Analysis of Gene-Gene Interactions Using Gene-Trait Similarity Regression

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Key Words

Gene-gene interactions · Gene-trait similarity · Similarity regression

Abstract

Objective: Gene-gene interactions (G×G) are important to study because of their extensiveness in biological systems and their potential in explaining missing heritability of complex traits. In this work, we propose a new similarity-based test to assess G×G at the gene level, which permits the study of epistasis at biologically functional units with amplified interaction signals. **Methods:** Under the framework of gene-trait similarity regression (SimReg), we propose a gene-based test for detecting G×G. SimReg uses a regression model to correlate trait similarity with genotypic similarity across a gene. Unlike existing gene-level methods based on leading principal components (PCs), SimReg summarizes all information on genotypic variation within a gene and can be used to assess the joint/interactive effects of two genes as well as the effect of one gene conditional on another. **Results:** Using simulations and a real data application to the Warfarin study, we show that the SimReg G×G tests have satisfactory power and robustness under different genetic architecture when compared to existing gene-based interac-

tion tests such as PC analysis or partial least squares. A genome-wide association study with approx. 20,000 genes may be completed on a parallel computing system in 2 weeks.

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Introduction

Gene-gene interactions (G×G) are important to study since they are believed to be widespread in biological systems [1, 2] and are likely involved in gene regulation, signal transduction, biochemical networks as well as other physiological and developmental pathways [3–5]. G×G can further provide insight into the missing heritability of complex traits [6–8] and explain replication failures of initial genome-wide association study (GWAS) findings [9, 10]. Regarding this latter point, G×G help explain between-study differences in marginal genetic effects, which can be due to between-study differences in the frequency of modifier genetic variant(s). The study of G×G has provided insight into biological mechanisms for many complex diseases, including Alzheimer's disease, diabetes, cardiovascular disease, autism, multiple sclerosis, and cancer [11–17].

Analysts often implement G×G tests using a regression model accounting for the main effects of two single nucleotide polymorphisms (SNPs) and also the two-way interaction between the two SNPs. The interaction effect can be assessed on the additive or the multiplicative scale – the former examines the effect on the phenotype on the linear scale, and the latter examines the effect on the log scale of the phenotype. The additive scale is often more relevant to public health importance [18], while the multiplicative scale more naturally corresponds to the biological mechanisms [19]. Regardless of the scale, one can test whether the interaction parameter is different from zero or, alternatively, consider a test similar to that of Chapman and Clayton [20] to assess the effect of a SNP in the presence of interaction with a second SNP. While analysts primarily applied such SNP-SNP interaction tests in small-scale candidate-gene studies, there has also been keen interest in performing exhaustive interaction testing of SNPs in a GWAS [21]. One can perform such genome-wide analyses using standard tools like regression [6]. However, one can also apply more innovative techniques such as two-stage screening procedures [22], Bayes networks [23], Bayesian model averaging [24], logistic regression [25] and data-mining procedures [26–29].

All of these existing interaction methods consider the analysis on the level of a SNP. However, there is increasing interest in performing such analyses on the broader level of a gene [30–32]. Several factors motivate the paradigm shift from SNP to gene. First, genes are the basic unit in the biological mechanism and SNPs within a gene tend to work concordantly. Thus, gene-level results may be more biologically insightful and easier to interpret. Second, a gene-level analysis incorporates linkage disequilibrium (LD) information from all SNPs simultaneously within the gene. Consequently, such a joint analysis of SNPs should have an improved ability to tag untyped causal variants compared to the analysis of individual SNPs, leading to improved power. Finally, if a gene harbors multiple causal variants, then a joint analysis of SNPs in aggregate should be more powerful than a separate analysis of each individual SNP (owing in part to the gene-based test often having less degrees of freedom than its individual-SNP counterpart).

