOVA test is performed with genotypes as grouping variable. Since both t-test and ANOVA can be seen as special cases of the linear regression model, several software packages implementing simple linear regression for eQTL mapping are available [7, 10, 14, 15].

Here we introduce the basic principles of the linear regression approach in eQTL mapping. Let's assume an expression profiling experiment with n samples that are genotyped at p markers which are the predictor variables. Without loss of generality, here we also assume that $n > p$. The expression of one transcript can be described as:

$$y = \alpha + x_1\beta_1 + \cdots + x_p\beta_p + \varepsilon, \qquad \varepsilon \sim N(0, \sigma^2),$$

where y is the $n \times 1$ vector of expression levels, $x_j = \left(x_{1,j}, \ldots, x_{n,j}\right)$, $j = 1, \ldots, p$, is the $n \times 1$ predictor vector which corresponds to the sample genotypes at the jth marker and ε is the normally distributed error term, centered in zero with residual variance σ^2. The regression coefficients $\beta = (\beta_1, \ldots, \beta_p)^T$, which encode the contribution of each marker to the gene expression y, can be estimated by minimizing the sum of squared residuals using Ordinary Least Squares (OLS), i.e., by solving:

$$\hat{\beta} = \arg\min_{\beta} \left\{ \sum_{i=1}^{n} \left(y_i - \alpha - \sum_{j=1}^{p} x_{ij}\beta_j \right)^2 \right\}.$$

A hypothesis test can be set-up testing whether all β regression coefficients are zero (null hypothesis) or at least one is not zero, in which case an eQTL association is detected

$$H_0: \beta = 0$$
$$H_1: \text{at least } \beta_j \neq 0, j = 1, \cdots, p.$$

Different statistics can be used to test this hypothesis, each employed by different methods in the frequentist eQTL literature: the *t*-statistic [24] if each β_j , $j = 1, \ldots, p$, is tested independently, the F-statistic [7, 15, 25] if all β are tested simultaneously, the Pearson's r [7] or the, closely related, Likelihood Ratio test [10, 12]. These linear regression models are quite flexible and can be extended in several ways, for example by including sex, age, batch effects,

population structure, *etc.*, or considering confounders as fixed effects (covariates), by combining additive, recessive and dominant effects of the genotypes or by adding a random effect that, for instance, can be used to account for family/pedigree structure [26]. Since in typical eQTL mapping experiments the number of markers is much larger than the number of observations, $p \gg n$, (also known as the "large p, small n" paradigm), linear regression models cannot be used straightforwardly with the whole set of markers. To overcome this problem, simple univariate strategies have been proposed where all possible transcript-markers pairs are tested for association. However, these procedures are sub-optimal since they are not able to control the number of false positive associations when an intricate Linkage Disequilibrium (LD) structure with correlated markers is present.

(b) *Non-parametric models*

When the assumptions of normality and/or linearity are not guaranteed, non-parametric models, based on the Wilcoxon rank-sum test [23, 27, 28], a non-parametric version of the t-test, or Spearman's rank correlation [9] have been proposed and employed to map eQTLs, in particular in simple model organisms [23]. Sometimes non-parametric models are used in conjunction with linear models to help establish a significance threshold, especially in the presence of outliers.

Both parametric and non-parametric frequentist approaches based on the "one at-a-time" strategy are widely adopted because of their computational performance – many employ efficient memory allocation techniques [11] or minimize the number of required operations [7, 29]. The appealing "simplicity" and widespread use of the *p*-value is another attractive feature of these approaches, as it allows for straightforward control of family-wise error rate (FWER) and FDR (e.g., using for instance the Benjamini-Hochberg method [30]) although both procedures assume the independence of the statistical tests that are rarely met in practice due to LD structure in the genetic markers.

Despite its extensive use, the *p*-value as a measure of association is based only on the null distribution and it cannot control the power, which depends on the alternative hypothesis. The lack of power control provided by *p*-values is particularly undesirable in typical eQTL studies based on linear regression models since it is hard to detect associations with small effect sizes, such as those observed for *trans*-eQTLs. For instance, it has been shown that with 5M SNPs a sample size of at least 200 is required to detect common (i.e., minor allele frequency, MAF > 20%) *trans*-eQTLs and over 500 with rare (MAF < 5%) variants [31]. Reaching this sample size requirement can be difficult in many eQTL-mapping experiments since relevant tissue for expression profiling is difficult to obtain, in particular in human eQTL analyses.

(c) *Penalized-regression models*

Penalized-regression methods such as ridge regression [14, 32], the LASSO [33–36], Elastic Net [37] and Group Lasso [38] have been proposed to address the limitations of classical regression-based eQTL mapping methods. This class of approaches tries to account for a sparse representation of the genetic markers that contribute to the expression of the gene when $p \gg n$ and

for the presence of blocks of LD between genetic markers. In penalized-regression approaches the output consists of a sparse set of predictors (genetic markers) that are obtained by shrinking the majority of regression coefficients towards zero. Here, we focus on the LASSO [33–36], as it is one of the most widely used method in eQTL mapping, and it is a key component of a larger class of penalized-based approaches [39–46]. In LASSO, shrinkage is achieved by restricting the OLS solution such that the absolute sum of the regression coefficients (L^1-norm) does not exceed a threshold t:

$$\sum_{j=1}^{p} |\beta_j| \leq t$$

which is equivalent to solve

$$\hat{\beta} = \arg\min_{\beta} \left\{ \sum_{i=1}^{n} \left(y_i - \alpha - \sum_{j=1}^{p} x_{ij}\beta_j \right)^2 + \lambda \sum_{j=1}^{p} |\beta_j| \right\}.$$

The parameter λ is called penalty, which is typically selected by cross-validation, such that it minimizes the off-sample prediction mean square error. However imposing a L^1-norm restriction on the effects, the non-zero regression coefficient estimates become biased.

The eQTL mapping by penalized regression-based approaches typically leads to the identification of a few genetic markers as eQTLs, implicitly assuming that the majority of markers in the genome have negligible effects of gene expression. While this hypothesis is plausible from a biological viewpoint, the interpretation of the results can be sometimes difficult, as the non-zero regression coefficients are not informative about the genome-wide significance of the eQTL results, and their estimate cannot be used straightforwardly to control the FWER or FDR.

To overcome this limitation, additional resampling-based approaches such as stability selection [47, 48] (which accounts for the number of times a genetic marker is selected by a LASSO-type algorithm during the resampling procedure) provides a selection frequency (posterior probability) for each predictor, that can be used to control the FWER, but not the FDR. Another limitation of this approach is that current strategies to calibrate the penalty parameter λ are not robust: in general there is no optimal strategy for the tuning of the parameter λ, while standard calibration strategies may lead to inconsistent prediction with either too many false positives or false negatives [49]. This is particularly important in the presence of moderately correlated predictors, which is usually the case in eQTL mapping studies due to the underlying LD structure in the genome [44].

In the presence of a group of highly correlated variables, the LASSO tends to select one variable from a group and ignore the others. To overcome this limitation, Elastic Net [37] has been proposed. This method adds an extra penalty (L^2-norm) which, when used alone, corresponds to the ridge regression:

$$\hat{\beta} = \arg\min_{\beta}\left\{\sum_{i=1}^{n}\left(y_i - \alpha - \sum_{j=1}^{p} x_{ij}\beta_j\right)^2 + \lambda_1\sum_{j=1}^{p}|\beta_j| + \lambda_2\sum_{j=1}^{p}\left(|\beta_j|^2\right)^{\frac{1}{2}}\right\}.$$

However including groups of correlated predictors in the sparse solution (i.e., the set of eQTLs) can produce large variance in the final parameter estimates since the determinant operator required in the OLS solution is close to zero, which makes the linear algebra operator “ill-conditioned”, and therefore the matrix inversion cannot be performed with as much precision (i.e., large variances). However, adding an extra penalty regularizes the matrix inversion, reducing the variance of the non-zero effects. Although the resulting non-zero regression coefficient estimates are biased, the expected mean squared error is lower than OLS since the bias is largely compensated by a smaller variance. Despite the theoretical and intuitive arguments in favor of the Elastic Net, the choice of the penalty parameters λ_1 and λ_2 by cross-validation is computationally time consuming since the optimization should be done in a two-dimensional grid. Moreover the optimal solution for λ_1 and λ_2 can lie in a very small interval that is not covered by the user-defined grid of penalty parameters, with the risk of producing a sub-optimal solution.

A concise list of the most commonly used frequentist eQTL mapping methods and their software implementation is reported in **Table 1**.

