A tissue-specific collaborative mixed model for jointly analyzing multiple tissues in transcriptome-wide association studies

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Abstract

Transcriptome-wide association studies (TWAS) integrate expression quantitative trait loci (eQTLs) studies with genome-wide association studies (GWASs) to prioritize candidate target genes for complex traits. Several statistical methods have been recently proposed to improve the performance of TWAS in gene prioritization by integrating transcriptome information imputed from multiple tissues, and made significant achievements in improving the ability to detect gene-trait associations. The major limitation of these methods is that they cannot be used to elucidate the specific functional effects of candidate genes across different tissues. Here, we propose a tissue-specific collaborative mixed model (TisCoMM) for TWAS, using a

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computational framework that integrates multi-tissue eQTL analysis. TisCoMM leverages the co-regulation of genetic variations across different tissues explicitly via a unified collaborative mixed model. With the probabilistic framework, TisCoMM not only performs hypothesis testing to prioritize gene-trait associations but also ascertains the tissue-specific role of candidate target genes in complex traits. To make use of widely available GWAS summary statistics, we extend TisCoMM to use summary-level data, namely, TisCoMM-S^2. Using extensive simulation studies, we demonstrate both the type I error control and the improved statistical power for the proposed methods. We further illustrate the benefits of our methods in applications to summary-level GWAS data for 35 traits. Notably, apart from better identifying potential trait-associated genes, we can elucidate the tissue-specific role of candidate target genes. The follow-up pathway analysis from tissue-specific genes for asthma shows that the immune system plays an essential function for asthma development in both thyroid and lung tissues.

Introduction

Over the last decade, GWASs have achieved remarkable successes in identifying genetic susceptible variants for a variety of complex traits [1]. However, the biological mechanisms to understand these discoveries remain largely elusive as majority of these discoveries are located in non-coding regions [2]. Recent expression quantitative trait loci (eQTLs) studies indicate that the expression regulatory information may play a pivotal role bridging both genetic variants and traits [3, 4, 5]. Cellular traits in comprehensive eQTL studies can serve as reference data, providing investigators with an opportunity to examine the regulatory role of genetic variants on gene expression. For example, the Genotype-Tissue Expression (GTEx) Project [6] has provided DNA sequencing data from 948 individuals and collected gene-expression measurements of 54 tissues from these individuals in the recent V8 release.

Transcriptome-wide association studies (TWAS) has been widely used to integrate the expression regulatory information from these eQTL studies with GWAS to prioritize genome-wide trait-associated genes [7, 8, 9]. A variety of TWAS methods have been proposed using different prediction models for expression imputation, including the parametric imputation models, e.g., PrediXcan [7], TWAS [8], CoMM [10] and CoMM-S^2 [11], and the nonparametric imputation model, e.g., Tigar [12]. These methods have been used for analyzing many complex traits with expression profiles from different tissues, successfully enhancing the discovery of genetic risk loci for complex traits [13, 9]. Moreover, previous studies have shown that multi-tissue eQTL analysis could improve statistical power by explicitly modeling shared patterns across tissues [14, 15, 16]. To enhance the power of identifying potential target genes, two recent studies were proposed by leveraging the substantial shared eQTLs across different tissues, i.e., MultiXcan [17] and UTMOST [18]. They use a step-wise procedure by first conducting imputation for gene expressions across multiple tissues and then performing subsequent association analysis using a multivariate regression that pools information across different tissues. Compared to single-tissue methods, these multi-tissue strategies were shown to enhance the imputation accuracy for transcriptomic data and further to improve the power of identifying potential target genes.

Despite their successes, the existing multi-tissue methods have several limitations. First, MultiXcan and UTMOST cannot be used to identify the tissue-specific gene-trait associations. Many studies have shown that significant genes are tissue-specific for complex traits [19, 20, 21, 9].

For example, a recent study for 18 complex traits across 44 tissues revealed a large number of modest-effect associations amongst eQTLs in a variety of tissues for many complex traits tested [22], implying the persuasive role of tissue-specific regulatory effects in a wide range of complex traits. Using a single-tissue test, one can easily reach a false conclusion regarding which tissue that a gene affects traits through. Second, both MultiXcan and UTMOST rely on a step-wise inference framework, ignoring the uncertainty in the process of expression imputation and thus leading to the loss of power, especially when cellular-heritability is small [10]. Recently, CoMM [10] and its variant for summary-level data, CoMM-S^2 [11], have been proposed to account for uncertainty in the process of expression imputation. Third, MultiXcan and UTMOST do not make efficient use of the shared patterns of eQTLs across tissues, where MultiXcan uses principal component analysis (PCA) regularization on the predicted expression data, and UTMOST uses penalized regularization on coefficients for eQTL effects. A study of GTEx revealed these shared patterns [23], and later many efforts have been made to take advantage of them in the analysis for GTEx data. For example, Urbut et al. proposed statistical methods for estimating and testing eQTL effects explicitly using this extensively tissue-shared patterns [24], shedding light on how to account for the tissue-shared eQTLs in statistical modeling successfully.

To overcome these limitations, we propose a tissue-specific collaborative mixed model (TisCoMM) for TWAS, providing a probabilistic model to perform gene-trait joint and conditional association tests across different tissues in a principled way. Our method allows us not only to perform hypothesis testing to prioritize gene-trait association but also to uncover the tissue-specific role of candidate genes. Using multiple tissues, one could largely remove the spurious associations due to confounding effects among highly correlated tissues. As a unified model, TisCoMM jointly conducts the "imputation" and the association analysis, pooling expression regulatory information across all relevant tissues. Furthermore, we extend TisCoMM to use summary statistics from a GWAS, namely, TisCoMM-S^2. In simulations, we show that both TisCoMM and TisCoMM-S^2 provide correctly controlled type I error and are more powerful than existing multi-tissue methods. More importantly, our methods can be used to test for the tissue-specific role of candidate genes. We illustrate the benefits of our methods using summary-level GWAS data in 35 complex traits. Results show that our novel findings have biologically meaningful implications. The follow-up pathway analysis from tissue-specific genes

for asthma shows that the immune system plays an essential function for asthma development in both thyroid and lung tissues.

Results

Method overview

Our method, TisCoMM, jointly integrates expression regulatory information across tissues by considering two models. The first one models transcriptome data across tissues in the reference data set,

$$\mathbf{Y}_g = \mathbf{X}_{1g}\mathbf{B}_g + \mathbf{E}_g, \tag{1}$$

where $\mathbf{Y}_g \in \mathbb{R}^{n_1 \times T}$ is expression matrix of n_1 samples across T tissues for gene g, $\mathbf{X}_{1g} \in \mathbb{R}^{n_1 \times M_g}$ is the standardized genotype matrix corresponding to M_g nearby single nucleotide polymorphisms (SNPs) of gene g in transcriptome study, $\mathbf{B}_g$ is an $M_g \times T$ matrix of eQTL effect sizes across T tissues and $\mathbf{E}_g$ is an $n_1 \times T$ matrix for random errors from a multivariate normal distribution $\mathcal{N}(0, \mathbf{V}_e)$. Here, $\mathbf{V}_e$ captures the correlations among tissues from the same individual. Then we assume that phenotypic value $\mathbf{z}$ and standardized genotype $\mathbf{X}_{2g}$ in GWAS are related by

$$\mathbf{z} = \mathbf{X}_{2g}\mathbf{B}_g\boldsymbol{\alpha}_g + \mathbf{e}_z, \tag{2}$$

where $\mathbf{z}$ is an $n_2 \times 1$ vector of phenotypic values, $\mathbf{X}_{2g} \in \mathbb{R}^{n_2 \times M_g}$ is the standardized genotype matrix corresponding to M_g nearby variants of gene g in GWAS, $\boldsymbol{\alpha}_g$ is a $T \times 1$ vector that represents the effect sizes of "imputed" gene expression across T tissues for gene g, and $\mathbf{e}_z \sim \mathcal{N}(0, \sigma^2)$ is an $n_2 \times 1$ vector of independent errors associated with the trait. Our TisCoMM can be depicted as Figure 1, within which Figure 1A illustrates the TisCoMM method combing both the transcriptomic prediction model (1) and the corresponding association model (2) together with data input and output.

To pool expression regulatory information across relevant tissues, we assume the factorizable assumption [25, 26] for $\mathbf{B}_g = [\beta_{jt}]$, $j = 1, \ldots, M_g, t = 1, \ldots, T$. This assumption has been empirically validated for GTEx data in an imputation study [27] and Park et al. further used this assumption in a multi-tissue TWAS [28]. Here, we assume that the effect size of cis-SNP j in tissue t can be factorized by genomic variant-dependent and tissue-dependent components: $\beta_{jt} = b_j w_{jt}$, where b_j (variant) is the eQTL effect of cis-SNP j shared in all the T tissues, and w_{jt} is the tissue-specific effect size. Thus, we have $\mathbf{B}_g = \text{diag}\{\mathbf{b}\}\mathbf{W}$ in a concise matrix form.