A few gene-based methods for interaction testing exist. Chatterjee et al. [33] proposed Tukey's 1-df method to investigate an interaction between two candidate genes. The approach calculates the sum of the main effects of SNPs contained within each gene and then uses the product of the two sums as the G×G term. Thus, the approach models one interaction parameter at the gene level rather

than several interaction parameters on the SNP level. Owing to the reduced degrees of freedom of the Tukey test, the authors showed that their approach lead to a great improvement in power to detect G×G compared to individual-SNP analysis. Motivated by this idea, Wang et al. [32] considered two different interaction tests that summarized SNP information within a gene. For the first test, the authors used principal component analysis (PCA) to summarize LD information of SNPs within a gene. They then created an interaction test using the top (first) principal component (PC) from each gene. For their second test, the authors applied partial least squares (PLS) to extract components that summarize both the LD information among SNPs in a gene as well as the correlation between such SNPs and the outcome of interest. The authors then constructed their interaction test using the top PLS component from each gene. Using simulated data, the PCA and PLS methods often had better performance than the Tukey 1-df method, particularly when causal SNPs had no or a negligible marginal effect.

In this article, we propose a new gene-based test for detecting G×G in complex traits using similarity regression (SimReg) [34]. SimReg is an analytic procedure that uses a regression model to correlate trait similarity with genotypic similarity across a gene. SimReg is inspired by Hase-man and Elston's regression from linkage analysis [35, 36] and haplotype similarity tests for regional association [37, 38]. In SimReg, the trait similarity is quantified by the trait covariance adjusting for the covariates. The multi-marker information of a gene is first summarized by genetic similarity, which is measured using a prespecified metric such as the proportion of alleles shared identical by state (IBS) across the gene. The G×G is then modeled by taking the product of the genetic similarities of the two genes, and its significance is assessed by testing the significance of the corresponding regression coefficient.

Compared to the Tukey-, PCA- or PLS-based methods, we believe SimReg has several advantages. First, SimReg utilizes all information within a gene, while the PCA or PLS procedures may lose some information due to considering only the top component of each gene within the interaction analysis. Second, as the top PCA or PLS component from each gene is a weighted sum of SNP information, the use of the product of the two linear combinations as the interaction term in the model only captures limited forms of non-additive effect. In contrast, SimReg provides a tool to model a variety of effects through different measures of summarized gene-similarity information. At the same time, SimReg does not involve a large number of parameters so that it has a good

power performance. Finally, as we show later, we can obtain an analytic p value for the SimReg procedure without the need for intensive resampling procedures like those required for the Tukey approach.

We arranged the remainder of the paper as follows. We first present the SimReg approach and describe how to use the framework to construct three score tests of interest (interaction test, joint test and conditional main effect test). Then, we derive the distributions of these tests under the null hypothesis by connecting the approach to a variance component model. We use simulated data to examine the performance of the proposed method against the PCA and PLS methods as well as a standard SNP-based test of interaction. Later, we also apply SimReg to the Warfarin data from the study of Wysowski et al. [39]. Finally, we discuss further research directions of the proposed approach.

Methods

The Gene-Trait Similarity Model

We assume a sample of N subjects. For subject i ($i = 1, \dots, N$), we denote Y_i as the trait value, X_i as a $K \times 1$ vector of covariates (including the intercept term), and $G_{m,i}$ as a coding of the subject's genotype at SNP m . We define S_{ij}^A to be the genetic similarity of gene A between subjects i and j ($i \neq j$). There are many ways to describe the genetic similarity between individuals. Here, we model similarity as the weighted sum of the proportion of alleles shared IBS across the M_A SNPs in gene A. That is, for subjects i and j , the similarity level is

$$S_{ij}^A = \sum_{m=1}^{M_A} w_m s_{m,ij},$$

where $s_{m,ij} = x/2$, if $G_{m,i}$ and $G_{m,j}$ share x alleles IBS [40, 41], and weight w_m can be used to up-weight or down-weight a variant based on allele frequencies, the degree of evolutionary conservation or the functionality of the variations [40, 42, 43]. In this work, to up-weight similarities that are contributed by rare alleles, we set $w_m = q_m^{-1}$, where q_m is the minor allele frequency (MAF) of marker m [44, 45]. We can define the similarity level in gene B S_{ij}^B in the same manner. The trait similarity between individuals i and j , denoted by Z_{ij} , is computed by $Z_{ij} = (Y_i - \mu_i)(Y_j - \mu_j)$, where $\mu_j = E(Y_i | X_i) = X_i\gamma$, which is the conditional mean of trait with no genotype effect, and where γ is the effect of the covariates. The SimReg for G×G is

$$E(Z_{ij} | X, G) = \tau_A S_{ij}^A + \tau_B S_{ij}^B + \tau_{AB} S_{ij}^A S_{ij}^B. \quad (1)$$

Note that the regression model has zero intercept because we incorporated the covariate effects when quantifying trait similarity [34].