Table 1. Frequentist eQTL mapping approaches

Strategy	Method, [ref] and availability	Statistic	Additional features	Multi-tissue	Genetic models
One at-a-time test	ANOVA	F-statistic	-	Yes	Additive
One at-a-time test	Krux [13] https://github.com/tmichoel/krux	Kruskal-Wallis	-	No	Additive
One at-a-time test	Matrix-eQTL [7] http://www.bios.unc.edu/research/genomic_software/Matrix_eQTL	Pearson's r	• Heteroskedastic error term (for correlated transcripts) • Slice computations in small matrices for computational efficiency	No	• Additive • Dominant
One at-a-time test	Genevar [9] https://www.sanger.ac.uk/resources/software/genevar/	Spearman's ρ	-	No	Additive
One at-a-time regression	R/QTL [10] http://www.rqtl.org	t-test	-	No	Additive
Multiple regression	snpMatrix [11] http://www.bioconductor.org/packages/2.3/bioc/html/snpMatrix.html	Chi-squared	• Generalized linear models • SNP conditioning search	No	Additive
Multiple regression	eMap [12] http://www.mybiosoftware.com/emap-1-2-eqtl-analysis.html	Likelihood Ratio	Inclusion of covariates *via* backward selection	No	• Additive • Dominant
Multiple regression	HEFT [14] http://mezeylab.cb.bscb.cornell.edu/Software.aspx	t-test	• Ridge regression • Detection of hidden covariates by factor analysis	No	Additive
One at-a-time regression	SNPTEST [15] https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html	F-statistic	Frequentist and Bayesian analysis (see **Table 2**)	Yes	• Additive • Dominant • Recessive • Heterozygote
Multiple regression	Glmnet [50] https://cran.r-project.org/web/packages/glmnet/index.html	-	• LASSO • Elastic Net	Yes	Additive

3. Bayesian eQTL mapping

Bayesian methods are becoming increasing popular in modern genetics [51], possibly as a consequence of recent more efficient algorithmic/computational implementations, cheaper high-performance computing solutions, and in general less computational constraints in their genome-wide applications. Unlike frequentist approaches, which try to infer the value of fixed models parameters from random data, in Bayesian inference the data are treated as a fixed quantity (since there is no randomness after observing the data) while the parameters are treated as random variables. This allows researchers to assign to parameters (and models) probabilities, making the inferential framework more intuitive and straightforward. Here we introduce a few general concepts that are at the core of the Bayesian paradigm. Denoting the parameters by θ and the observed data by D, the Bayes theorem allows to write:

$$\pi(\theta|D) = \frac{\ell(D|\theta)\pi(\theta)}{\ell(D)} = \frac{\ell(D|\theta)\pi(\theta)}{\int \ell(D|\theta)\pi(\theta)d\,\pi} \ ,$$

where $\pi(\theta|D)$ is the posterior distribution, $\ell(D|\theta)$ is the likelihood (conditionally on some parameters' value), $\pi(\theta)$ is the prior distribution on the parameters and $\ell(D)$ the marginal likelihood. In a nutshell, the equation above states that the Bayesian paradigm provides a distribution regarding what it has been learned about the parameter from the data. In contrast to the frequentist approach, where only a point estimate (Maximum Likelihood Estimation of MLE) and a standard error (SE) are obtained from the inferential process, in the Bayesian paradigm the whole distribution of the parameters is available.

Similarly, the Bayesian model selection is obtained by assigning a distribution of probability over alternative competing models and, after observing the data, selecting the most promising model as the one with the largest posterior probability. The assignment of probabilities to model parameters is made using both the information captured by the data D and prior knowledge (or beliefs) about the structure of the model, which is encoded by the prior probability $\pi(M)$ of a model M. Then, a typical Bayesian experiment updates the prior distribution $\pi(M)$ to the posterior $\pi(M|D)$ by multiplying the likelihood $\ell(D|M)$ with the prior probability of the model $\pi(M)$, using the Bayes theorem:

$$\pi(M|D) = \frac{\ell(D|M)\pi(M)}{\pi(D)} = \frac{\ell(D|M)\pi(M)}{\sum_i \ell(\,D|M_i)\pi(M_i)} \ ,$$

where $\ell(D|M)$ is the conditional probability of observing the data under the model and $\pi(D)$ is the probability of the data, which can be computed by summing over the conditionals of all possible models.

An alternative way to evaluate which model is most supported by the data D, between two alternative models $M_1$and M_2, is to calculate the so-called Bayes Factor (BF) [52]:

$$BF(M_1, M_2) = \frac{\ell(D|M_1)}{\ell(D|M_2)} = \frac{\frac{\pi(M_1|D)}{\pi(M_1)}}{\frac{\pi(M_2|D)}{\pi(M_2)}} = \frac{\frac{\pi(M_1|D)}{\pi(M_2|D)}}{\frac{\pi(M_1)}{\pi(M_2)}}$$

which is the ratio between posterior odds $\pi(M_1|D)/\pi(M_2|D)$ and prior odds $\pi(M_1)/\pi(M_2)$. The BF can also be interpreted as a Likelihood Ratio test between two competing models M_1 and M_2 when all the uncertainty about nuisance parameters η (i.e., parameters that are of no direct interest but are specified in the model) have been marginalised (integrated) out

$$\frac{\ell(D|M_1)}{\ell(D|M_2)} = \frac{\int \ell(D|M_1, \eta)\pi(\eta)d\eta}{\int \ell(D|M_2, \eta)\pi(\eta)d\eta}$$

without conditioning as in frequentist approaches.

In Bayesian eQTL mapping the observed data typically include a $n \times 1$ vector of outcomes y (i.e., gene expression levels) and a $n \times p$ matrix of predictor variables X (i.e., genetic markers). The set of model parameters, their prior distribution and hence the joint posterior distribution may vary between approaches [53]. The Bayesian models presented here attempt to infer the posterior distribution of the vector of regression coefficients $\beta = (\beta_1, \ldots, \beta_p)^T$, which encodes the effect of markers to the gene expression level, i.e., the eQTLs.

(a) *Univariate regression models*

One class of Bayesian eQTL approaches associates the outcome with one marker "at-a-time", by computing the BF for each SNP (instead of the frequentist *p*-value) [15, 19, 20, 54]. This approach is computationally efficient since only two alternative models M_1 and M_2 are compared each time, i.e., M_1 and M_2, encoding for the inclusion/exclusion of the marker, respectively. In this framework the BF is further simplified

$$BF(M_1, M_2) = \frac{\pi(M_1|D)}{1 - \pi(M_1|D)} \frac{1 - \pi(M_1)}{\pi(M_1)} ,$$

where $\pi(M_1)$ and $\pi(M_1|D)$ are the prior and posterior probability, respectively, that the marker is an eQTL. Markers whose BF exceeds a certain threshold are therefore defined as eQTL (the general criteria for setting the optimal BF threshold based on number of predictors can be found in [15, 55]). Beyond setting the BF threshold, it has been shown that using the BF is superior to conventional *p*-value since the Bayesian-inferred associations can benefit from the elicitation of "biologically primed" informative priors [56, 57], which in some cases can improve power [58].

SNPTEST [59] performs a single-marker eQTL association analysis, i.e., implementing a "one at-a-time" strategy, which incorporates both frequentist and Bayesian association tests. In its Bayesian form, SNPTEST fits a linear regression model that computes the posterior odds of including marker j in the linear regression model $y = \alpha + \beta x_j + \varepsilon$. The error term is normally

distributed $\varepsilon \sim N(0, \sigma^2)$, while the model parameters β, σ^2 are given a conjugate Normal-Inverse-Gamma prior set-up. This regression approach can be extended to map eQTL under dominant or recessive inheritance models: the prior distributions remain the same, but the genotype vector is modified and recoded to reflect dominant or recessive inheritance model. A normal prior is used on β with larger variance, reflecting the assumption that dominant or recessive alleles contribute differently to the phenotypic (i.e., gene expression) variance.

(b) *Bayesian Variable Selection methods*

Initially, most "one at-a-time" Bayesian strategies were applied to GWAS of clinical traits or disease, in which the phenotype (disease trait) was analyzed against a genome-wide panel of genetic predictors (SNPs). When applied to these data and especially to data problem that is typical of eQTL mapping (i.e., large number of *both* expression phenotypes *and* genetic markers), these methods display similar problems as the simple frequentist models described in Section 2; namely, an inflated number of false positive associations and loss of power due to setting arbitrary study-wide significance thresholds [60].

Similarly to penalized-regression models, Bayesian Variable Selection (BVS) methods have been developed for eQTL mapping to analyze jointly the whole set of markers. However, differently from penalized-regression, BVS is able to perform model choice (select the markers that are likely to influence the expression of the gene) and provide parameters estimate (the regression coefficients of the active markers) at the same time [16–18, 61–65]. With both these quantities available, genome-wide significance can be obtained by controlling the FDR level [66]. Here, we describe the major components of BVS and introduce a few computational implementations of this class of approaches.