This factorization allows us to model the co-regulation of cis-SNPs shared across different tissues explicitly (Figure 1A, right).

As the number of parameters in $\mathbf{B}_g$ is large, and both $\mathbf{b}$ and $\mathbf{W}$ are not identifiable, we need to make further assumptions to allow TisCoMM identifiable. Due to polygenicity, we assume that each b_j independently follows a normal distribution $\mathcal{N}(0, \sigma_b^2)$. Following the adaptive weighting strategy used in [27], we regress the gene expression in tissue type t on the j-th eQTL and let the marginal effect be the adaptive weight w_{jt}. In the case that a sparse multi-tissue eQTL model is preferred, $\mathbf{W}$ could be chosen as a sparse matrix to introduce sparsity for $\mathbf{B}_g$.

Our key parameter of interest in TisCoMM is the effect size $\boldsymbol{\alpha}_g$. To prioritize candidate target genes, we conduct hypothesis testing for a joint null, $H_0 : \boldsymbol{\alpha}_g = 0$ (Figure 1C); to explore the tissue-specific role of candidate genes, we further conduct hypothesis testing for each tissue, $H_0 : \alpha_{gt} = 0$, $t = 1, \ldots, T$ (Figure 1D). We refer to the two inference tasks as the TisCoMM joint test and TisCoMM conditional test, respectively. In contrast to the existing two-step TWAS methods, we perform TisCoMM analysis within a unified model by treating $\mathbf{b}$ as a hidden random variable. The conditional test for each tissue could largely remove the spurious discoveries due to the correlations of expression levels among different tissues for a single gene. We develop an expectation-maximization (EM) algorithm for parameter estimation by maximizing the complete-data likelihood. A parameter expansion technique is further adopted to accelerate computational efficiency (see details in Supplementary Text).

[Figure 1 about here.]

Inferring TisCoMM results from GWAS summary statistics

Here, we propose TisCoMM-S^2 to expand the applicability of TisCoMM to cover the use of widely available GWAS summary statistics. TisCoMM-S^2 infers the individual-level TisCoMM results, using summary statistics from GWASs and LD information from a reference panel data set. Derivation details are shown in Supplementary Text.

We observe high concordance between TisCoMM and TisCoMM-S^2 results. Figure 2 shows the comparison of TisCoMM and TisCoMM-S^2 test statistics for ten traits from the Northern Finland Birth Cohorts program 1966 (NFBC1966) data set [29] (see Methods section). The reference panel was 400 subsamples from the NFBC1966 data set. The high correlation between TisCoMM and TisCoMM-S^2 suggests the goodness of gene-level association detections using

summary-level GWAS data.

[Figure 2 about here.]

To test the robustness of TisCoMM-S^2, we applied European subsamples from 1000 Genomes as the reference panel. Note that the NFBC1966 data set is Finns study, and it is well known that Finns have significant genetic differences with other Europeans [30]. Hence, the estimated LD did not well match that of the GWAS study. Supplementary Figure S1 shows the performance of TisCoMM-S^2 using European subsamples as a reference panel data set. Despite the high concordance between TisCoMM and TisCoMM-S^2 in the null region ($\Lambda > 34.67 = p$-values $> 5 \times 10^{-6}$), the test statistics of TisCoMM-S^2 in the non-null region are much more significant than TisCoMM. This experiment demonstrates the importance of using closely matched LD information, which is also highlighted by other summary-based approaches [17].

To explore false positives due to the LD discrepancy in the analysis for the NFBC1966 data set, we did additional simulations under the null hypothesis by permuting the ten traits randomly in the NFBC1966 data set. Supplementary Figure S2 compares TisCoMM and TisCoMM-S^2 under mismatched LD information (European samples from 1000 Genomes). We observe that the test statistics of TisCoMM-S^2 across all permuted traits are highly concordant with those from TisCoMM. This experiment demonstrates the robustness of our method in the null hypothesis to mismatches between reference and study samples.

Simulation

Methods for comparison We compared the performance of three methods in the main text: (1) our TisCoMM and TisCoMM-S^2 implemented in the R package *TisCoMM*; (2) MultiXcan and S-MultiXcan implemented in the MetaXcan package available at http://gene2pheno.org/; (3) UTMOST available at https://github.com/Joker-Jerome/UTMOST/. All methods were used with default settings. We conducted comprehensive simulation studies to gauge the performance of each method better by performing gene-trait joint and conditional tests across different tissues.

Simulation settings In detail, we considered the following simulation settings. We set $\{n_1, n_r, n_2\} = \{400, 400, 5000\}$ as the sample size for transcriptome data, GWAS data and reference panel data. We first generated the genotype data for $M_g = 400$ cis-SNPs from a multivariate normal distribution assuming an autoregressive correlation with parameter ρ.

We then discretized each SNP to a trinary variable $\{0, 1, 2\}$ by assuming Hardy-Weinberg equilibrium and a minor allele frequency randomly selected from a uniform $[0.05, 0.5]$ distribution. The genotype correlation was varied at $\rho = \{0.2, 0.5, 0.8\}$. All three genotype matrices, $\mathbf{X}_{1g}, \mathbf{X}_{rg}$, and $\mathbf{X}_{2g}$, for transcriptome data, GWAS data and reference panel data, respectively, are generated in this manner.

To generate multi-tissue gene expressions from genotype data, we considered different cellular-level heritability levels (h_c^2) and sparsity levels (s). These are key parameters to describe the genetic architecture of gene expression [31]. The cellular-level heritability represents the proportion of variance of the eQTL that can be explained by genotype, while sparsity represents the proportion of genetic variants that are associated with the gene expression. First, SNP effect size $\mathbf{B}_g = \text{diag}\{\mathbf{b}\}\mathbf{W}$ is generated. Specifically, we simulated SNP effect size $\mathbf{b}$ from a standard normal distribution, and randomly selected 10%, 50% or 100% of the SNPs to have non-zero tissue-specific effect $\mathbf{W}$ for gene expressions in all T tissues, while simulated their effects from a standard normal distribution. We then simulated errors $\mathbf{E}_g$ from a normal distribution, where their variances were chosen according to h_c^2, and the covariance structure was autoregressive with $\rho_e = 0.5$. Here we set $h_c^2 = 0.025, 0.05, 0.1, 0.2, 0.4$. Afterward, we simulated a multi-tissue eQTL data set assuming $\mathbf{Y}_g = \mathbf{X}_{1g}\mathbf{B}_g + \mathbf{E}$.

To simulate a quantitative trait, we generated nonzero entries of $\boldsymbol{\alpha}_g$ from a uniform distribution and $\mathbf{e}_z$ from a normal distribution. The variance σ^2 was chosen according to the tissue-level heritability $h_t^2 = \frac{\text{Var}(\mathbf{X}_{2g}\mathbf{B}_g\boldsymbol{\alpha}_g)}{\text{Var}(\mathbf{z})}$. Here we set $h_t^2 = 0$ for null simulations and type I error control examination and $h_t^2 = 0.01$ for non-null simulations and power comparisons.

Simulation I: Testing gene-trait associations We focus on candidate gene detection in the first set of simulations. Here, we compared TisCoMM and TisCoMM-S^2 with three different multi-tissue methods that include MultiXcan, S-MultiXcan, and UTMOST. We set $T = 10$, and all tissues are causal. For each scenario, we run 5,000 replicates. We first examined type I error control of different methods under the null. Results are shown in Supplementary Figures S3 – S7. By comparing the distribution of p-values with the expected uniform distribution, we observe that all methods provide well-controlled type I errors .

Next, we examined the power of different methods under the alternative hypothesis (Figure 3). We observe that the performance of all five methods improves with the increment of cellular heritability. In general, the summary-level methods (TisCoMM and MultiXcan) perform

similarly to their counterparts in individual-level data. TisCoMM and TisCoMM-S^2 have better performance than other alternative methods when cellular heritability is relatively small ($h_c^2 = 0.025, 0.05, 0.1$), and comparable performance when cellular heritability is large. Finally, we observe that although our model favors dense eQTLs, it was robust to the sparsity level s. Specifically, the power of TisCoMM and TisCoMM-S^2 in the setting where 10% of cis-SNPs have non-zero effects on gene expression are similar to the setting where all cis-SNPs have non-zero effects.

[Figure 3 about here.]

Simulations II: Testing tissue specific effects We focus on tissue-specific effect detection in the second set of simulations. Here, we compared TisCoMM conditional test with CoMM under the alternative hypothesis with fixed tissue heritability $h_t^2 = 0.01$. We considered three tissues $T = 3$ and varied the number of causal tissues to simulate different levels of tissue specificity of a trait. Specifically, we considered settings with one ($\alpha_{g2} = \alpha_{g3} = 0$) and two causal tissues ($\alpha_{g3} = 0$), respectively. Other sittings are similar to Simulation I.