The Interaction Test

The interaction test examines the null hypothesis $H_{0,Int}: \tau_{AB} = 0$. We derive the score test of this hypothesis from equation 1 by taking advantage of the connection between the similarity model and a variance component model [34]. Below, we first show that the regression coefficients in equation 1 can be viewed as the variance

components under a mixed model. We then construct the test of τ_{AB} using the same test from the corresponding variance component model. Specifically, consider the following working mixed model

$$Y_i = X_i\gamma + g_{A,i} + g_{B,i} + g_{AB,i} + e_i \quad (2)$$

where $e_i \sim N(0, \sigma)$ and $g_{A,i}$, $g_{B,i}$ and $g_{AB,i}$ are subject-specified genetic effects for gene A, gene B and G×G, respectively. Let g_A equal $[g_{A,1}, \dots, g_{A,n}]$, g_B equal $[g_{B,1}, \dots, g_{B,n}]$ and g_{AB} equal $[g_{AB,1}, \dots, g_{AB,n}]$, and assume that $g_A \sim MN(0, \nu_A S_A)$, $g_B \sim MN(0, \nu_B S_B)$ and $g_{AB} \sim MN(0, \nu_{AB} S_{AB})$, where $S_A = \{S_{ij}^A\}$, $S_B = \{S_{ij}^B\}$ and $S_{AB} = \{S_{ij}^A \times S_{ij}^B\}$. Under equation 2, we can obtain the marginal trait covariance by

$$\begin{aligned} cov(Y_i, Y_j | X, G) &= cov_g\{E(Y_i | X, G, g_A, g_B, g_{AB}), \\ &\quad E(Y_j | X, G, g_A, g_B, g_{AB})\} \\ &= cov_g\{X_i\gamma + g_{A,i} + g_{B,i} + g_{AB,i}, X_j\gamma \\ &\quad + g_{A,j} + g_{B,j} + g_{AB,j}\} \\ &= \nu_A S_{ij}^A + \nu_B S_{ij}^B + \nu_{AB} S_{ij}^A S_{ij}^B. \end{aligned} \quad (3)$$

Comparing equation 3 with equation 1, we have $\tau_A = \nu_A$, $\tau_B = \nu_B$ and $\tau_{AB} = \nu_{AB}$.

We derive the score function of the restricted maximum likelihood (REML) log-likelihood function of equation 2 in Appendix A, and obtain the score statistic under $H_{0,Int}$ as

$$T_{Int} = 1/2 Y' P_{Int} S_{AB} P_{Int} Y | \tau_A = \tau_A^*, \tau_B = \tau_B^*, \sigma = \hat{\sigma}$$

where $(Y_1, \dots, Y_n)$, $P_{Int} = V_{Int}^{-1} - V_{Int}^{-1} X(X'X)^{-1} X' V_{Int}^{-1}$, $V_{Int} = \tau_A S_A + \tau_B S_B + \sigma I$ and $(\tau_A^*, \tau_B^*, \hat{\sigma})$ are the maximum REML estimates obtained under $H_{0,Int}: \tau_{AB} = 0$. We describe an expectation-maximization (EM) algorithm to obtain $(\tau_A^*, \tau_B^*, \hat{\sigma})$ in Appendix B.

Under the alternative hypothesis $\tau_{AB} \neq 0$, T_{Int} is a strictly increasing function of τ_{AB} . Therefore larger values of T_{Int} provide stronger evidence against $H_{0,Int}$. This suggests that the testing procedure should be one sided. As shown in Appendix A, the distribution of T_{Int} follows a weighted χ^2 distribution. That is, define

$$C_{Int} = 1/2 V_{Int}^{1/2} P_{Int} S_{AB} P_{Int} V_{Int}^{1/2},$$

and then

$$T_{Int} \sim \sum_{j=1}^c \lambda_{j,Int} \chi_1^2,$$

where $\lambda_{j,Int}$ is the ordered nonzero eigenvalue of matrix C_{Int} . We can calculate the p values analytically using moment-matching approximations [46].