BVS - Prior set-up: similar to penalized-regression methods, BVS models try to choose few important markers with large effects. Unlike LASSO-type regressions, in BVS sparsity is not only controlled by the prior distribution on the regression coefficients (i.e., the L^p-norm penalty in the frequentist approach), but by specifying an *a priori* number of eQTLs encoded in a latent binary vector $\gamma = (\gamma_1, \dots, \gamma_p)^T$, where $\gamma_j = 1$ if $\beta_j \neq 0$ and $\gamma_j = 0$ if $\beta_j = 0$. In a nutshell, BVS can control *both* the level of shrinkage and *the* number of non-zero eQTL effects that can be detected. These two tasks are tightly connected in BVS given the prior specification of the regression coefficients [67]:

$$\beta | \gamma, \sigma^2 \sim N\left(0, g\sigma^2 \left({X_\gamma}^T X_\gamma\right)^\lambda\right).$$

The equation above states that for the selected markers, i.e., for markers with $\gamma_j = 1$ since $\beta_j \neq 0$, the prior distribution on vector of regression coefficients is normal distributed and centered in zero. The covariance matrix can be the unit diagonal matrix, giving rise to the so-called independent prior, if $\lambda = 0$ or the inverse of the covariance matrix which characterize the so-called *g*-prior if $\lambda = -1$, multiplied by a constant g and the residual variance σ^2. Under this specification the linear regression model becomes $y = \alpha + X_\gamma \beta_\gamma + \varepsilon$,

$\varepsilon \sim N(0, \sigma^2)$, where γ, the vector of binary values indicating which markers are selected and therefore their number, receives a Binomial prior distribution

$$\pi(\gamma) = Bin(p, \theta)$$

where θ can be a fixed parameter or a further level of hierarchy can be specified [1]. The prior distribution for the model parameters β, σ^2 usually follows a Normal-Inverse-Gamma set-up [16, 17, 21] with a different specification for the power-prior λ. The choice of $\lambda = -1$ is particularly appealing in eQTL studies since an *a priori g*-prior "discourages" highly collinear predictors to enter the models simultaneously by inducing a negative correlation between the coefficients, therefore controlling LD structure automatically. On the opposite side with $\lambda = 0$, the regression coefficients are *a priori* mutually independent [59] although, given the influence of the likelihood, this does not hold *a posteriori*. It turns out that *a priori* independent prior is less capable in handling intricate correlations between markers, but its use is encouraged because it induces, like the ridge estimator, an absolute shrinkage to the regression coefficients, i.e., it shrinks greatly in directions of small eigenvalues, whereas the *g*-prior proportional shrinkage retains much more of the OLS estimator in ill-conditioned directions [68].

The specification of the prior distribution for the model parameters β, σ^2 is an active area of research in Bayesian statistics, since different prior set-ups imply different levels of shrinkage. Sparsity-inducing prior set-ups include the Laplace prior [69], the spike-and-slab priors [62, 63], the horseshoe shrinkage prior [70] and local adaptation priors [17, 64] – analyzing the different features of these set-ups in detail goes beyond the scope of this review. However, here we mention that in the piMASS eQTL mapping method [17] a novel prior set-up is used on the expected genetic effect sizes linking them with the model size. In particular, the effect size prior demonstrates the biologically primed idea that if the model size is small, then the few associated markers will have large effect sizes – the opposite is expected when then model size is large. This exemplifies how the Bayesian setting can effectively leverage "biologically informed" priors to improve ad refine eQTL detection.

BVS - Model selection and posterior computation: in typical genomics and eQTL mapping experiments the number of predictor variables is too large to enumerate all possible combinations of latent binary vector γ. Therefore search algorithms are used to explore the model space. Most methods use Markov Chain Monte Carlo (MCMC) [17, 18, 62–64], a sampling technique in which the posterior distribution $\pi(\gamma|D)$ is simulated using Markov Chain algorithms. The idea behind is that not all the 2^p possible models (i.e., combination of markers) need to be simulated, since the majority of them are unable to explain the data with $\pi(\gamma|D) \approx 0$. On the contrary, it is more efficient to concentrate the markers' space exploration on important models with large $\pi(\gamma|D)$. In a post-processing analysis, $\pi(\gamma|D)$ can be used to rank the visited models and decide which one to report. The output provided by MCMC algorithm is very rich since the sampled distribution of $\pi(\gamma|D)$ is available (apart from non-interesting models with $\pi(\gamma|D) \approx 0$). However, this comes at a price since MCMC algorithms are computational intensive and, as for frequentist penalized-regression methods, rather time consuming. If the goal

of the analysis is to report only the top visited model, alternative faster sampling algorithms based on the expectation maximization (EM) algorithm [71] have been recently proposed [72].

There is a very large literature on the MCMC sampling schemes that can be used to sample realizations of $\pi(\gamma|D)$. The simplest MCMC algorithm that can be implemented is the Gibbs sampling [73], which is particular suitable when spike-and-slab priors are specified for the regression coefficients [18, 62, 63]. Since spike-and-slab priors can be seen as two-point mixture distribution, once conditioning on value of *j*th binary latent variable γ_j, the posterior distribution of the *j*th "spike" or the "slab" is ready available and it is relatively simple to simulate from. The drawback of this approach is that it tends to mix slowly when there are correlated predictors (e.g., in the presence of LD between the markers), since the posterior distribution of regression coefficient for the *j*th predictor depends on the neighbour predictors and if a marker has been selected, $\gamma_j = 1$, markers in strong LD with it will be selected as well. It turns out that the algorithm may be stuck in a particular configuration of γ for many iterations of the MCMC algorithm (slow mixing) without being able to detect the optimal combination of predictive markers. To overcome this problem, MCMC algorithms that explore more efficiently the model space have been proposed. For instance, using a "shotgun" stochastic search [74] one can explore the entire neighbour of the current model and randomly pick up with a non-uniform probability a model from that list. For instance in piMASS [17], once a model has been selected, the next active marker that can be included in the model is the one that shows the (residual) highest absolute correlation with the phenotype so that correlated predictors are less likely to be included in the model. The Evolutionary Stochastic Search method [16, 61, 75, 76], which we will discuss in detail below, has been designed for a more efficient and far-reaching exploration of the model space. It runs several parallel MCMC samplers that swap information about the different configurations of markers selected in each chain and therefore avoiding the slow mixing phenomenon described above.

BVS - Posterior summary and interpretation: a large number of MCMC iterations are generally required in order to match the frequency a particular model has been sampled, $\hat{\pi}(\gamma|D)$, with the theoretical posterior probability of that model, $\pi(\gamma|D)$. In that case the algorithm is said to have reached convergence. From a practical point of view, assessing convergence of MCMC is not easy and many diagnostic measures can be applied to detect any anomalous behavior of the algorithm. Moreover the initial draws of the algorithm (burn-in phase) are usually discarded because it may be possible that the models are sampled with the wrong frequency compared with the correct theoretical probability with some models over-represented or *vice versa* during the initial phase. All the models visited by the search algorithm after the burn-in are kept and summarized into a marginal posterior probability of inclusion (MPPI) $\hat{\pi}\left(\gamma_j = 1 \middle| D, \gamma_{\setminus j}\right)$, which indicates the frequency the *j*th marker has been selected in the models visited by the search algorithm. Despite its straightforward interpretation (MPPI = the probability that the marker *j* explains the variation of the gene expression given all other markers), the use of MPPI alone in variable selection by setting a threshold is not recommended apart for prediction [77], as there is no direct interpretation

of it with respect to effect size or for controlling the FDR. However the classification of the MPPI into two groups will allow the assessment of their genome-wide significance. Specifically, one can employ the EM algorithm to fit a mixture of two beta distributions and then use the classification probabilities to derive the FDR, as described in [78]. Alternatively, versatile R packages that can estimate local (tail-area) FDR from the posterior distribution [79], are also available [80].

piMASS [17] is a BVS algorithm for eQTL mapping with a new regression coefficients' prior variance that allows either models with a large number of predictors with a small proportion of variance explained (PVE) or a small number of predictors with a large PVE. This prior set-up is in tune with what is expected in typical eQTL mapping experiments, where few *cis*-eQTLs are present with large effects and large PVE, whereas many *trans*-eQTLs have relatively smaller effects and smaller PVE. Its implementation is based on a single MCMC chain with a sampling strategy that explores models made by faraway and/or uncorrelated genetic markers.

Another BVS algorithm is Evolutionary Stochastic Search (ESS) [16, 21, 61, 76] in which the level of sparsity can be controlled directly by the user specifying the *a priori* expected number of predictors to be included in the model and its variance. Moreover given the prior structure on the regression coefficients that can be thought as a mixture of *g*-priors and an Inverse-Gamma prior [81], the level of proportional shrinkage automatically adapts to different real data scenarios. ESS uses an advanced stochastic search algorithm in which multiple models are explored by parallel MCMC samplers. Specifically, at each iteration, each chain locally selects a different model using local moves based on the Gibbs sampler [73] or a fast version of the Metropolis-Hasting algorithm [82]. Global moves, which allow the exchange of information between parallel chains about the models selected, are also implemented, using a MCMC version of genetic algorithms [83]. The combination of local and global moves allows the efficient exploration of the model space and prevents the algorithm from getting stuck to a sub-optimal model made by highly correlated predictors (i.e., genetic markers in high LD).

A concise list of the most commonly used Bayesian eQTL mapping methods and their software implementation is reported in **Table 2**.