We repeated the whole process 1,000 times. We calculated statistical power (Figure 4A) and false positive rate (Figure 4B) as the proportion of p-values reaching the significance level in causal tissues and non-causal tissues, respectively. Specifically, we set the significance level at 0.05/3 for TisCoMM conditional test, and 0.05/30000 for CoMM while accounting for the total number of genes [18]. We observe that in all cases, TisCoMM conditional test had well behaved false positive rate (FPR). Moreover, as the cell heritability increases, its corresponding FPR reduces. However, this is not true for CoMM, except that $s = 0.1$. Additionally, in all the cases that the two methods lead to comparable FPR, TisCoMM conditional test was more powerful.

[Figure 4 about here.]

Real Data Applications

We performed multi-tissue TWAS analysis for summary-level GWAS data in 35 complex traits (see Supplementary Table S1 for details), including 15 traits from [22] and 20 traits from UK Biobank. Hereafter we refer to as NG traits and UKB traits, respectively. These traits can be roughly divided into four categories, including metabolites (e.g., HDL-C, LDL-C and

Fasting glucose), autoimmune diseases (e.g., asthma, Crohn's disease and macular degeneration), psychiatric/neurodegenerative disorders (e.g., Alzheimer's disease, major depression disorder, and psychiatric disorder), and cardiovascular disorders (e.g., coronary artery disease and peripheral vascular disease). The transcriptome data is from the Genotype-Tissue Expression (GTEx) Project [6] that has gene expressions for 48 tissues, where the number of genes in each tissue ranges from 16,333 to 27,378. In the analysis, we extracted cis-SNP that are within either 500 kb upstream of the transcription start site or 500 kb downstream of the transcription end site.

We used two different strategies to select tissues used in TisCoMM-S^2 for NG and UKB traits. For the NG traits, we used trait-relevant tissues for different complex traits from Supplementary Table 2 in [22]. eQTLEnrich was used to identify trait-relevant tissues, in which a set of best cis-eQTL per gene was tested for enrichment of highly ranked trait associations compared to an empirical null distribution. For each trait, a set of tissues with significant enrichment p-values (after Bonferroni correction) was identified, and a subset with more than 100 overlapped samples [32] was chosen for further use in TisCoMM-S^2. For the UKB traits, we assume no prior information regarding tissue-trait relevance and used six tissues with the largest number of overlapped samples in GTEx.

The analysis for each summary statistics together with the transcriptome data for multiple tissues can be done around 100 min on a Linux platform with 2.6 GHz Intel Xeon CPU E5-2690 with 30 720 KB cache and 96 GB RAM (0nly 10$\sim$12 GB RAM used) on 24 cores.

TisCoMM-S^2 provides statistically powerful inference results

For prioritizing trait-associated genes, we compared TisCoMM-S^2 with other two multi-tissue TWAS methods, i.e., S-MultiXcan and UTMOST. Both alternative methods take advantage of prediction models to impute gene expressions. The prediction models used here were Elastic Net models trained on 48 GTEx tissues. See Table 1 and 2 for the summary of detections across different approaches for the 15 NG and 20 UKB traits, respectively. Generally, TisCoMM-S^2 identifies more associations than S-MultiXcan and UTMOST in most traits. We observe similar patterns in simulation studies. In detail, TisCoMM-S^2/S-MultiXcan/UTMOST identified 3,058/2,008/1,769, and 493/376/325 genome-wide significant genes in all the NG traits and UKB traits, respectively. Their qq-plots of p-values are shown in Supplementary Figures S8 – S12. As illustrative examples, we carefully examined the results for late-onset Alzheimer's

disease (LOAD) and asthma.

For LOAD, after Bonferroni correction, TisCoMM-S^2/S-MultiXcan/UTMOST identified 92/71/70 genome-wide significant, respectively, with 45 overlapping genes (17 of them were previously linked to LOAD). The qq-plots for associations in these three approaches are shown in Figure 5A. Among the 92 candidate target genes identified by TisCoMM-S^2, 24 of them were previously linked to LOAD [33], which are annotated in the Manhattan plot in Figure 5A. These include genes on the chromosome (CHR) 2 (*BIN1*), CHR 6 (*CD2AP*), CHR 7 (*EPHA1*), CHR 8 (*CLU*), CHR 11 (*PICALM, CCDC89, MS4A2, MS4A6A*), CHR 16 (*IL34*) , and CHR 19 (*STK11* and *APOE* region). Moreover, TisCoMM-S^2 also identified 35 genes that were not significant in neither S-MultiXcan nor UTMOST, and four of them were from previously identified LOAD risk loci, including *IL34* (p-value $=1 \times 10^{-6}$), *PTK2b* (p-value $=1.4 \times 10^{-9}$), *EPHX* (p-value $=4.7 \times 10^{-8}$) and *STK11* (p-value $= 7.2 \times 10^{-7}$). TisCoMM-S^2 also identified several novel loci for LOAD. *MAP3K2* is 200kb downstream of *B1N1*, a reported LOAD risk gene [34] that was also genome-wide significant in our joint test (p-values for both *B1N1* and *MAP3K2* $< 10^{-10}$). *MAP3K2* belongs to the serine/threonine protein kinase family and has been previously identified as a member of the Alzheimer's disease susceptibility network [35]. *STMN4* (p-value $< 10^{-10}$) encodes the known protein that exhibits microtubule-destabilizing activity. The expression levels of this gene in mouse neurons have been shown to change significantly after different exposure of cortical nerve cells to the Aβ peptide [36]. The expression of *STMN4* in zebrafish has also been shown to have an important role in regulating neurogenesis in the neural keel stage [37]. *EED* (p-value $=5.7^{-7}$) encodes a Polycomb protein, which plays a starring role as an important modulator of hippocampal development [38]. APC2 (p-value $= 1.3^{-6}$) is preferentially expressed in postmitotic neurons and involved in brain development through its regulation of neuronal migration and axon guidance [39]. Validation of these potential target genes requires further functional studies. The list of significant gene-trait associations of TisCoMM-S^2, S-MultiXcan, and UTMOST can be found in Supplementary Table S2. To replicate our findings in another independent data set, we used the summary statistics from the GWAS by proxy (GWAX [40], the sample size is 114,564). Our replication rate was high (Supplementary Table S3), where 31 out of 92 genes were successfully replicated under the Bonferroni-corrected significance threshold and the numbers of replicated genes raised to 44 under a relaxed p-value cutoff of 0.05.

For asthma, after Bonferroni correction, TisCoMM-S^2/S-MultiXcan/UTMOST identified 200/157/140 genome-wide significant genes, respectively, with 98 overlapping genes in all three methods (and 21 of them were previously reported). The qq-plots for associations in these three approaches are shown in Figure 5B. Among all 200 candidate target genes identified by TisCoMM-S^2, 31 of them were previously reported [41], which is annotated in the Manhattan plot in Figure 5B, including genes on CHR 2 (*IL1RL1/IL18R1*), CHR 5 (*TSLP/WDR36*, *RAD50*), CHR 6 (*HLA-DR/DQ* regions, *MAP3K7*), CHR 9 (*IL33*), CHR 11 (*C11orf30*, *LRRC32*), CHR 15 (*SMAD3*), and CHR 17 (genes from the 17q21 asthma locus). Also, TisCoMM-S^2 identified 56 genes that were not significant in neither S-MultiXcan nor UTMOST, and two of them have been previously suggested to associate with asthma, which are *PSORS1C1* (p-value $=2.2 \times 10^{-7}$), and *MAP3K7* (p-value $=3 \times 10^{-7}$). TisCoMM-S^2 also identified two novel loci for asthma. *HLA-DOB* belongs to the HLA class II beta chain paralogues. HLA-DO, a known protein encoded by *HLA-DOB*, has been shown to play essential roles in B-cell development and maturation [42]. *PDCD1LG2*, also known as *PD-L2*, was shown to have essential roles in modulating and polarizing T-cell functions in airway hyperreactivity [43]. Validating causal roles of these genes in asthma requires further instigation. The list of significant gene-trait associations of TisCoMM-S^2, S-MultiXcan, and UTMOST can be found in Supplementary Table S4. To replicate our findings in another independent data set, we used the summary statistics from TAGC European-ancestry GWAS [44] (the sample size is 127,669). Our replication rate was high (Supplementary Table S5), where 179 out of 200 genes were successfully replicated under the Bonferroni-corrected significance threshold and the numbers of replicated genes raised to 189 under a relaxed p-value cutoff of 0.05.

TisCoMM-S^2 infers tissue-specific effects of genes

To demonstrate the utility of the TisCoMM-S^2 conditional test, we applied the conditional test to all identified 92 candidate genes of LOAD and 200 candidate genes of asthma prioritized by the TisCoMM-S^2 joint test (details are in Supplementary Table S6 and S7), and compared analysis results with those from CoMM [10, 11]. Table 3 shows distributions of tissues in which the associations of candidate genes arise from LOAD and asthma (see details in Supplementary Table S8 and S9). For TisCoMM-S^2, 76.1% and 81.5% of candidate genes were significantly associated with LOAD and asthma in less than two tissues, respectively. However, for CoMM-S^2, 70.7% and 60% candidate genes of the two traits were significant in all the tissues. The most

plausible explanation is that compared to the multivariate perspective of our TisCoMM-S^2 conditional test, single tissue approaches, e.g., CoMM-S^2, have more considerable tissue bias and effect size inflation [9]. Suppose there is a causal gene in tissue A but not tissue B. In a single-tissues test, the association can be spuriously significant for tissue B. By performing a conditional test for this gene in tissue B, the significant spurious association will be largely excluded given the causal tissue A.