The Joint Test

Instead of performing a test specifically for interactions, one may be interested in assessing whether the two genes have any marginal or interactive effects on the trait [47]. This can be done using the joint test to examine $H_{0,Joint}: \tau_A = \tau_B = \tau_{AB} = 0$ under the full model $E(Z_{ij} | X, G) = \tau_A S_{ij}^A + \tau_B S_{ij}^B + \tau_{AB} S_{ij}^A S_{ij}^B$. As shown in Appendix A, the test statistic is given as

$$T_{Joint} = 1/2 Y' P_{Joint} (S_A + S_B + S_{AB}) P_{Joint} Y | \sigma = \hat{\sigma},$$

where $P_{Joint} = \sigma^{-1} \{I - X(X'X)^{-1} X'\}$ and $\hat{\sigma} = Y' \{I - X(X'X)^{-1} X'\} Y / (n - K)$. The distribution of T_{Joint} also follows a weighted χ^2 distribution, i.e.

$$T_{Joint} \sim \sum_{j=1}^c \lambda_{j,Joint} \chi_1^2,$$

with $\lambda_{j,Joint}$ being the ordered nonzero eigenvalue of $C_{Joint} = 1/2 V_{Joint}^{1/2} P_{Joint} (S_A + S_B + S_{AB}) P_{Joint} V_{Joint}^{1/2}$. As with the interaction

test, we calculate the p values of the joint test analytically using moment-matching procedures.

The Conditional Main Effect Test

We also construct the score test for the main effect of a gene conditioning on the effect of the other gene, assuming no G×G. This is because in scenarios where the interaction effects do exist, the main effects are typically not well defined, and its significance depends on the scale of the interacting variables. When there is no G×G, the full model is

$$E(Z_{ij} | X, G) = \tau_A S_{ij}^A + \tau_B S_{ij}^B$$

Here to test the main effect of Gene A accounting for the effect of Gene B, we evaluate $H_{0,A}: \tau_A = 0$. We can similarly test the main effect of Gene B accounting for the effect of Gene A by evaluating $H_{0,B}: \tau_B = 0$. Similar to the score tests in the interaction test and joint test, we derive the score test statistic for the conditional main effect of Gene A conditional on Gene B as

$$T_A = 1/2 \mathbf{Y}' P_A S_A P_A \mathbf{Y} |_{\tau_B = \bar{\tau}_B, \sigma = \bar{\sigma}},$$

where $P_A = V_A^{-1} - V_A^{-1} \mathbf{X} (\mathbf{X}' V_A^{-1} \mathbf{X})^{-1} \mathbf{X}' V_A^{-1}$, $V_A = \tau_B S_B + \sigma I$ and $(\bar{\tau}_B, \bar{\sigma})$ are the maximum REML estimates obtained under $H_{0,A}: \tau_A = 0$. We describe the EM algorithms to obtain $(\bar{\tau}_B, \bar{\sigma})$ in Appendix B. The test statistic T_B can be defined similarly for examining $H_{0,B}: \tau_B = 0$ under the constrain of $\tau_{AB} = 0$. As shown in Appendix A, T_A has the same distribution as

$$\sum_{j=1}^c \lambda_{j,A} X_1^2,$$

where $\lambda_{j,A}$ is the ordered nonzero eigenvalue of $C_A = 1/2 V_A^{1/2} P_A S_A P_A V_A^{1/2}$. As with the interaction and joint tests, we analytically derive the p values of the test statistic using moment-matching procedures.

Simulation Study

Design

We study the performance of the proposed methods using simulated data, and benchmark them against 3 approaches: (1) LR: linear regression; (2) PCA: the principal component method of Wang et al. [32], and (3) PLS: the partial least square of Wang et al. [32]. The LR incorporates all SNPs from each of the 2 genes as well as all pairwise interactions of SNPs across genes. When performing conditional main effect testing, we exclude the interaction terms from the LR analysis. We also only consider the proposed test and LR for the conditional main effect test, since PCA and PLS are identical to LR when there is no interaction term.