Table 2. Bayesian eQTL mapping approaches

Strategy	Method [ref] and availability	Statistic	Additional features	Multi-tissue	Genetic models
Univariate	SNPTEST [15] https://mathgen.stats.ox.ac.uk/	BF	• Bayesian and frequentist and analysis (see **Table 1**) • Covariates can be included in the model • Imputation of missing genotypes	Yes	• Additive • Dominant • Recessive • Heterozygote • General
	Sherlock [19] http://sherlock.ucsf.edu/	BF	• Integration of known GWAS hits	No	Additive
	eQTLBMA [20] https://github.com/timflutre/eqtlbma	BF	Multiple tissues, while allowing different eQTLs per tissue	Yes	Additive
BVS	ESS [16, 21, 61, 76] www.bgx.org.uk/software/guess.html (command-line implementation) https://cran.r-project.org/package=R2GUESS (R implementation)	• MPPI • Best models visited	• Covariates can be included in the model • FDR control • Extension for eQTLs hotspots [75] • Extension for eQTLs hotspots in multiple tissues [84]	Yes	Additive
	piMASS [17] http://www.haplotype.org/pimass.html	MPPI	• Linear and logistic regression	No	Additive
	iBMQ [18] https://www.bioconductor.org/packages/release/bioc/html/iBMQ.html	MPPI	• FDR control • Extension for eQTLs hotspots	No	Additive

4. Multi-tissue extensions

Transcriptomic studies can assess gene expression levels in multiple tissues or cell-types in order to understand the mechanism of gene regulation at the systems-level, including mapping of eQTLs in multiple systems [85]. While expression of certain genes and pathways can be conserved across different tissues, intersecting results from several single-tissue eQTL analyses (for instance by imposing the same FDR threshold in each eQTL study) may be too conservative and can lead to inflated false negative rate [84]. In contrast, utilizing a cross-tissue analysis of eQTLs by jointly mapping gene expression profiles from multiple tissues, has been shown to increase power to detect small effect eQTLs (specifically, *trans*-eQTLs) [20–22].

Several eQTL mapping approaches, including some discussed above, have been extended to allow eQTL mapping of tissue-consistent QTLs (i.e., eQTLs that are detected across multiple tissues), by allowing BVS models to analyze multivariate outcomes. Thus, assuming an experiment with n samples, p predictors and q outcomes (tissues or cell-types), the multiple outcome linear regression model can be written as:

$$Y = A + x_1 B_1 + \cdots + x_p B_p + E, \qquad E \sim MN(0, I_n, \Sigma),$$

where Y is a $n \times q$ matrix of outcomes, A is a $n \times q$ matrix of intercepts, x_j is the *j*th predictor encoded in a $n \times 1$ vector and $B_j = (\beta_{j,1}, \ldots, \beta_{j,q})$ is the vector of regression coefficients that links the *j*th predictor with the multiple outcomes Y. Finally, E is the $n \times q$ matrix of errors that is distributed as a matrix-variate normal distribution centered in zeros, with the matrix Σ that controls the residual correlation between the q outcomes.

The above equation can be seen as the multiple-outcome extension of the linear model and both SNPTEST [15] and ESS [21, 76] come with this multivariate outcome extensions. Both algorithms use a similar prior set-up, modeling the matrix of regression coefficients $B = (B_1, \ldots, B_p)^T$ by a matrix-variate normal prior $|\Sigma \sim MN\left(g\left({X_\gamma}^T X_\gamma\right)^\lambda, \Sigma\right)$, where $\left({X_\gamma}^T X_\gamma\right)^\lambda$ is the correlation matrix between the selected markers with $\lambda = 0$ in SNPTEST and $\lambda = -1$ in ESS and Σ is the $q \times q$ matrix modelling the correlation between outcomes (i.e., gene expression levels in different tissues). The model is further specified by placing an Inverse-Wishart prior on Σ, $\Sigma \sim IW(c, Q)$, where c indicates the degrees of freedom and Q is proportional to the expected residual variance. eQTLBMA [20] is another eQTL mapping method that is designed to handle multi-tissue eQTLs, again using a matrix-variate normal prior set-up – but it also uses a hierarchical model which permits heterogeneity between tissues, to allow the estimate of genetic effects both between- and within-tissues.

Frequentist approaches for multiple-tissue eQTL analysis have also been implemented: for example the multivariate version of the ANOVA model (MANOVA) or the Wilks' test statistic [86], a generalization of the F-statistic for multivariate random variables [87]. Multiple-outcome penalized-regression approaches have also been proposed [43, 88], while the R package glmnet [50] includes options that fit multiple-outcome Gaussian models. However,

controlling for FWER and FDR is more challenging than in the case of univariate penalized linear regression, and extensions of stability selection [47] for the multivariate problem are still in the stage of development. As a result, interpretation of the multi-tissue eQTL results from multivariate penalized-regression has to be based on the value of regression coefficients, and so thresholding can be a challenge.

5. Empirical comparison of frequentist and Bayesian eQTL mapping

In this section we present an illustrative example of previously reported eQTL mapping for the *Hopx* gene, which in the rat has been shown to be under control by two loci on chromosome 14 (*cis*-eQTL) and chromosome 2 (*trans*-eQTL), respectively; where both *cis*- and *trans*-eQTLs have been experimentally validated [21]. Rather than providing a comprehensive comparison of eQTL mapping methods (systematic simulation studies that compare methods in a variety of scenarios can be found in [76]), our purpose here is to use this empirical eQTL mapping example to facilitate discussion on the comparison between frequentist and Bayesian eQTL mapping approaches. In this eQTL mapping exercise, we have used microarray gene expression data for the *Hopx* gene in two tissues (heart and fat) from 29 recombinant inbred (RI) rat strains (generated by sibling-mating the offspring of a genetic cross until the progenies are inbred), genotyped at 1,307 SNPs. Since rats within an RI strain have complete homozygosity at each locus in the genome, each genetic marker allows splitting the rat population in two groups. We considered *(i)* a single-tissue example using gene expression data from the heart only and *(ii)* a multi-tissue example using gene expression data from both tissues.

(i) *Single-tissue example*

We mapped genome-wide eQTLs for the heart gene expression data using three frequentist (Matrix-eQTL [7], Kruskal-Wallis test [8] and LASSO from the R package glmnet [50]) and three Bayesian methods (SNPTEST [15], ESS [16, 61], piMASS [17]) - see **Tables 1-2** for reference. The parameters and eQTL analysis details are provided in the table below and the eQTL results from all methods are reported in **Figure 1**.

Method	Genome-wide eQTL analysis details
Matrix-eQTL	We used the linear additive model as the genotypes are binary. *p*-values were adjusted using the Benjamini-Yekutieli FDR method [89]. We selected eQTL associations at 1% FDR.
Kruskal-Wallis test	The test is the non-parametric equivalent of a one-way ANOVA. We used the kruskal.test function in R to extract *p*-values, and selected eQTLs at 1% FDR employing Benjamini-Yekutieli method.
Glmnet-LASSO	We performed 9-fold cross-validation using the function cv.glmnet, setting alpha = 1 and family = “gaussian”. After obtaining estimates on the regression coefficients, these were transformed in posterior probabilities by using stability selection method, implemented in the R package stabs [90]. We declared significance with a threshold of 0.2 on the posterior probabilities.

SNPTEST	We ran the Bayesian version of SNPTEST-v.2.5.2 with $\beta \sim N(0,0.02\sigma^2)$, $\sigma^2 \sim IG(3,2)$ as priors (-prior_qt_mean_b 0 -prior_qt_V_b 0.02 -prior_qt_a 3 -prior_qt_b 2). We called eQTLs at $\log_{10} BF \geq 0.25$.
piMASS	We ran piMASS-v.0.90 setting the prior probability that a SNP is truly associated with the phenotype to range between 1 and 56 (-pmin 1 –pmax 56) and the model size to range from 1 to 100 (-smin 1 –smax 100). We did not impose constraints on the hyperparameter h and to the minor allele frequency (-exclude_maf 0). The burn-in phase was set to 10^6 iterations, followed by 10^7 sampling iterations, while only one every 10 models considered by the sampling steps was recorded (-w 1,000,000 –s 10,000,000 –num 10). We computed the FDR on the marginal posterior probabilities of inclusion (MPPI) by fitting a mixture of beta distributions, as described in [91].
ESS	We ran GUESS-v.1.1, setting the a priori expected model size to $E = 5, S = 3$ (-Egam 5 –Sgam 3) and ran 25,000 steps of which the first 5,000 as burn-in (-nsweep 25,000 –burn_in 5,000). We computed FDR on the MPPI provided by ESS in the same way as described above for piMASS algorithm.

All six approaches detected a clear *cis*-QTL signal on rat chromosome 14 (close to the location of *Hopx* gene), although for the ANOVA and SNPTEST the level of significance reached at the *cis*-eQTL is only a little higher than the rest of the genome. In this example, Glmnet-LASSO and ESS are the only methods that unambiguously detect a *trans*-eQTL signal on chromosome 2. However, Glmnet-LASSO is also picking an additional eQTL signal on chromosome 3. Therefore, in this example, the classic method that implements penalization (Glmnet-LASSO) and one of the Bayesian approaches that uses sparsity (ESS), show good performance in detecting both the *cis*- and *trans*-eQTL signals (however, Glmnet-LASSO is also picking an comparable eQTL signal on chromosome 3). The most striking observation that we can derive from this empirical analysis is that widely used frequentist methods (e.g., Matrix-eQTL) which employ a “one at-a-time” strategy were not able to detect the *trans*-eQTL signal on rat chromosome 2 (with both the *cis*- and *trans*-eQTL signals experimentally validated, as previously reported in [21]), therefore highlighting an important limitation of this approach.