[Table 1 about here.]

To demonstrate the tissue-specific role of candidate genes inferred by TisCoM-S^2 conditional test for LOAD and asthma, respectively, we plot the volcano plots in Supplementary Figure S14, where the x-axis is the effect size showing in log scale, the y-axis is $-log10$ of the p-value from conditional test, and the size of points reflect the cellular-heritability in each tissue. Genes previously linked to traits studied are also annotated. Next, we explored the tissue-specific effects of some well-replicated genes that are identified by the TisCoMM-S^2 joint test for LOAD and asthma, respectively.

For LOAD, the well-replicated risk gene *APOE* [45] and its 50Kb downstream *CLPTM1* have been identified by the TisCoMM-S^2 joint test. TisCoMM-S^2 conditional test identified *CLPTM1* to be significantly associated with LOAD in all the four tissues (artery aorta, esophagus mucosa, nerve tibial, and skin sun-exposed lower leg with conditional p-values $< 4.9 \times 10^{-7}$), but *APOE* to be only significantly associated with LOAD in artery aorta (conditional p-value $=8.3 \times 10^{-9}$) and nerve tibial (conditional p-value $=1.2 \times 10^{-8}$). Also, the effect directions of *APOE* in artery aorta and nerve tibial were the same while those of *CLPTM1* were different (see the volcano plot in Supplementary Figure S14A). On the other hand, CoMM-S^2 significantly identified both *APOE* and *CLPTM1* in all four tissues (p-values $\leq 10^{-10}$) but failed to identify the difference of tissue-specific role for these two genes. We further tried to investigate the tissue-specific role of disease-related genes identified by the TisCoMM-S^2 conditional test. For LOAD, there are about 40 LOAD-associated genes in each tissue (artery aorta, skin sun-exposed lower leg, esophagus mucosa, and nerve tibial). It is difficult to carry out a proper pathway analysis with such limited gene sets. So we classified the genes into seven functional groups based on which molecular functions they belong. As shown in Figure 6A, majority ($> 62\%$) of LOAD-associated genes belonged to binding and catalytic activity, and a small portion of significant LOAD genes were transcription factors suggesting that many regulation processes are going on at both

protein and mRNA levels in different tissues. Although LOAD is a neurological disorder, we can identify relevant gene expression changes in non-brain tissues, indicating that the genes associated with LOAD could also be regulated differently in a tissue-specific manner.

For asthma, we take identified genes *ORMDL3* and *GSDMB* in the 17q21 asthma locus as an example. These two genes have been identified as asthma susceptibility locus by many studies [46], and their functions in mouse and human lung structural cells have been reported [47]. The TisCoMM-S^2 conditional test identified both *ORMDL3* and *GSDMB* to be significantly associated with asthma only in lung tissue (see the volcano plot in Supplementary Figure 14B, conditional p-values for these two genes are 1.7×10^{-3} and 7.1×10^{-7}, respectively). However, CoMM identified both *ORMDL3* and *GSDMB* in all six tissues (p-values $\leq 10^{-10}$) but failed to identify the relevant tissues with which these two genes are causally related to asthma. We further conducted pathway analysis using DAVID [48] on six sets of asthma-associated genes in six tissues (thyroid, lung, artery tibial, muscle skeletal, adipose subcutaneous, and skin sun-exposed lower leg). As listed in Figure 6B, all three significant pathways in thyroid belonged to the immune system, and the only significant pathway in lung tissue was immune response. However, no significant pathways were detected in the other four tissues. Among asthma-associated genes in immune response (first row in Figure 6B), the majority of them were shared between thyroid and lung, and located in the MHC region on the CHR 6 including several *HLA* genes and *LST1*. Our pathway analysis suggests that nearly the same set of immune genes in thyroid and lung are responsible for asthma development.

[Figure 5 about here.]

[Figure 6 about here.]

Discussion

Despite the substantial successes of TWAS and its variants, the existing multi-tissue methods have several limitations, e.g., incapability to identify the tissue-specific effect of a gene, failure to use tissue-shared patterns in eQTLs, and ignorance of imputation uncertainty. To overcome these limitations and provide additional perspectives over tissue-specific roles of identified genes, we have proposed a powerful multi-tissue TWAS model, together with a computationally efficient inference method and software implementation in TisCoMM. Specifically, we have developed

a joint test for prioritizing gene-trait associations and a conditional test for identifying the tissue-specific role of candidate genes. The conditional test in TisCoMM can mostly remove the spurious associations in a single-tissue test due to high correlations among gene expression across tissues. We have also developed a summary-statistic-based model, TisCoMM-S^2, extending the applicability of TisCoMM to available public summary-level GWAS data. Using both simulations and real data, we examined the relationship between TisCoMM and TisCoMM-S^2. Our results (Figure 2) show that the test statistics from TisCoMM and TisCoMM-S^2 are highly correlated ($R^2 > 0.95$). We further analyzed summary-level GWAS data from 35 traits with replication data for Alzheimer's disease and asthma. Overall, the findings from TisCoMM-S^2 are around 30% more than those from S-MultiXcan or UTMOST while qq-plots from these studies show that there are no apparent inflations. To replicate our findings for Alzheimer's disease and asthma, we applied TisCoMM-S^2 to independent data sets for each disease. Results show that replication rates for Alzheimer's disease and asthma are high.

We further inferred the tissue-specific effects of identified genes using the TisCoMM-S^2 conditional test. The findings are intriguing, e.g., *APOE* and *CLPTM1* were identified to be associated with LOAD in artery aorta and nerve tibial with different effect directions. By classifying these genes into seven functional groups, we observed that majority (62%) of LOAD-associated genes were related to binding and catalytic activity while a small portion was from transcription factors suggesting active regulation processes at both protein and mRNA level in different tissues. We also observe there are about 40 LOAD-associated genes in non-brain tissues. The significance of these genes could be due to not inclusion of LOAD-relevant tissues, e.g., brain tissues. For asthma, genes *ORMDL3* and *GSDMB* were identified to be significantly associated with asthma only in lung tissue using TisCoMM-S^2. However, single-tissue analysis (CoMM-S^2) identified both genes significant in all six tested tissues. Further pathway analysis shows that all three significant pathways for thyroid tissue belong to the immune system and the only significant pathway for lung tissue was immune response. The majority of shared genes between thyroid and lung tissues are located in the MHC region on CHR 6, including several *HLA* genes and *LST1*. The proteins encoded by *HLA* genes are known as antigens. In combination with antigen-presenting cells (e.g., macrophages and dendritic cells), they play an essential role in the activation of immune cells as well as airway inflammation in response to asthma-related allergens [49, 50]. Based on our multi-tissue conditional test, *TNF* that is

a well-studied asthma gene [51, 52] was explicitly identified to be associated with asthma in lung tissue. The positive correlation between *TNF* expression and asthma in lung confirmed our previous understanding of *TNF* activation in asthma, promoting airway inflammation and airway hyperresponsiveness. On the other hand, *LTA* was specifically regulated in thyroid tissue. It is a cytokine produced by lymphocytes, and also known as a regulator of lipid metabolism [53]. Another immune gene regulated individually in thyroid tissue is *NCR3*, which mediates the crosstalk between natural killer cells and dendritic cells [54]. However, it remains unclear how the alteration of *LTA* and *NCR3* in thyroid could lead to asthma development.

Despite the utility of TisCoMM to perform gene-trait association analysis in a tissue-specific manner, it is primarily designed to test genes with direct effects from cis-eQTL. Recently, an omnigenic model was proposed to better understand the underlying mechanism of so-called polygenicity in complex traits [55]. Liu et al. [56] further provided a theoretical model to understand complex trait architecture by partitioning genetic contributions into direct effects from core genes and indirect effects from peripheral genes acting in trans. Most works from TWAS identify core genes with direct effects. How to effectively interrogate peripheral genes with indirect effects essentially remains an open question. As high-throughput data are continuously generating for a much larger sample size with more precision, TisCoMM sheds light on how to integrate useful data for the desired analysis effectively.