To simulate genotype data with realistic LD patterns, we use genotype data of gene RBJ (8 SNPs) and gene GPRC5B (15 SNPs) of the phase III CEU samples downloaded from the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>). We show the LD structure of the two genes in online supplementary figure 1 (see www.karger.com/doi/10.1159/000360161 for all online suppl. material) and the MAF and the average R^2 of each SNP in online supplementary table 1. The average R^2 is calculated by averaging the R^2 between the target SNP and the remaining SNPs in the same gene. We consider two causal SNPs from each gene and simulate the trait values based on the model listed below.

Table 1. The causal SNPs for Gene A and Gene B for the three scenarios considered in the power analysis

Scenario	SNP_1^A		SNP_2^A		SNP_1^B		SNP_2^B	
	avg R^2	MAF	avg R^2	MAF	avg R^2	MAF	avg R^2	MAF
1	0.58	0.12	0.13	0.48	0.26	0.46	0.23	0.48
2	0.57	0.12	0.16	0.06	0.14	0.16	0.23	0.48
3	0.58	0.12	0.16	0.06	0.07	0.04	0.14	0.16

$$Y = \beta_A \times (SNP_1^A + SNP_2^A + SNP_1^A \times SNP_2^A) + \beta_B \times (SNP_1^B + SNP_2^B + SNP_1^B \times SNP_2^B) + \beta_{AB} \times (SNP_1^A \times SNP_1^B + SNP_2^A \times SNP_2^B) + e, \quad (4)$$

where SNP_1^A and SNP_2^A are the number of the minor alleles carried by a subject at the first and second causal loci in Gene A, SNP_1^B and SNP_2^B are defined similarly and e follows a normally distributed variable with mean 0 and variance 1. The trait value Y is defined based on 3 parts: the gene effect from Gene A, the gene effect from Gene B and the interaction effect between Gene A and Gene B. We assume no LD between the genes RBJ and GPRC5B, and consequently sample the genotypes of the former gene independent of the genotypes for the latter gene.

To evaluate the type I error rate of the interaction test, we considered 3 situations of no G×G effects: (1) both genes have no genetic effect ($\beta_A = \beta_B = \beta_{AB} = 0$); (2) only Gene A has a main effect to the trait ($\beta_A \neq 0, \beta_B = \beta_{AB} = 0$), and (3) both Gene A and Gene B have a main effect to the trait ($\beta_A \neq 0, \beta_B \neq 0, \beta_{AB} = 0$). To evaluate the type I error rate for the joint test, we set $\beta_A = \beta_B = \beta_{AB} = 0$. To evaluate the type I error rate for the conditional main effect test, we examined the size strictly for Gene B ($H_0: \tau_B = 0$). We considered two scenarios of no effect of Gene B: (1) both Gene A and Gene B have no main effect ($\beta_A = \beta_B = \beta_{AB} = 0$) and (2) Gene A has a main effect but not Gene B ($\beta_A \neq 0, \beta_B = 0, \beta_{AB} = 0$). For each scenario, we simulated datasets comprised of 300 subjects and evaluated the type I error rates using 1,000 replicates of the data.

For power analysis, we consider three different scenarios based on the causal SNPs. Specifically, we pick up ‘representative’ SNPs from all SNPs with similar LD and MAF patterns and form 3 different causal SNP combinations (table 1). When generating trait values using the model above, we consider different β values so that the power of the different methods at a significance threshold of 5% is between 20 and 80%. The power was calculated based on 200 replications.

Results

Table 2 shows the empirical type I error rates for all methods at a significance level of $\alpha = 0.05$. The type I error rates of all approaches are around the nominal level in all different settings. For the interaction test, the type I error rates of the proposed method are slightly conservative but we observed that, as the variance due to the main effects

Table 2. The type I error rate at significance level 0.05 for the three tests

Method	Interaction test			Joint test	Conditional main effect test (B A)	
	(0, 0, 0)	(0.1, 0, 0)	(0.1, 0.1, 0)		(0, 0, 0)	(0.3, 0, 0)
SimReg	0.038	0.04	0.046	0.049	0.048	0.052
LR	0.060	0.062	0.064	0.060	0.060	0.056
PCA	0.056	0.06	0.058	0.055		
PLS	0.046	0.048	0.049	0.054		

$(\beta_A, \beta_B, \beta_{AB})$ is the effect size.