(ii) *Multiple-tissue example*

For the second illustrative example, we ran multivariate ANOVA, Glmnet-LASSO and ESS to jointly map eQTLs for *Hopx* gene expression levels across heart and fat tissues from the 29 rat RI strains used for single-tissue eQTL analysis. The parameters and eQTL analysis details are provided in the table below and the eQTL results in heart and fat tissues from all methods are reported in **Figure 2.**

Method	**Genome-wide eQTL analysis details**
MANOVA	We ran a Wilks’ test using the R function wilks.test setting method = “rank”, and selected associations at 1% FDR employing Benjamini-Yekutieli method.
Glmnet-LASSO	We set parameters to cv.glmnet in the same way as in the single-tissue analysis, but specified family = “mgaussian” to perform multivariate analysis.
ESS	The prior set-up was the same as in single-tissue analysis described in the table above, but we instead ran 110,000 sampling steps, of which 10,000 were burn-in. No further specification for multi-outcome analysis is required by ESS

	that automatically recognises the multivariate nature of the matrix Y.

Similarly to the results of eQTL analysis in the single tissue, all three methods unambiguously identified a strong *cis*-effect on rat chromosome 14, which therefore suggests the presence of a common *cis*-eQTL in heart and fat tissues. However, only the Glmnet-LASSO and ESS methods were able to identify an additional *trans*-eQTL on rat chromosome 2, suggestive of common *trans*-regulation between the two tissues (as previously shown for other *trans*-eQTL signals conserved across multiple tissues in this genetic system [91]). Glmnet-LASSO identifies the two eQTLs without identifying false-positives, although the signal from the *trans*-effect is much weaker than that of the *cis-effect*. One important issue with Glmnet-LASSO is in the output provided by the algorithm: although the regression coefficients of the selected markers for the two tissues are clearly reported there is not a simple way to combine them and to transform the tissue-specific effects into a posterior probability, for instance, by the stability selection procedure. In the same eQTL example ESS picks up with a very low MPPI an additional signal from chromosome 10, which is likely to be a false positive. A noteworthy observation that can be derived from the results of the ESS analysis is that the MPPI of the *trans*-effect is almost doubled in multiple-tissues compared to the single tissue analysis, highlighting the advantage of combining information from multiple sources (in this case tissues). From a biological viewpoint, when compared the MPP of the same *trans*-eQTL detected in the single-tissue analysis (**Figure 1**), the signal in the multi-tissue maybe reflects a potential pleiotropic nature of this eQTL.

6. Discussion and outlook

We discussed the challenges in eQTL mapping and reviewed several commonly used approaches, including their advantages and advantages. In particular, we emphasized the useful features provided by the Bayesian methods. Using a simple yet informative example of polygenic regulation of gene expression in the rat, we illustrated the major differences between frequentist and Bayesian eQTL mapping approaches. In this, we first focused on single-tissue eQTL mapping (**Figure 1**), where both *cis*- and *trans*-signals have been previously experimentally validated [21]. We used this demonstrative example to show that frequentist approaches based on a computational efficient strategy that tests for association all transcript-marker pairs (“one at-a-time”) were not suitable to detected polygenic control of gene expression. In contrast, methods based on multivariate models, either frequentist (LASSO) or Bayesian (ESS), were able to detect both eQTLs, although ESS performed marginally better as it eliminated possible false positive associations identified by the LASSO-based approach. We then extended this example to include gene expression data from two tissues for the same gene: the eQTL results were very similar to what observed in the two single tissue cases, with the Bayesian variable selection method detecting unambiguously both *cis*- and *trans*-eQTLs (**Figure 2**). This example also highlighted the benefits of using multiple tissues for simultaneous eQTL mapping since, by joint modelling the dependence between tissues, it further increased the power to detect (small-effect) *trans*-eQTLs compared to the

single-tissue experiment [20–22].

For Bayesian approaches, the ability to handle the whole set of predictors (genome-wide genetic markers) and model their correlation (i.e., accounting for LD structure) as well as providing the whole posterior distribution of the parameters come at a price. The more traditional (frequentist) eQTL approaches (such as Matrix-eQTL [7]) have the attractive feature of computational efficiency compared to the more demanding BVS methods. This might account for the common application of frequentist eQTL mapping methods in biomedical research. However, as highlighted in our illustrative examples, the high computational efficiency of frequentist approaches might come at the expenses of missing polygenic control of gene expression. This can have important implications when both *cis*- and *trans*-eQTLs are investigated at the genome-wide level, usually resulting in a smaller fraction of "replicable" *trans*-eQTLs as compared with *cis*-eQTLs, and advocating the use of larger populations to boost detection of small *trans*-effects [92].

However, recent advancements in high-performance computing have rendered the application of MCMC methods feasible even for hundreds of thousands of predictors in hundreds (if not thousands) of individuals [76] – which now justifies the increasing popularity of the Bayesian eQTL mapping methods. In contrast, although recent advances in the computational aspects of the LASSO solution [93], frequentist penalized-regression methods still need time-consuming cross-validation procedure to estimate the penalty parameter λ. In the case of Elastic Net a two-dimensional grid is required in order to select the optimal λ_1, λ_2 penalties. Selecting the optimal parameters however, necessitates a very fine-grained grid of penalties to be analyzed, which is even more computationally expensive.

Regarding interpretation of the eQTL results, in BVS approaches all the models visited by the search algorithm (after the burn-in) are kept and summarized into a marginal posterior probability of inclusion (MPPI). Penalized-regression models usually output estimates of regression coefficient values, which can vary largely between experiments and therefore are less safe for declaring eQTL associations consistently across studies. Moreover, estimation of the FDR from the regression coefficients is not possible, so one is limited to controlling family-wise error rates, a more conservative approach that can lead to false negatives. In contrast, several techniques that control the FDR from the MPPI are now available, making the genome-wide control of the significance level less of a problem for Bayesian eQTL methods. In addition, although not directly investigated in our illustrative examples, the Bayesian prior set-up offers more flexibility to consider (and explore) different eQTL models, for example by specifying the number of expected eQTLs and their effect size or by using genomic locations of the transcripts to improve the accuracy of the posterior distribution for the location of the eQTL [94].

In summary, we advocate that Bayesian approaches are in general more flexible to analyze complex genetic regulation of expression than frequentist methods. In particular, Bayesian eQTL mapping strategies can adapt naturally to a wider range of applications, such as (*i*) detection of polygenic effects on gene expression [21], (*ii*) epistatic eQTL interactions [63], (*iii*) eQTLs hotspots

[75] and (*iv*) eQTLs and eQTLs hotspots across multiple-tissues [21, 75, 84]. We also argue that using Bayes Factors might provide a more objective way to call statistically significant eQTLs [55, 95] and compare them across studies. Conversely, using computationally inexpensive *p*-values generated by frequentist approaches to call significant eQTLs requires a threshold for genome-wide significance that can varies largely with sample size as well as with other study-specific factors. While this issue is well known and yet often ignored, it is likely to be highly relevant to the development of reference eQTL databases and resources. Since eQTL analyses have been proved useful in the identification of molecular pathways affecting disease susceptibility, e.g., [6, 91, 96, 97], it is generally advisable to use truly multivariate eQTL mapping strategies that can provide more flexibility in modeling complex data structures and can have enhanced interpretability of the results. In this respect, Bayesian mapping approaches now provide a valid alternative to traditional "one at-a-time" frequentist methods and a richer and easy to interpret output than penalized-regression methods.

Figure legends

Figure 1. For each SNP genotyped in the rat genome (*x*-axis), for each method we report the evidence in support of genetic regulation of *Hopx* gene expression in the heart tissue (*y*-axis). The input consisted of $n \times 1$ expression values and a $n \times p$ matrix of predictors (genome-wide SNPs), where n = 29 and p = 1,307. Black dots, associations called at 1% FDR. Boxes highlight the chromosomal locations where the *cis*- and *trans*-eQTLs are located, respectively.

Figure 2. For each SNP genotyped in the rat genome (*x*-axis), for each method we report the evidence in support of genetic regulation of *Hopx* gene expression simultaneously in the heart and fat tissues (*y*-axis). The input consisted of $n \times 2$ expression values (fat and heart, respectively) and a $n \times p$ matrix of predictor variables (genome-wide SNPs), where n = 29 and p = 1,307. Black dots, associations called at 1% FDR. For the Glmnet-LASSO, the blue and black dots indicate the absolute values of β-coefficients estimated in fat and heart tissues, respectively. Boxes highlight the chromosomal locations where the *cis*- and *trans*-eQTLs are located, respectively.