Methods

Model settings

Conventionally, both single-tissue and multi-tissue TWAS methods proceed by conducting a prediction model in Equation (1) followed by a subsequent association analysis in Equation (2), where a steady-state gene expression is imputed from $\mathbf{X}_{2g}\widehat{\mathbf{B}}_g$ and $\widehat{\mathbf{B}}_g$ is estimated in the first prediction model, e.g., PrediXcan, MultiXcan, S-MulitXcan, and UTMOST. However, this imputation strategy ignores the uncertainty in the process of expression imputation. Here, we describe the individual-level data version of TisCoMM by jointly analyzing models (1) and (2), and extensions to summary statistics will be discussed in the Supplementary Text. Assume $\mathcal{D}_1 = \{\mathbf{Y}_g, \mathbf{X}_{1g}\}$ denote the reference transcriptome data set of gene g for n_1 samples over T tissues, where $\mathbf{Y}_g$ is the $n_1 \times T$ expression matrix for this gene over T tissues, and $\mathbf{X}_{1g}$ is the corresponding $n_1 \times M_g$ standardized genotype matrix for M_g cis-SNPs within this gene.

Denote the GWAS data $\mathcal{D}_2 = \{\mathbf{z}, \mathbf{X}_{2g}\}$, where $\mathbf{z}$ is an $n_2 \times 1$ vector of phenotypic values, $\mathbf{X}_{2g}$ is the corresponding $n_2 \times M_g$ standardized genotype matrix for M_g cis-SNPs. Since we conduct hypothesis testing sequentially or parralelly for each gene, we will omit the subscript g in all the expression that has dependence on gene g to simplify notations. Our model becomes

$$\mathbf{Y} = \mathbf{X}_1\mathbf{B} + \mathbf{E}, \quad \mathbf{z} = \mathbf{X}_2\mathbf{B}\boldsymbol{\alpha} + \mathbf{e}_z, \tag{3}$$

where $\boldsymbol{\alpha} \in \mathbb{R}^T$, $\mathbf{E} \sim \mathcal{MN}(0, \mathbf{I}_m, \mathbf{V}_e)$, and $\mathbf{e}_z \sim \mathcal{N}(0, \sigma^2\mathbf{I}_n)$. Note that we assume $\mathcal{D}_1$ and $\mathcal{D}_2$ are centered and thus intercepts can be omitted.

To estimate the tissue-specific eQTL effects, we need to first estimate an $M \times T$ coefficient matrix $\mathbf{B}$. To reduce the number of parameters, we follow an adaptive weighting scheme [25, 26, 27]: we regress the gene expression in tissue type t on the jth eQTL and let the marginal eQTL effect be the adaptive weight, w_{jt}. Specifically, we assume the joint eQTL effect size β_{jt} can be decomposed into variant-dependent components b_j and tissue-specific components w_{jt}: $\beta_{jt} = b_j w_{jt}$. That is, $\mathbf{B} = \text{diag}\{\mathbf{b}\}\mathbf{W}$. Similar strategies have been applied to model tissue-shared patterns [27, 24]. Let $\mathbf{y}_i$, $\mathbf{x}_{1i}$ and $\mathbf{w}_j$ denote the ith row of $\mathbf{Y}$, $\mathbf{X}_1$ and $\mathbf{W}$, respectively. Our model can be written as

$$\begin{aligned}
\mathbf{y}_i|\mathbf{b} &\sim \mathcal{N}\big(\sum_j x_{1ij} b_j \mathbf{w}_j, \mathbf{V}_e\big), \\
z_i|\mathbf{b} &\sim \mathcal{N}\Big(\boldsymbol{\alpha}^\top\big(\sum_j x_{2ij} b_j \mathbf{w}_j\big), \sigma^2\Big), \\
b_j &\sim \mathcal{N}(0, \sigma_b^2).
\end{aligned}$$

Denote $\boldsymbol{\theta} = (\boldsymbol{\alpha}, \sigma_b^2, \sigma^2, \mathbf{V}_e)^T$ the vector for all model parameters. We need to estimate parameters and maker inference for $\boldsymbol{\alpha}$. Both the TisCoMM joint test and conditional test are based on likelihood ratio tests. The joint test for gene-trait associations is $H_0 : \boldsymbol{\alpha} = 0$ verses $H_1 : \boldsymbol{\alpha} \neq 0$. The likelihood ratio test statistic is given by

$$\Lambda = 2\left[\log \Pr(\mathbf{y}, \mathbf{z}|\mathbf{X}_1, \mathbf{X}_2; \hat{\boldsymbol{\theta}}) - \log \Pr(\mathbf{y}, \mathbf{z}|\mathbf{X}_1, \mathbf{X}_2; \hat{\boldsymbol{\theta}}_{\boldsymbol{\alpha}=0})\right],$$

where $\hat{\boldsymbol{\theta}}$ is the vector of parameter estimates under the full model, and $\hat{\boldsymbol{\theta}}_{\boldsymbol{\alpha}=0}$ is the vector of estimates under the constrain $\boldsymbol{\alpha} = 0$. Similarly, the conditional test for the tissue-specific effect is $H_0 : \alpha_t = 0$ verses $H_1 : \alpha_t \neq 0$. The likelihood ratio test statistic is given by

$$\Lambda_t = 2\left[\log \Pr(\mathbf{y}, \mathbf{z}|\mathbf{X}_1, \mathbf{X}_2; \hat{\boldsymbol{\theta}}) - \log \Pr(\mathbf{y}, \mathbf{z}|\mathbf{X}_1, \mathbf{X}_2; \hat{\boldsymbol{\theta}}_{\alpha_t=0})\right],$$

where $\hat{\boldsymbol{\theta}}_{\alpha_t=0}$ is the vector of parameter estimates under $\alpha_t = 0$.

For statistical inference, we developed an expectation-maximization (EM) algorithm accelerated by expanding parameters [57]. Details of updating equations for each parameter and the corresponding algorithm can be found in Supplementary Text.

GWAS data

The NFBC1966 data set

The NFBC1966 data set consists of ten traits and 364 590 SNPs from 5402 individuals [29], including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), inflammatory marker C-reactive protein, markers of glucose homeostasis (glucose and insulin), body mass index (BMI) and blood pressure (BP) measurements (systolic and diastolic BP). Quality control procedures are conducted following similar steps to Shi et al. [58]. After quality control filtering, 172,412 SNPs from 5123 individuals were available for downstream analysis.

The tissues used in TisCoMM and TisCoMM-S^2 were the same, and the six tissues with the largest number of overlapped individuals were used. The summary statistics for TisCoMM-S^2 were calculated using PLINK [59].

Summary-level GWAS data

We obtained summary statistics from GWASs for 35 traits, including 15 traits from [22] and 20 traits from UK Biobank. These traits can be roughly divided into four categories, including metabolites (e.g., HDL-C, LDL-C and Fasting glucose), autoimmune diseases (e.g., asthma, Crohn's disease and macular degeneration), psychiatric/neurodegenerative disorders (e.g., LOAD, major depression disorder and psychiatric disorder), and cardiovascular disorders (e.g., coronary artery disease and peripheral vascular disease). Details of these traits can be found in Supplementary Table S1. In the main text, we discussed LOAD and asthma. Analyses results for other traits can be found in Supplementary Text.

GTEx eQTL Data

We obtained genotype and normalized gene expression data collected by the GTEx project and used the GTEx project's V7p data. We collected 48 different tissues. As previously recommended [19], normalized gene expression data in each tissue was adjusted for covariates

downloaded from the GTEx portal website https://gtexportal.org/home/. For genotype data, SNPs with ambiguous alleles or minor allele frequency (MAF) less 0.01 were removed.

We used two different strategies to select tissues used in our real data analysis. For the 15 NG traits, we obtained the top enriched tissues for each trait according to Supplementary Table 2 in [22], and a subset of tissues with sample sizes larger than 100 was kept. For the UKB traits, we assumed no prior information regarding tissue-trait relevance and used the six tissues with the largest number of overlapped individuals.

Reference panel

Due to the absence of genotype data using summary statistics, we will use reference samples to estimate the LD structures R among SNPs in the study samples. Since diseases and traits considered in our real data application are for European population cohorts, we choose to use European subsamples from the 1000 Genome Project.

Let $\mathbf{X}_r$ denote the genotype matrix for cis-SNPs in the reference panel. To estimate the LD matrix R, we adopt a simple shrinkage method as follows. We first calculate the empirical correlation matrix $\hat{R}^{\text{emp}} = [r_{jk}] \in \mathbb{R}^{M\times M}$ with $r_{jk} = \frac{X_{rj}^\top X_{rk}}{\sqrt{(X_{rj}^\top X_{rj})(X_{rk}^\top X_{rk})}}$, where X_{rj} the jth column of $\mathbf{X}_r$. To make the estimated correlation matrix positive definite, we apply a simple shrinkage estimator [60]: $\hat{R} = \tau R^{\text{emp}} + (1-\tau)\mathbf{I}_M$, where $\tau \in [0,1]$ is the shrinkage intensity. In real data application, we fixed the shrinkage intensity at 0.95 both for simplicity and computational stability.

Web Resources

TisCoMM is available at Github (https://github.com/XingjieShi/TisCoMM/).

MultiXcan and *S-MultiXcan* are available at http://gene2pheno.org/.

UTMOST is available at https://github.com/Joker-Jerome/UTMOST/.