Table 3. Power analysis for different approaches at significance level 0.05 under the scenarios listed in table 1

	SimReg	LR	PCA	PLS
<i>Interaction test</i>				
Scenario 1 (0.1, 0.1, 0.3)	0.690 (0.626, 0.754) ^a	0.375 (0.308, 0.442)	0.715 (0.652, 0.778)	0.745 (0.685, 0.805)
Scenario 2 (0.1, 0.1, 0.5)	0.460 (0.391, 0.529)	0.285 (0.222, 0.348)	0.350 (0.284, 0.416)	0.490 (0.421, 0.559)
Scenario 3 (0.1, 0.1, 1.5)	0.815 (0.761, 0.869)	0.760 (0.701, 0.819)	0.120 (0.075, 0.165)	0.470 (0.401, 0.539)
<i>Joint test</i>				
Scenario 1 (0.05, 0.05, 0.05)	0.670 (0.605, 0.735) ^a	0.325 (0.260, 0.390)	0.535 (0.466, 0.604)	0.545 (0.476, 0.614)
Scenario 2 (0.1, 0.1, 0.1)	0.875 (0.829, 0.921)	0.690 (0.626, 0.754)	0.765 (0.706, 0.824)	0.785 (0.728, 0.842)
Scenario 3 (0.3, 0.3, 0.3)	0.580 (0.512, 0.648)	0.325 (0.260, 0.390)	0.500 (0.431, 0.569)	0.505 (0.436, 0.574)
<i>Conditional main effect test</i>				
Scenario 1 (0.07, 0.07, 0)	0.650 (0.584, 0.716) ^a	0.415 (0.347, 0.483)		
Scenario 2 (0.1, 0.1, 0)	0.685 (0.621, 0.749)	0.475 (0.406, 0.544)		
Scenario 3 (0.3, 0.3, 0)	0.495 (0.426, 0.564)	0.485 (0.416, 0.554)		

In each scenario, the effect size $(\beta_A, \beta_B, \beta_{AB})$ is set so that the power range is between 20 and 80%. The values in italics and bold indicate the method with the largest power under a certain scenario; the values in bold indicate the methods with overlapping confidence intervals with the best methods.

^a 95% confidence interval of the power, obtained by $\hat{p} \pm 1.96 \times \sqrt{\hat{p} \times (1 - \hat{p}) / 200}$, with $\hat{p}$ being the empirical power estimated based on 200 replications.

of Gene A and/or Gene B increases, the type I error rate generally approaches the nominal level. As discussed in the Appendix, the conservative nature of the interaction test arises because of the bias in the EM algorithm estimates of the variance components when their true values are 0. The type I error rates for the interaction test using the competing methods (PCA, PLS, LR) are generally appropriate. For joint and conditional main effect tests, we observed the type I error rates of all methods considered in our analyses to be quite similar to the nominal levels.

We next performed a power analysis under the models considered in table 1. Because different scenarios have different causal SNP combinations, we adjust the different β values so that the power of the different methods is around 0.2–0.8. The results are summarized in table 3.

Overall, we observed that the proposed method generally has optimal power relative to the other methods considered under various genetic architectures of the causal SNPs. For the interaction test, the causal SNPs have relatively high LD and large MAFs in Scenario 1. PCA and PLS perform the best, closely followed by the proposed method. LR has the least power due to the large degree of freedom used. In Scenario 2, where the causal SNPs have a relatively smaller LD and MAF, PLS still has the best power, closely followed by the proposed method. The power of PCA drops a lot in this scenario. LR again has the least power. In Scenario 3, the proposed method has the best power, followed by LR, PLS and PCA. PCA and PLS have a significantly low power performance in Scenario 3. As the LD and MAF become smaller, the