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References

1. Guo H, Fortune MD, Burren OS, et al. (2015) Integration of disease association and eQTL data using a Bayesian colocalisation approach highlights six candidate causal genes in immune-mediated diseases. Hum Mol Genet 24:3305–13. doi: 10.1093/hmg/ddv077

2. Pierce BL, Tong L, Chen LS, et al. (2014) Mediation analysis demonstrates that trans-eQTLs are often explained by cis-mediation: a genome-wide analysis among 1,800 South Asians. PLoS Genet 10:e1004818. doi: 10.1371/journal.pgen.1004818

3. Kang H, Kerloc'h A, Rotival M, et al. (2014) Kcnn4 is a regulator of macrophage multinucleation in bone homeostasis and inflammatory disease. Cell Rep 8:1210–24. doi: 10.1016/j.celrep.2014.07.032

4. Rotival M, Zeller T, Wild PS, et al. (2011) Integrating genome-wide genetic variations and monocyte expression data reveals trans-regulated gene modules in humans. PLoS Genet 7:e1002367. doi: 10.1371/journal.pgen.1002367

5. Fehrmann RSN, Jansen RC, Veldink JH, et al. (2011) Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. PLoS Genet 7:e1002197. doi: 10.1371/journal.pgen.1002197

6. Small KS, Hedman AK, Grundberg E, et al. (2011) Identification of an imprinted master trans regulator at the KLF14 locus related to multiple metabolic phenotypes. Nat Genet 43:561–4. doi: 10.1038/ng.833

7. Shabalin A a (2012) Matrix eQTL: ultra fast eQTL analysis via large matrix operations. Bioinformatics 28:1353–8. doi: 10.1093/bioinformatics/bts163

8. MacDonald JH (2009) Kruskal-Wallis Test. Biol Handb Stat 165–172. doi: 10.1002/9780470479216.corpsy0491

9. Yang T-P, Beazley C, Montgomery SB, et al. (2010) Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 26:2474–2476. doi: 10.1093/bioinformatics/btq452

10. Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–90.

11. Clayton D, Leung H-T (2007) An R Package for Analysis of Whole-Genome Association Studies. Hum Hered 64:45–51. doi: 10.1159/000101422

12. Sun W (2009) eQTL Analysis by Linear Model. In: http://www.bios.unc.edu/~weisun/software/eMap.pdf. http://www.bios.unc.edu/~weisun/software/eMap.pdf. Accessed 20 Oct 2015

13. Qi J, Asl HF, Björkegren J, Michoel T (2014) kruX: matrix-based non-parametric eQTL discovery. BMC Bioinformatics 15:11. doi: 10.1186/1471-2105-15-11

14. Gao C, Tignor NL, Salit J, et al. (2014) HEFT: eQTL analysis of many thousands of expressed genes while simultaneously controlling for hidden factors. Bioinformatics 30:369–76. doi: 10.1093/bioinformatics/btt690

15. Marchini J, Howie B, Myers S, et al. (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 39:906–13. doi: 10.1038/ng2088

16. Bottolo L, Chadeau-hyam M, Hastie DI, et al. (2011) ESS++: A C++ objected-oriented algorithm for Bayesian stochastic search model exploration. Bioinformatics 27:587–588. doi: 10.1093/bioinformatics/btq684

17. Guan Y, Stephens M (2011) Bayesian variable selection regression for genome-wide association studies and other large-scale problems. Ann Appl Stat 5:1780–1815.

18. Scott-Boyer MP, Imholte GC, Tayeb A, et al. (2012) An integrated hierarchical Bayesian model for multivariate eQTL mapping. Stat Appl Genet Mol Biol. doi: 10.1515/1544-6115.1760

19. He X, Fuller CK, Song Y, et al. (2013) Sherlock: detecting gene-disease associations by matching patterns of expression QTL and GWAS. Am J Hum Genet 92:667–80. doi: 10.1016/j.ajhg.2013.03.022

20. Flutre T, Wen X, Pritchard J, Stephens M (2013) A Statistical Framework for Joint eQTL Analysis in Multiple Tissues. PLoS Genet. doi: 10.1371/journal.pgen.1003486

21. Petretto E, Bottolo L, Langley SR, et al. (2010) New insights into the genetic control of gene expression using a Bayesian multi-tissue approach. PLoS Comput Biol 6:e1000737. doi: 10.1371/journal.pcbi.1000737

22. Sul JH, Han B, Ye C, et al. (2013) Effectively Identifying eQTLs from Multiple Tissues by Combining Mixed Model and Meta-analytic Approaches. PLoS Genet 9:e1003491. doi: 10.1371/journal.pgen.1003491

23. Brem RB, Yvert G, Clinton R, Kruglyak L (2002) Genetic dissection of

transcriptional regulation in budding yeast. Science 296:752–5. doi: 10.1126/science.1069516

24. Dudoit S, Yang YH, Callow MJ, Speed TP (2002) Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. Stat Sin 12:111–139. doi: 10.1146/annurev.psych.53.100901.135153

25. Gerrits A, Li Y, Tesson BM, et al. (2009) Expression quantitative trait loci are highly sensitive to cellular differentiation state. PLoS Genet 5:e1000692. doi: 10.1371/journal.pgen.1000692

26. Zhou X, Stephens M (2014) Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nat Methods 11:407–9. doi: 10.1038/nmeth.2848

27. Narahara M, Higasa K, Nakamura S, et al. (2014) Large-Scale East-Asian eQTL Mapping Reveals Novel Candidate Genes for LD Mapping and the Genomic Landscape of Transcriptional Effects of Sequence Variants. PLoS One 9:e100924. doi: 10.1371/journal.pone.0100924

28. Duggal G, Wang H, Kingsford C (2014) Higher-order chromatin domains link eQTLs with the expression of far-away genes. Nucleic Acids Res 42:87–96. doi: 10.1093/nar/gkt857

29. Gatti DM, Shabalin AA, Lam T-C, et al. (2009) FastMap: fast eQTL mapping in homozygous populations. Bioinformatics 25:482–9. doi: 10.1093/bioinformatics/btn648

30. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289–300. doi: 10.2307/2346101

31. (2013) The Genotype-Tissue Expression (GTEx) project. Nat Genet 45:580–5. doi: 10.1038/ng.2653

32. Hoerl AE, Kennard RW (1970) Ridge Regression: Biased Estimation for Nonorthogonal Problems. Technometrics 12:55–67. doi: 10.1080/00401706.1970.10488634

33. Tibshirani R (2011) Regression shrinkage and selection via the lasso: A retrospective. J R Stat Soc Ser B Stat Methodol 73:273–282. doi: 10.1111/j.1467-9868.2011.00771.x

34. Wu TT, Chen YF, Hastie T, et al. (2009) Genome-wide association analysis by lasso penalized logistic regression. Bioinformatics 25:714–721. doi: 10.1093/bioinformatics/btp041

35. Zou H (2006) The Adaptive Lasso and Its Oracle Properties. J Am Stat Assoc 101:1418–1429. doi: 10.1198/016214506000000735

36. Tibshirani R, Saunders M, Rosset S, et al. (2005) Sparsity and smoothness via the fused lasso. J R Stat Soc Ser B Stat Methodol 67:91–108. doi: 10.1111/j.1467-9868.2005.00490.x

37. Zou H, Hastie T (2005) Regularization and variable selection via the elastic net. J R Stat Soc Ser B Stat Methodol 67:301–320. doi: 10.1111/j.1467-9868.2005.00503.x

38. Yuan M, Lin Y (2006) Model selection and estimation in regression with grouped variables. J R Stat Soc Ser B (Statistical Methodol 68:49–67. doi: 10.1111/j.1467-9868.2005.00532.x

39. Kim S, Xing EP (2009) Statistical estimation of correlated genome associations to a quantitative trait network. PLoS Genet. doi: 10.1371/journal.pgen.1000587

40. Wang W, Zhang X (2011) Network-based group variable selection for detecting expression quantitative trait loci (eQTL). BMC Bioinformatics 12:269.