Summary statistics from UK Biobank is available at http://geneatlas.roslin.ed.ac.uk/.

Summary statistics from Gamazon et al. [22] is available at X.

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Table 1: Numbers of significant gene-trait associations across 15 NG traits. The reference penal is European subsamples from 1000 Genome. The number in the parenthesis denoted genes reported on the GWAS catalog. The full names of traits can be found in Supplementary Table S1.

	TisCoMM-S^2	S-MultiXcan	UTMOST
2hrGlu	1(0)	0(0)	5(0)
AD	92(24)	71(20)	70(19)
BMI	82(30)	59(21)	68(25)
CAD	69(9)	28(5)	36(11)
CD	468(52)	339(50)	291(55)
FG	94(11)	66(11)	54(8)
FI	3(0)	1(0)	2(0)
HDL	464(14)	268(13)	237(12)
HOMAB	10(0)	7(0)	4(0)
HOMAIR	1(0)	1(0)	1(0)
LDL	498(5)	273(5)	228(5)
TC	603(88)	376(77)	330(86)
TG	360(58)	250(48)	192(40)
UC	301(30)	262(31)	243(41)
WHR	12(3)	7(1)	8(1)

Table 2: Numbers of significant gene-trait associations across 20 UKB traits. The reference penal data is European subsamples from 1000 Genome. The number in the parenthesis denoted genes reported on the GWAS catalog. The full names of traits can be found in Supplementary Table S1.

	TisCoMM-S^2	S-MultiXcan	UTMOST
ALLERGIC_RHINITIS	25(3)	23(2)	12(4)
ASTHMA	200(31)	157(29)	140(36)
CARD	2(0)	2(0)	4(0)
DEPRESS	2(0)	1(0)	0(0)
DIA2	36(4)	25(4)	30(7)
DYSLIPID	166(0)	120(0)	91(0)
HEMORRHOIDS	0	1(0)	0
HERNIA_ABDOMINOPELVIC	2(1)	2(1)	1(1)
INSOMNIA	0	0	0
IRON_DEFICIENCY	0	0	0
IRRITABLE_BOWEL	0	1(0)	0
MACDEGEN	0	0	0
OSTIOA	1(0)	2(0)	2(1)
OSTIOP	0	1(0)	0
PEPTIC_ULCERS	1(0)	1(0)	0
PSYCHIATRIC	1	1	3
PVD	2(0)	3(0)	3(0)
STRESS	1(0)	1(0)	1(0)
SUM_DISEASES	14	13	18
VARICOSE_VEINS	40(2)	22(2)	20(2)

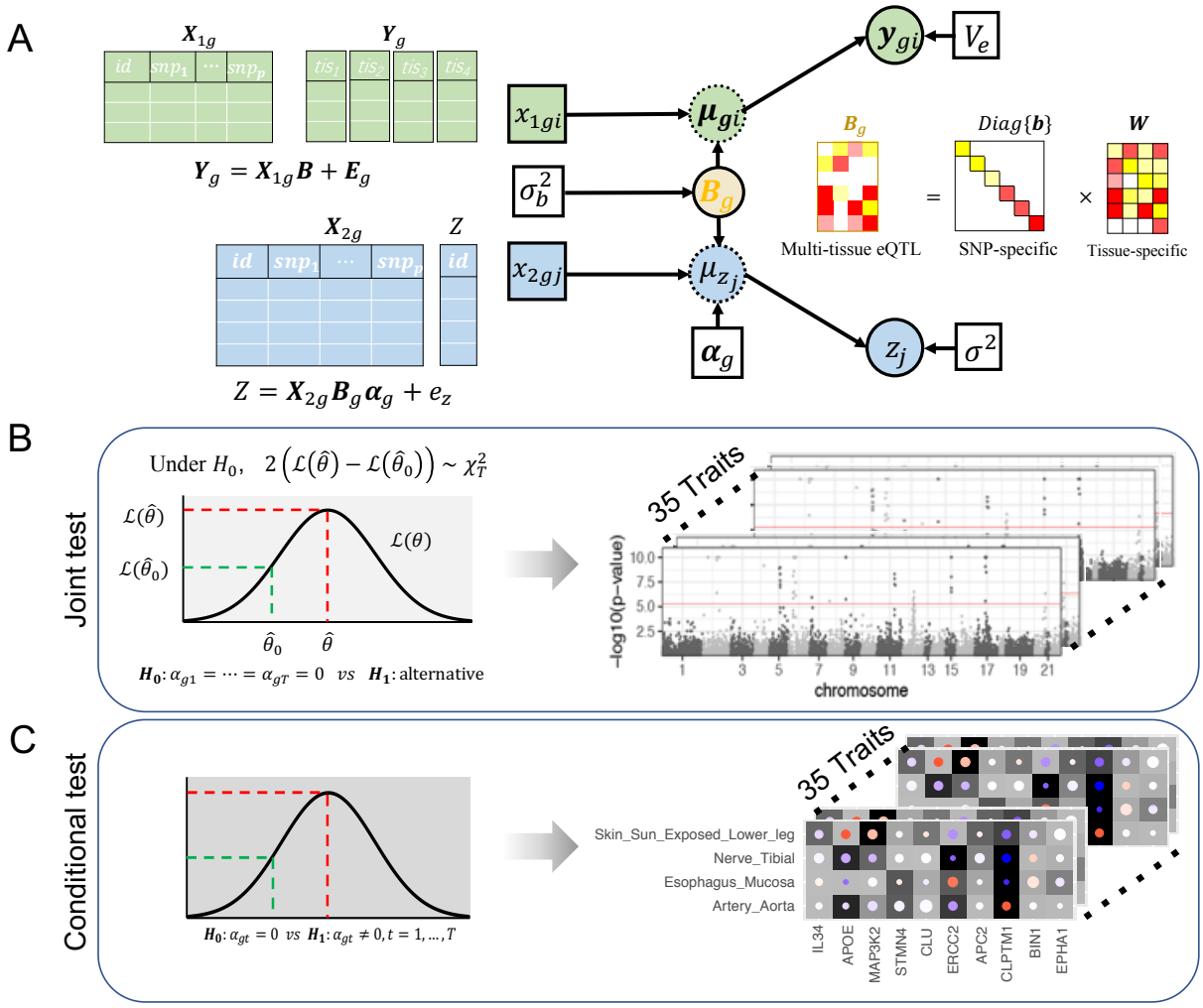


Figure 1: TisCoMM workflow. **A.** Two sets of TisCoMM input matrices are highlighted in green and blue separately (left). The probabilistic graphical model for TisCoMM is shown in the middle, which integrates gene expressions and models the co-regulation of cis-SNPs across different tissues explicitly. The decomposition of the **B** matrix is illustrated on the right-hand side of the figure. **B.** The TisCoMM joint test for all genes to prioritize candidate causal genes. The example outputs (right) are shown as 35 Manhattan plots for 35 traits. **C.**The TisCoMM conditional test for all candidate genes to explore the tissue-specific roles of candidate genes. The example outputs (right) are shown as heatmaps which summarize the tissue-specific effect of each gene. Significance level, effect size, and heritability are converted into background color, circle color, and circle size.