Table 4. p values of the 4 approaches in the warfarin analysis

Method	Joint test	Interaction test	Conditional main effect test	
			VKORC1	CYP2C9
SimReg	5.6×10^{-20}	0.450	1.67×10^{-20}	1.02×10^{-7}
LR	1.92×10^{-16}	0.753	1.94×10^{-16}	2.65×10^{-8}
PCA	8.07×10^{-5}	0.530	–	–
PLS	7.43×10^{-5}	0.470	–	–

power of PCA drops dramatically, because the first PC can only capture a limited amount of information on the causal SNPs. The first PC aims to maximize the SNP variation captured and tends to be dominated by SNPs with high LD or common MAFs, which are non-causal SNPs in Scenario 3. PLS shares the same issue as PCA and hence it also suffers from power loss when the LD and MAFs of the causal SNPs become smaller. However, PLS also accounts for the trait information, which makes PLS perform better than PCA. The power advantage of PLS over PCA is more substantial in Scenarios 2 and 3, where an increased proportion of causal SNPs have low MAFs. Consequently, the first PC of PCA (which explains the largest variance among the genotypes) is less likely to harbor such causal SNPs and therefore PCA has a reduced power for interaction testing compared to PLS [32].

For the joint test, under the three different scenarios, the proposed method performs the best. PCA and PLS perform similarly with a slightly better power of PLS. The observation is consistent with the results of Wang et al. [32], because the first PC from PLS considers LD and a correlation between trait and gene. LR has the lowest power, which may be due to the large degree of freedom used in the joint test.

For the conditional main effect test, the proposed method always has a better power than linear models, but the difference between the proposed method and the linear models becomes smaller when the LD and MAFs of the causal SNPs become smaller. The finding is consistent with the finding in the interaction test.

Real Data Analysis

Warfarin is a widely used oral anticoagulant. In 2004, more than 30 million prescriptions contained this drug in the USA [39]. The optimal dose of warfarin is different from patient to patient, and an inappropriate dosage can

lead to severe consequences such as bleeding and swelling of face or throat. Extensive research has been conducted to develop methods for predicting the appropriate dose.

We have conducted a genetic analysis using the data from the Warfarin study [48]. In this dataset, we studied the relationship between a stable warfarin dose and two genes: VKORC1 (containing 7 SNPs) and CYP2C9 (a tri-allelic locus). We further adjusted for 4 covariates associated with warfarin therapy: age, sex, height, and weight. After quality control, the dataset consisted of 301 individuals. We applied the proposed method and the benchmark methods to evaluate the association between warfarin dose and the two genes, adjusting for covariates. The results are summarized in table 4. All methods identified a significant association between warfarin dose and the two genes. The smallest p value of the joint test was obtained from SimReg (i.e. $p = 5.6 \times 10^{-20}$), closely followed by LR (i.e. $p = 1.92 \times 10^{-16}$). The p values from PCA and PLS are similar to each other and are many orders of magnitude larger (i.e. 10^{-5}) than those derived from SimReg and LR. We next examined the interaction effect between VKORC1 and CYP2C9 and observed no significant interaction using any of the methods considered. Finally, we performed the conditional main effect test for each gene using SimReg and LR. The result suggests that both genes had significant effects on warfarin dose, with VKORC1 demonstrating a stronger effect (i.e. p values on the order of 10^{-16} – 10^{-20}) than CYP2C9 (i.e. p values on the order of 10^{-7} – 10^{-8}).

The main effect results were consistent with those in the current literature [49–51]. Previous studies indicate contradictory results regarding the interaction between VKORC1 and CYP2C9 on anticoagulant effect and hence the dose requirement [52–56]. Our results agree with recent reports [55, 56], which suggest no evidence of G×G between CYP2C9 and VKORC1.

Discussion

In this article, we describe a novel similarity-based test to assess G×G in large-scale association studies. Unlike the majority of existing procedures in this area, our approach considers the analysis at the level of the gene rather than of the SNP, which we show can lead to considerable improvements in power as a result. We also compare the performance of our similarity approach to existing gene-based interaction tests (PCA and PLS) and show that our joint, interaction and conditional main effect tests nearly always had optimal power across the simula-

tion models. Relative to the PCA and PLS approaches, we believe our approach can improve power since it summarizes all information on genotypic variation within a gene. PCA and PLS, on the other hand, only use a subset of this variation within their analytic frameworks. The R code that implements the SimReg testing procedure is available from the authors' website (<http://www4.stat.ncsu.edu/~jytzeng/Software/SimReg-GxG-QT/>). Using a personal computer equipped with Intel Core i7-3770, 40 GHz CPU and 20 GB RAM, 200 runs of the interaction test (which is the slowest test because it requires estimating two nuisance variance components) with the simulated datasets required 767 s. While the approach is not scalable to genome-wide interaction testing using a single processor, we believe the procedure can be applied to genome-wide data in reasonable time using a parallel-computing cluster that possesses a large number (1,000) of CPUs.