41. Lee S, Xing EP (2012) Structured Input-Output Lasso, with Application to eQTL Mapping, and a Thresholding Algorithm for Fast Estimation. arXiv.org

42. Cheng W, Zhang X, Guo Z, et al. (2014) Graph-regularized dual Lasso for robust eQTL mapping. Bioinformatics 30:139–148. doi: 10.1093/bioinformatics/btu293

43. Kim S, Xing EP (2012) Tree-guided group lasso for multi-response regression with structured sparsity, with an application to eQTL mapping. Ann Appl Stat 6:1095–1117. doi: 10.1214/12-AOAS549

44. Leng C, Lin Y, Wahba G (2006) A note on the lasso and related procedures in model selection. Stat Sin 16:1273–1284.

45. Rakitsch B, Lippert C, Stegle O, Borgwardt K (2013) A Lasso multi-marker mixed model for association mapping with population structure correction. Bioinformatics 29:206–214. doi: 10.1093/bioinformatics/bts669

46. Brown AA, Richardson S, Whittaker J (2011) Application of the Lasso to Expression Quantitative Trait Loci Mapping. Stat Appl Genet Mol Biol 10:1–35. doi: 10.2202/1544-6115.1606

47. Meinshausen N, Bühlmann P (2010) Stability selection. J R Stat Soc Ser B (Statistical Methodol 72:417–473. doi: 10.1111/j.1467-9868.2010.00740.x

48. Shah RD, Samworth RJ (2013) Variable selection with error control:

another look at stability selection. J R Stat Soc Ser B (Statistical Methodol 75:55–80. doi: 10.1111/j.1467-9868.2011.01034.x

49. Waldmann P, Mészáros G, Gredler B, et al. (2013) Evaluation of the lasso and the elastic net in genome-wide association studies. Front Genet 4:270. doi: 10.3389/fgene.2013.00270

50. Friedman J, Hastie T, Tibshirani R (2010) Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw 33:1–22. doi: 10.18637/jss.v033.i01

51. Beaumont MA, Rannala B (2004) The Bayesian revolution in genetics. Nat Rev Genet 5:251–61. doi: 10.1038/nrg1318

52. Kass RE, Raftery AE (1995) Bayes Factors. J Am Stat Assoc 90:773–795. doi: 10.2307/2291091

53. O'Hara RB, Sillanpää MJ (2009) A review of Bayesian variable selection methods: what, how and which. Bayesian Anal 4:85–117.

54. Servin B, Stephens M (2007) Imputation-based analysis of association studies: Candidate regions and quantitative traits. PLoS Genet 3:1296–1308. doi: 10.1371/journal.pgen.0030114

55. Stephens M, Balding DJ (2009) Bayesian statistical methods for genetic association studies. Nat Rev Genet 10:681–690. doi: 10.1038/nrg2615

56. Lee S-I, Dudley AM, Drubin D, et al. (2009) Learning a prior on regulatory potential from eQTL data. PLoS Genet 5:e1000358. doi: 10.1371/journal.pgen.1000358

57. Das A, Morley M, Moravec CS, et al. (2015) Bayesian integration of genetics and epigenetics detects causal regulatory SNPs underlying expression variability. Nat Commun 6:8555. doi: 10.1038/ncomms9555

58. Balding DJ (2006) A tutorial on statistical methods for population association studies. Nat Rev Genet 7:781–91. doi: 10.1038/nrg1916

59. Marchini J, Howie B, Myers S, et al. (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 39:906–13. doi: 10.1038/ng2088

60. Kendziorski CM, Chen M, Yuan M, et al. (2006) Statistical methods for expression quantitative trait loci (eQTL) mapping. Biometrics 62:19–27. doi: 10.1111/j.1541-0420.2005.00437.x

61. Bottolo L, Richardsony S (2010) Evolutionary stochastic search for bayesian model exploration. Bayesian Anal 5:583–618. doi: 10.1214/10-BA523

62. Zhang M, Montooth KL, Wells MT, et al. (2005) Mapping multiple Quantitative Trait Loci by Bayesian classification. Genetics 169:2305–18. doi: 10.1534/genetics.104.034181

63. Zhang M, Zhang D, Wells MT (2008) Variable selection for large p small n regression models with incomplete data: mapping QTL with epistases. BMC Bioinformatics 9:251. doi: 10.1186/1471-2105-9-251

64. Liu J, Liu Y, Liu X, Deng H-W (2007) Bayesian mapping of quantitative trait loci for multiple complex traits with the use of variance components. Am J Hum Genet 81:304–320. doi: 10.1086/519495

65. Chun H (2009) Expression quantitative trait loci mapping with multivariate sparse partial least squares regression. Genetics 182:79–90. doi: 10.1534/genetics.109.100362

66. Chen W, Ghosh D, Raghunathan TE, Sargent DJ (2009) Bayesian variable selection with joint modeling of categorical and survival outcomes: an application to individualizing chemotherapy treatment in advanced colorectal cancer. Biometrics 65:1030–40. doi: 10.1111/j.1541-0420.2008.01181.x

67. Chipman H, George EI, McCulloch RE (2001) The Practical Implementation of Bayesian Model Selection. 65–116.

68. Brown PJ, Vannucci M, Fearn T (2002) Bayes model averaging with selection of regressors. J R Stat Soc Ser B (Statistical Methodol 64:519–536. doi: 10.1111/1467-9868.00348

69. Park T, Casella G (2008) The Bayesian Lasso. J Am Stat Assoc 103:681–686. doi: 10.1198/016214508000000337

70. Carvalho CM, Polson NG, Scott JG (2010) The horseshoe estimator for sparse signals. Biometrika 97:465–480. doi: 10.1093/biomet/asq017

71. Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. J R Stat Soc Ser B 39:1–38. doi: 10.1.1.133.4884

72. Ročková V, George EI (2014) EMVS: The EM Approach to Bayesian Variable Selection. J Am Stat Assoc 109:828–846. doi: 10.1080/01621459.2013.869223

73. Gelfand AE, Smith AFM (2012) Sampling-Based Approaches to Calculating Marginal Densities. J. Am. Stat. Assoc.

74. Hans C, Dobra A, West M (2007) Shotgun Stochastic Search for “Large p ” Regression. J Am Stat Assoc 102:507–516. doi: 10.1198/016214507000000121

75. Bottolo L, Petretto E, Blankenberg S, et al. (2011) Bayesian detection of expression quantitative trait loci hot spots. Genetics 189:1449–59. doi: 10.1534/genetics.111.131425

76. Bottolo L, Chadeau-Hyam M, Hastie DI, et al. (2013) GUESS-ing polygenic associations with multiple phenotypes using a GPU-based evolutionary stochastic search algorithm. PLoS Genet 9:e1003657. doi: 10.1371/journal.pgen.1003657

77. Barbieri MM, Berger JO (2015) Optimal predictive model selection. Ann Stat 32:870–897.

78. Broët P, Lewin A, Richardson S, et al. (2004) A mixture model-based strategy for selecting sets of genes in multiclass response microarray experiments. Bioinformatics 20:2562–71. doi: 10.1093/bioinformatics/bth285

79. Efron B (2008) Microarrays, Empirical Bayes and the Two-Groups Model. Stat Sci 23:1–22. doi: 10.1214/08-STS236REJ

80. Strimmer K (2008) fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics 24:1461–2. doi: 10.1093/bioinformatics/btn209

81. Zellner A, Siow A (1980) Posterior odds ratios for selected regression hypotheses. Trab Estad Y Investig Oper 31:585–603. doi: 10.1007/BF02888369

82. Hastings WK (1970) Monte Carlo Sampling Methods Using Markov Chains and Their Applications. Biometrika 57:97–109. doi: 10.2307/2334940

83. Eiben AE, Raué, Ruttkay Z (1994) Genetic algorithms with multi-parent recombination Parallel Problem Solving from Nature — PPSN III. In: Parallel Probl. Solving from Nat. — PPSN III. pp 78–87

84. Lewin A, Saadi H, Peters JE, et al. (2015) MT-HESS: an efficient Bayesian approach for simultaneous association detection in OMICS datasets, with application to eQTL mapping in multiple tissues. Bioinformatics. doi: 10.1093/bioinformatics/btv568

85. Ardlie KG, Deluca DS, Segre A V., et al. (2015) The Genotype-Tissue Expression (GTEx) pilot analysis: Multitissue gene regulation in humans. Science (80-) 348:648–660. doi: 10.1126/science.1262110

86. Todorov V, Filzmoser P (2010) Robust statistic for the one-way MANOVA. Comput Stat Data Anal 54:37–48. doi: 10.1016/j.csda.2009.08.015

87. Kim S, Becker J, Bechheim M, et al. (2014) Characterizing the genetic

basis of innate immune response in TLR4-activated human monocytes. Nat Commun 5:5236. doi: 10.1038/ncomms6236

88. Chen X, Shi X, Xu X, et al. (2012) A Two-Graph Guided Multi-task Lasso Approach for eQTL Mapping. ece.ubc.ca XX:208–217.

89. Benjamini Y, Yekutieli D (2001) THE CONTROL OF THE FALSE DISCOVERY RATE IN MULTIPLE TESTING UNDER DEPENDENCY By Yoav Benjamini 1 and Daniel Yekutieli 2. Ann Stat 29:1165–1188. doi: 10.1214/aos/1013699998

90. Hofner B, Boccuto L, Göker M (2014) Controlling false discoveries in high-dimensional situations: Boosting with stability selection.