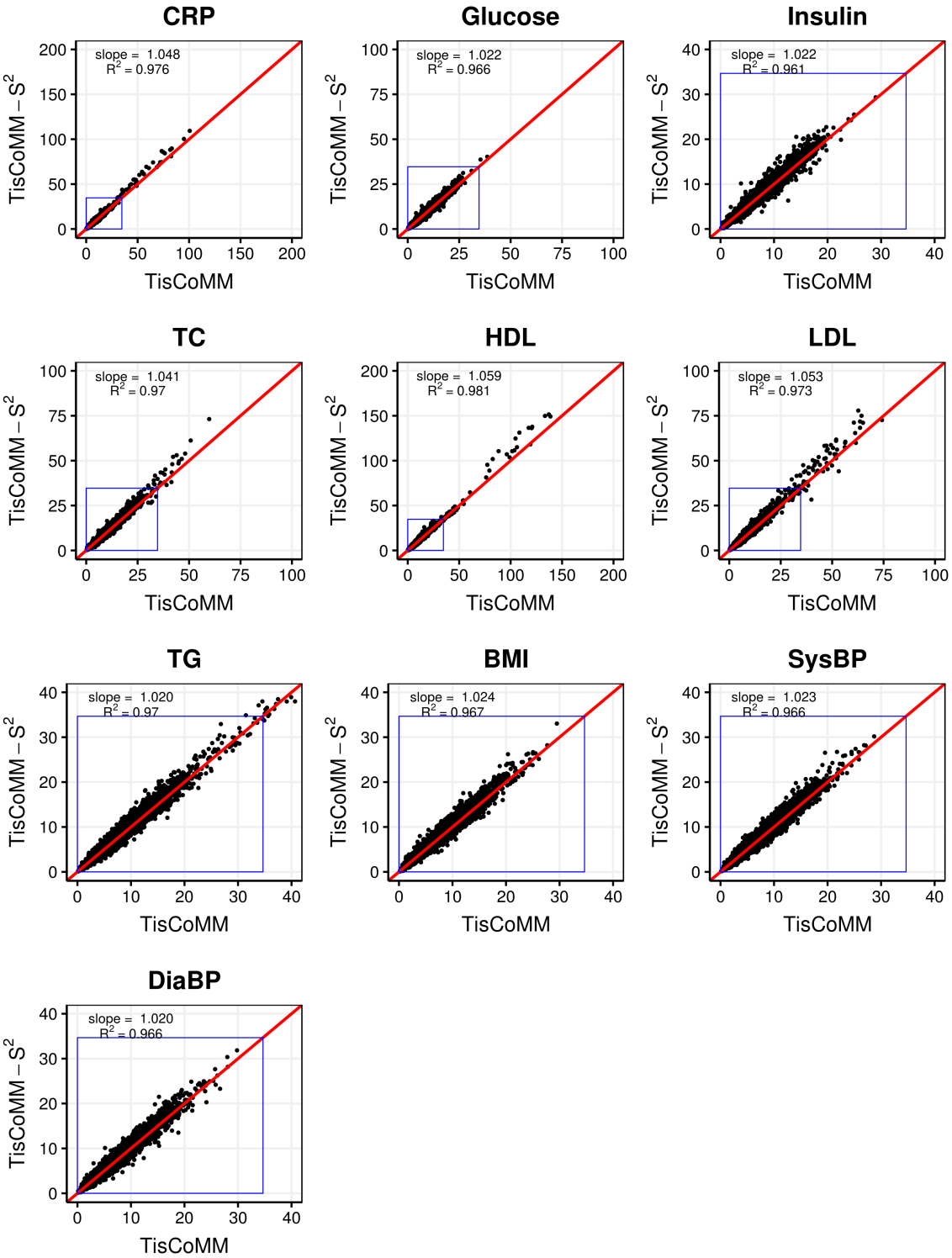


Figure 2: Comparison of TisCoMM and TisCoMM-S^2 results in NFBC1966 traits. The reference panel is subsamples from the NFBC1966 data set. The summary-based method shows similar results to the individual-based method. The blue rectangle indicates the null region.

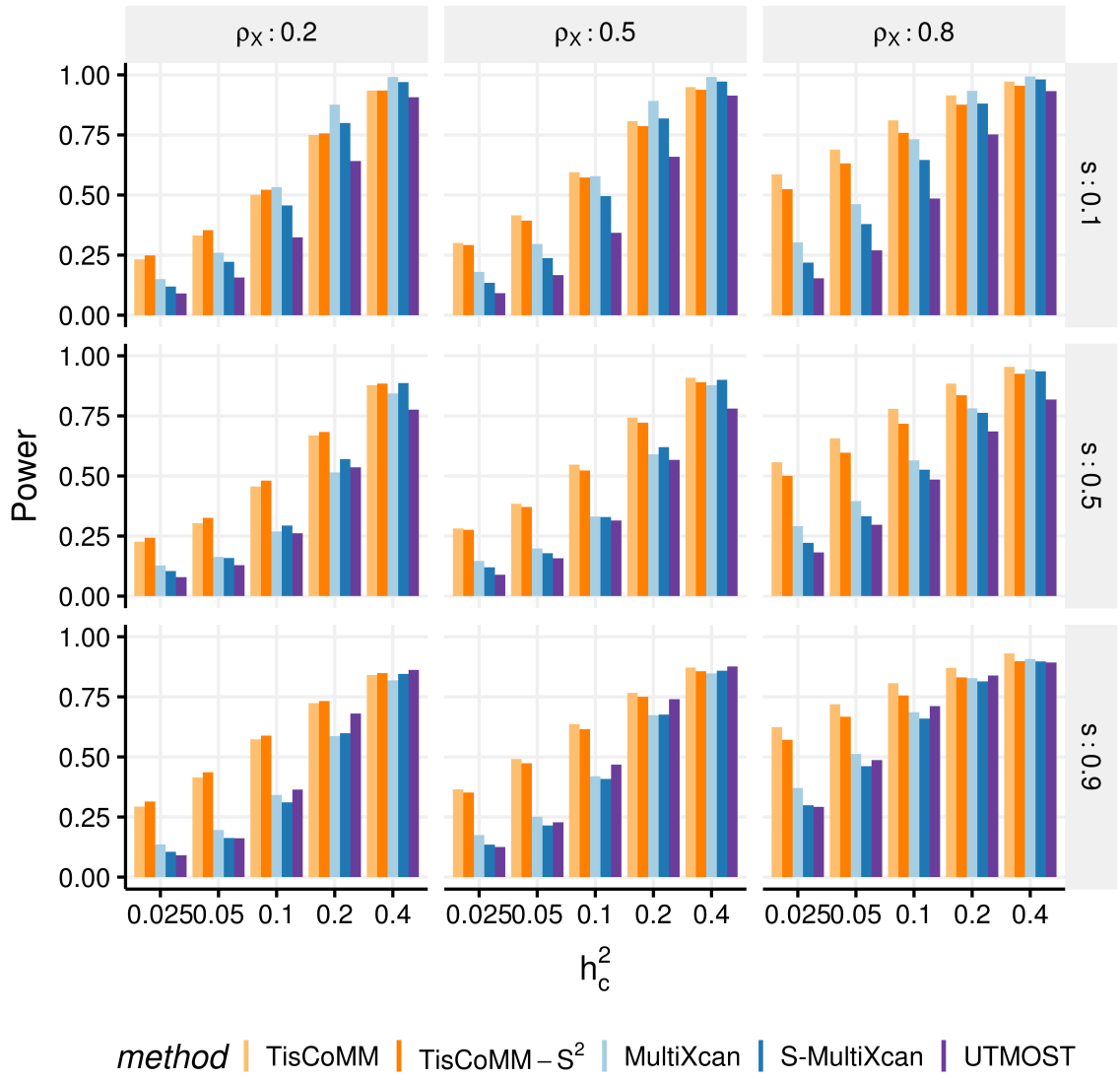
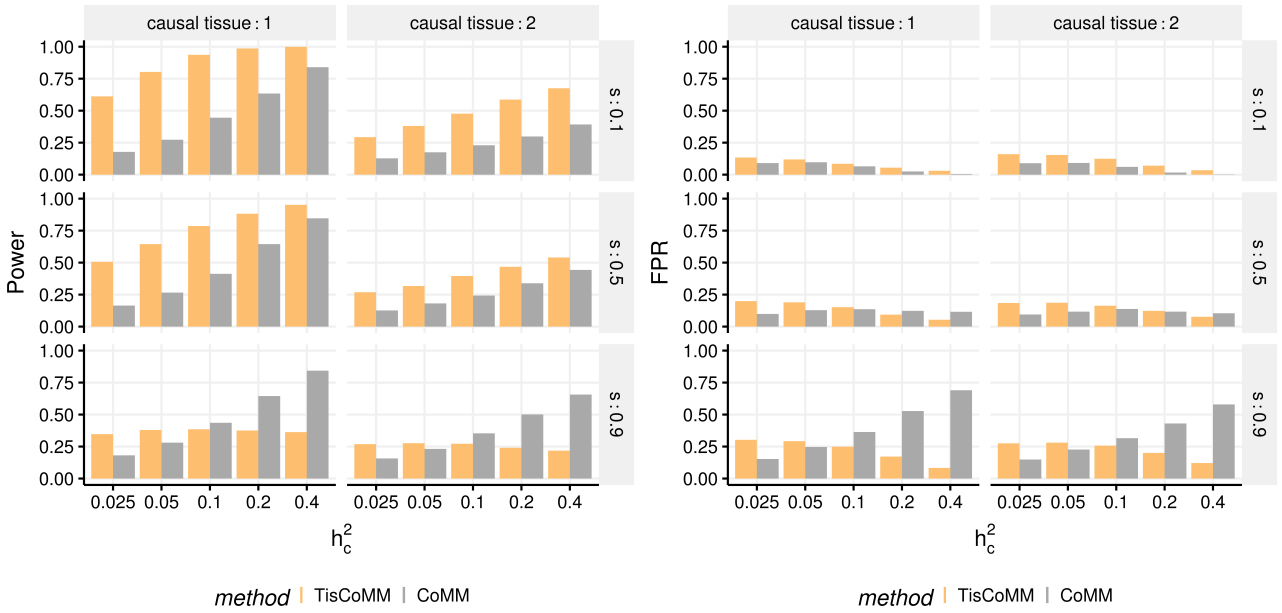


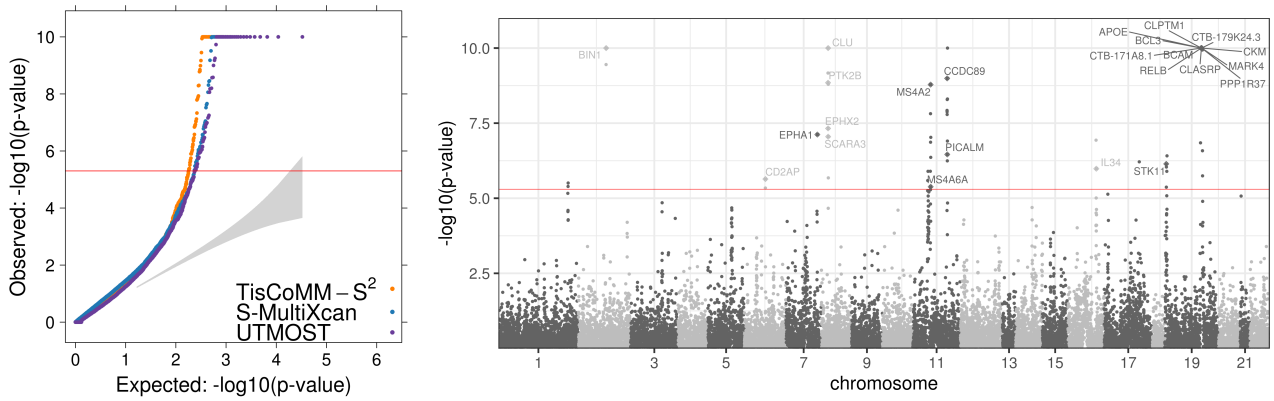
Figure 3: TisCoMM joint test outperforms the other multi-tissue methods. The number of replicates is 5000. In each subplot, the x-axis stands for the SNP heritability level, and the y-axis stands for the proportion of significant genes within 5000 replicates



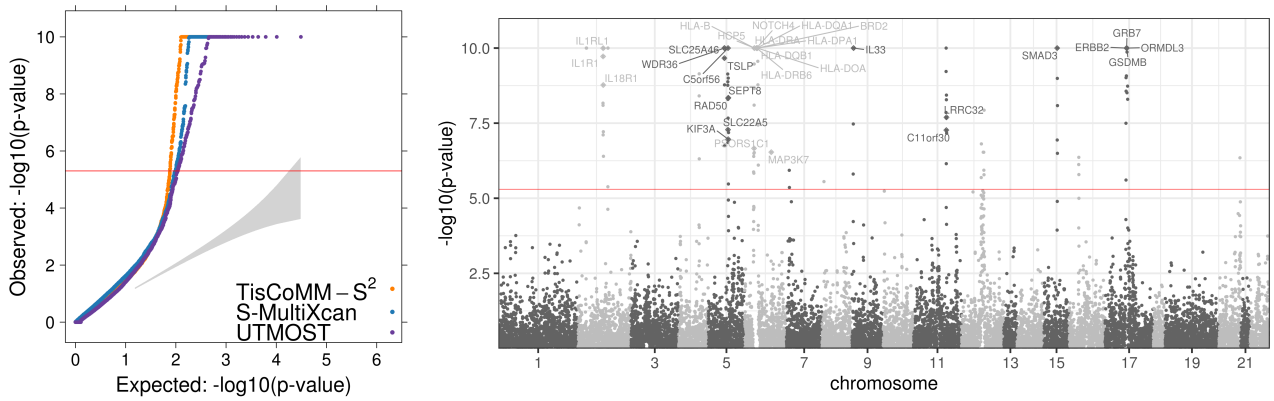
(A) Power

(B) false positive rate (FPR)

Figure 4: TisCoMM conditional test outperforms the single-tissue association test CoMM. **A.** the power of TisCoMM conditional test and the single tissue method CoMM with Bonferroni correction applied. **B.** the corresponding false positive rates under each setting.



(A) Alzheimer's disease



(B) Asthma

Figure 5: TisCoMM-S^2 results for Alzheimer's disease and asthma. The reference panel is European subsamples from 1000 Genome. In each row, the two panels show the qq-plot (left) and Manhatton plot (right).

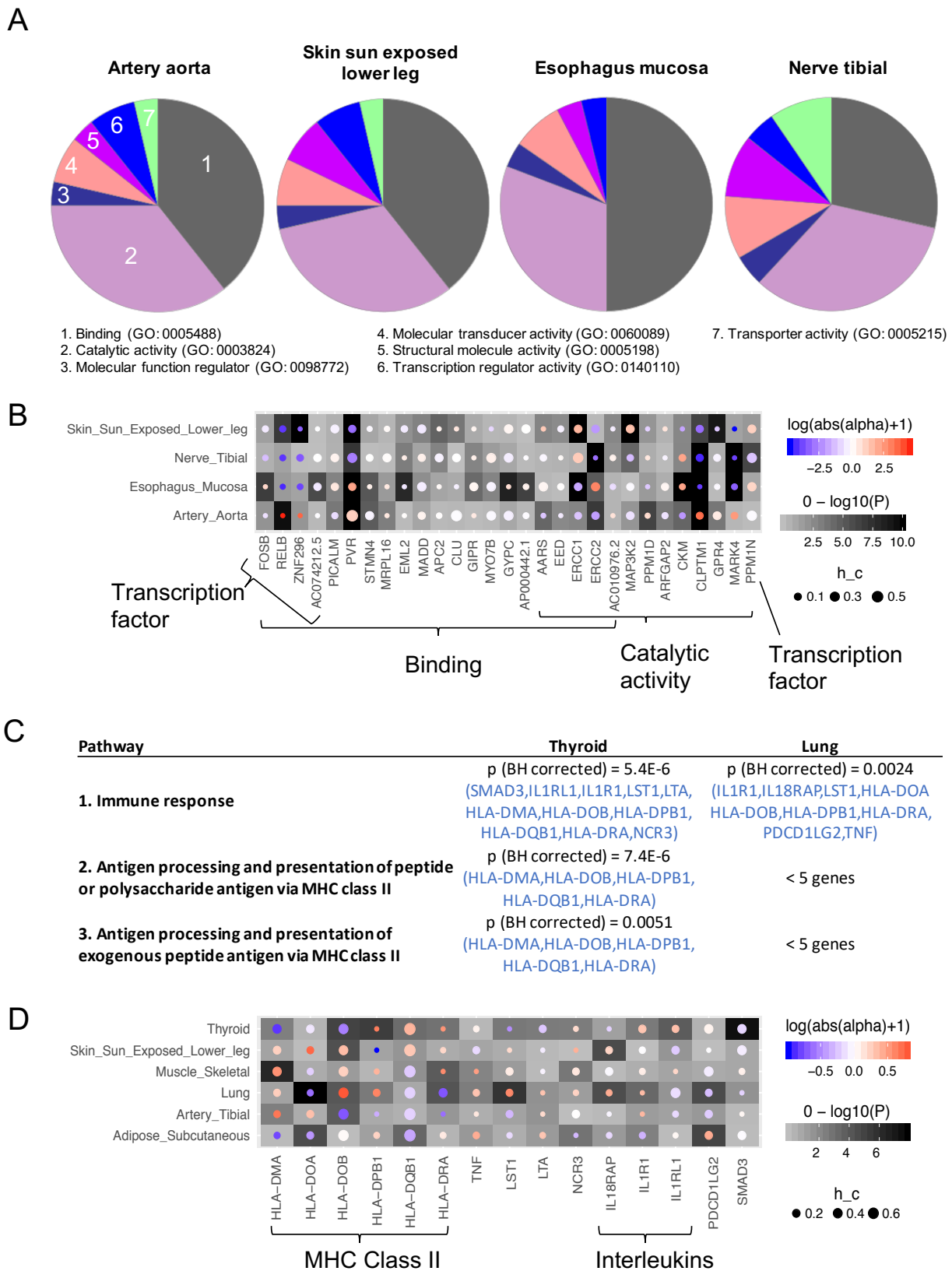


Figure 6: **A.** Each pie chart corresponding to a different tissue shows the percentage of AD-associated genes in each molecular function group (from gene ontology). **B.** The x-axis of the heatmap represents the union of AD-associated genes in 3 function groups (binding, catalytic activity, and transcription factor). The y-axis represents different tissue types. In each cell, the background color (shades of gray) indicates the significance level, the circle size indicates the heritability, and the color inside each circle indicates the effect size. **C.** Pathway analysis of asthma-associated genes in thyroid and lung. Pathway analysis was done using a web-based software DAVID, testing the enrichments of asthma-associated genes in biological processes (from gene ontology). Significant pathways were selected if gene count 5 and Benjamini-Hochberg (BH) corrected p-value 0.05. The asthma-associated genes are highlighted in blue. **D.** The x-axis of the heatmap represents the asthma-associated genes in the immune response pathway. And all the other settings are the same as the one used in part B.

Table 3: Distributions of tissues in which the candidate genes' associations arise in AD and asthma.

trait	#tissues	0	1	2	3	4	5	6
AD	TisCoMM-S^2	5	28	37	17	5	-	-
	CoMM-S	6	5	7	9	65	-	-
Asthma	TisCoMM-S^2	37	68	58	28	6	3	0
	CoMM-S	20	11	5	5	9	30	120