91. Heinig M, Petretto E, Wallace C, et al. (2010) A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. Nature 467:460–4. doi: 10.1038/nature09386

92. Grundberg E, Small KS, Hedman ÅK, et al. (2012) Mapping cis- and trans-regulatory effects across multiple tissues in twins. Nat Genet 44:1084–1089. doi: 10.1038/ng.2394

93. Wu TT, Lange K (2008) Coordinate descent algorithms for lasso penalized regression. Ann Appl Stat 2:224–244.

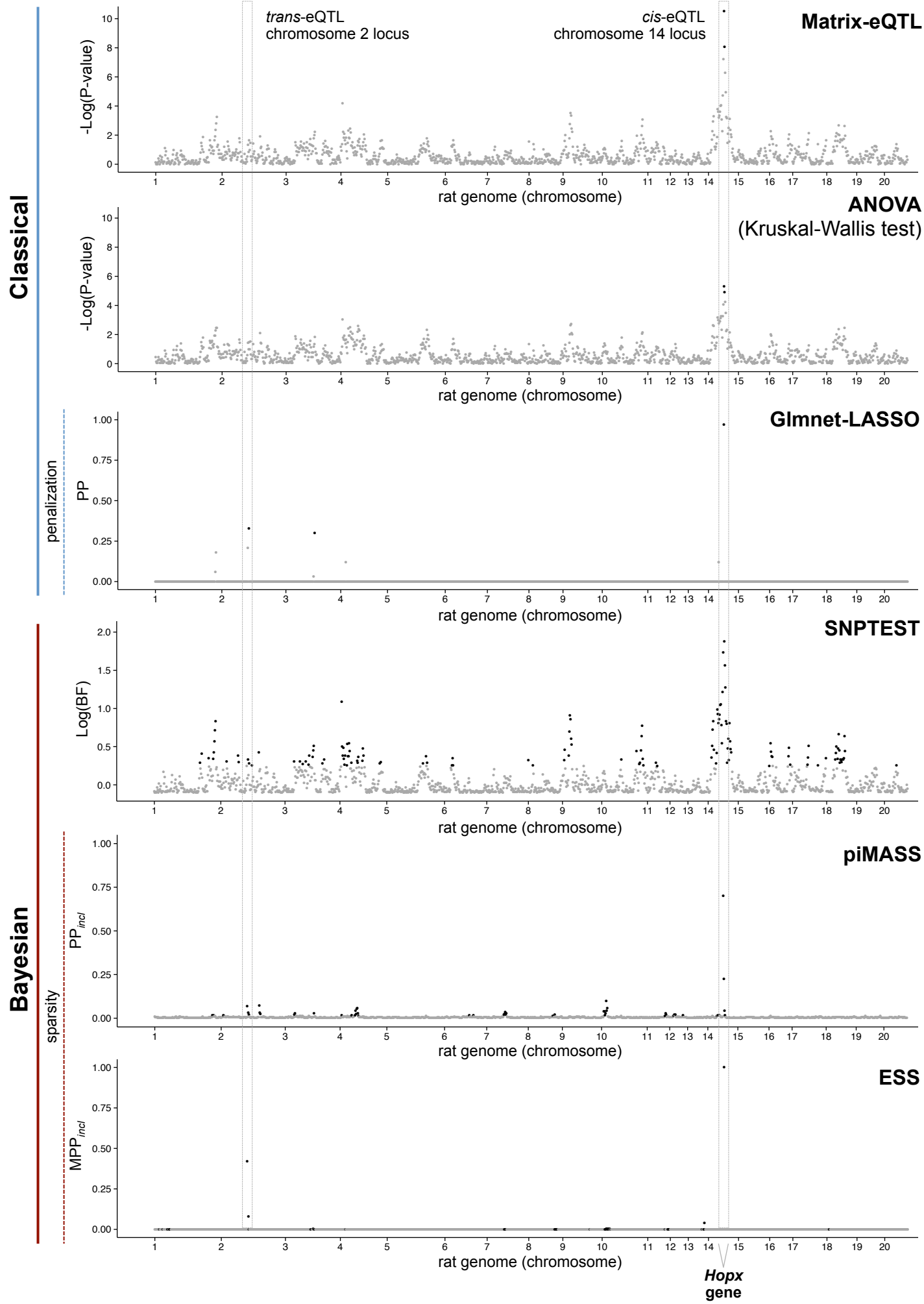
94. Gelfond JAL, Ibrahim JG, Zou F (2007) Proximity model for expression quantitative trait loci (eQTL) detection. Biometrics 63:1108–16. doi: 10.1111/j.1541-0420.2007.00778.x

95. Wakefield J (2009) Bayes factors for genome-wide association studies: comparison with P-values. Genet Epidemiol 33:79–86. doi: 10.1002/gepi.20359

96. Emilsson V, Thorleifsson G, Zhang B, et al. (2008) Genetics of gene expression and its effect on disease. Nature 452:423–8. doi: 10.1038/nature06758

97. Westra H-J, Peters MJ, Esko T, et al. (2013) Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 45:1238–1243. doi: 10.1038/ng.2756

Figure 1


Classical
Bayesian
penalization
sparsity
trans-eQTL
chromosome 2 locus
cis-eQTL
chromosome 14 locus
Matrix-eQTL
-Log(P-value)
rat genome (chromosome)
ANOVA
(Kruskal-Wallis test)
-Log(P-value)
rat genome (chromosome)
Glmnet-LASSO
PP
rat genome (chromosome)
SNPTEST
Log(BF)
rat genome (chromosome)
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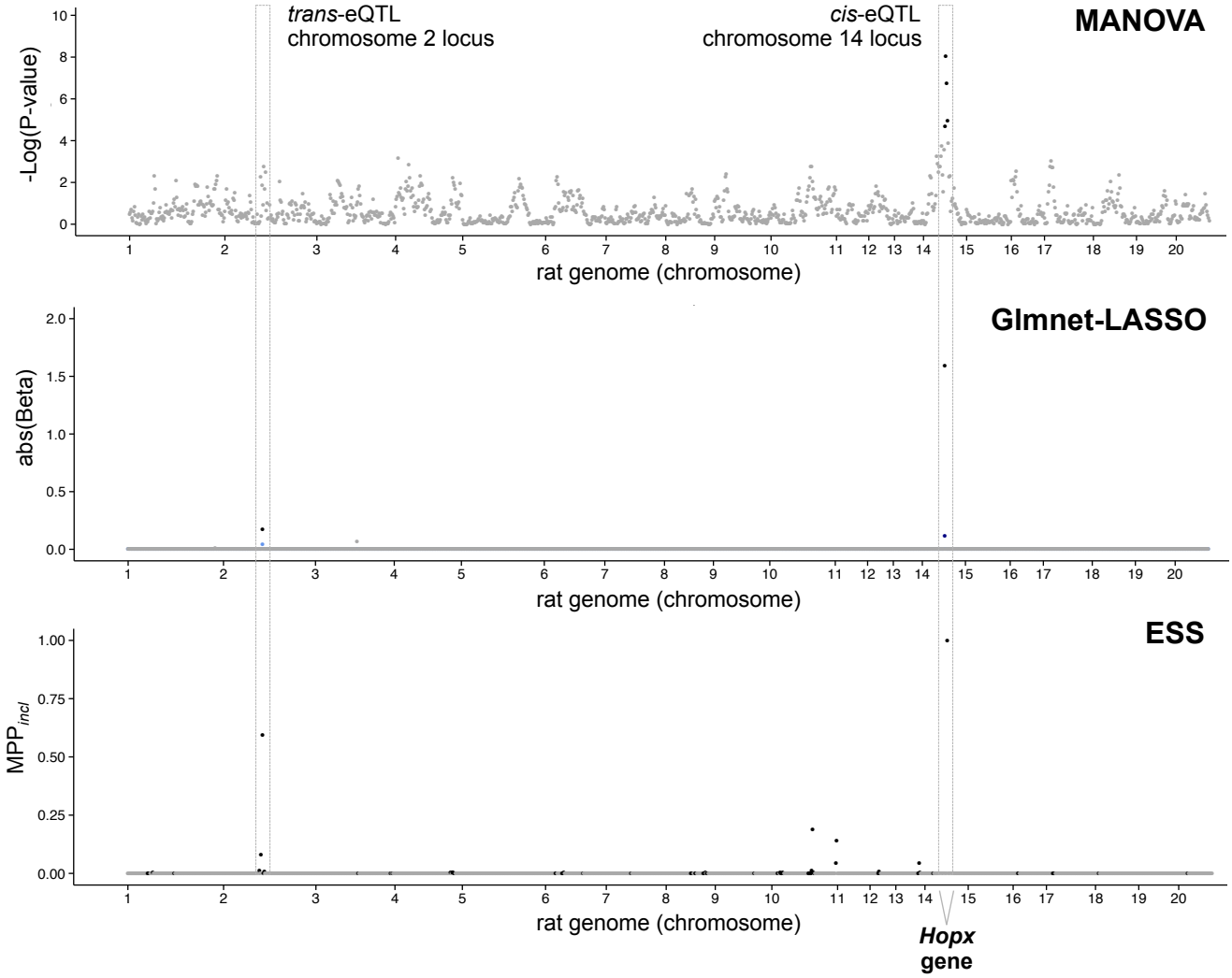
ESS
MPP_{incl}
rat genome (chromosome)
Hopx
gene

Figure 2


MANOVA
trans-eQTL
chromosome 2 locus
cis-eQTL
chromosome 14 locus
-Log(P-value)
rat genome (chromosome)
Glmnet-LASSO
abs(Beta)
rat genome (chromosome)
ESS
MPP*incl*
rat genome (chromosome)
Hopx
gene