In the SimReg model, we define the similarity function for two subjects as the weighted average of SNP alleles shared IBS by the pair across a gene. The weights are SNP specific and user defined. It is common to weight SNPs based on MAF such that rarer variants are assigned more weight than common variants; common weights in this setting include the reciprocal of a SNP's MAF or its square root [40, 41]. Additionally, we can weight SNPs using functional information; perhaps providing more weight to SNPs that demonstrate evidence of being a cis-acting expression quantitative trait locus [57]. While we considered only common SNP variants in this work, we note that our SimReg approach can also integrate information from rare variants ascertained using next-generation sequencing technology. While weighting rare variants by MAF has obvious value, additional weighting of such variants using some measure that predicts the probability that the variant is deleterious and lowers fitness likely has value as well. A variety of computational algorithms exists for such predictions based on evolutionary, biochemical and/or structural information [for an overview, see 58].

We applied our similarity approach in the context of a candidate-gene study, but we can further expand the approach to consider interactions on a genome-wide scale. A gene-based interaction test has appealing features over SNP-based interaction tests when applied to a GWAS. Given that the number of genes is many-fold smaller than the typical number of SNPs in a GWAS, the number of tests that need to be evaluated using SimReg would be substantially smaller than a SNP-based interaction test like LR. This could substantially reduce the computational burden. Additionally, the issue of adjusting for multiple

testing when conducting genome-wide interaction testing of SNPs is challenging. Within GWAS, most studies adjust for multiple testing using permutations, since a Bonferroni correction leads to conservative inference due to LD among SNPs. However, while permutations are valid for multiple testing adjustment in main effect tests, they are not valid when applied to interaction tests because such random shuffling of phenotypes does not preserve the observed main effects in the sample [59]. In contrast, multiple testing adjustment of interaction tests based on SimReg is more straightforward. While the application of gene-based SimReg to GWAS data will lead to correlated tests (due to repeated testing of the same gene) such that a Bonferroni correction is inappropriate, we can adjust for multiple testing using a computationally efficient perturbation procedure similar to that proposed by Wu et al. [60] that preserves the main effects of the genes under consideration. We will explore this work in a future paper.

Appendix A

Derivation of the Score Tests and Their Distributions

Consider the matrix presentation of equation 2

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\gamma} + \mathbf{g}_A + \mathbf{g}_B + \mathbf{g}_{AB} + \mathbf{e}.$$

The corresponding REML log-likelihood function, denoted as $L(\tau_A, \tau_B, \tau_{AB}, \sigma)$, is

$$L(\theta) = -1/2 [\log |\mathbf{V}| + \log |\mathbf{X}'^{-1}\mathbf{V}\mathbf{X}| + \mathbf{Y}'\mathbf{P}\mathbf{Y}],$$

where $\mathbf{V} = \text{Var}(\mathbf{Y}) = \tau_A \mathbf{S}_A + \tau_B \mathbf{S}_B + \tau_{AB} \mathbf{S}_{AB} + \sigma \mathbf{I}$ is the marginal variance of $\mathbf{Y}$ and $\mathbf{P} = \mathbf{V}^{-1} - \mathbf{V}^{-1}\mathbf{X}(\mathbf{X}'^{-1}\mathbf{V}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}^{-1}$ is the projection matrix for the model. The score functions of τ_A , τ_B , and τ_{AB} based on $L(\theta)$ are

$$U_{\tau_A}(\tau_A, \tau_B, \tau_{AB}, \sigma) = \frac{\partial L(\tau_A, \tau_B, \tau_{AB}, \sigma)}{\partial \tau_A}$$

$$= \frac{1}{2} [\mathbf{Y}'\mathbf{P}\mathbf{S}_A\mathbf{P}\mathbf{Y} - \text{tr}(\mathbf{P}\mathbf{S}_A)],$$

$$U_{\tau_B}(\tau_A, \tau_B, \tau_{AB}, \sigma) = \frac{\partial L(\tau_A, \tau_B, \tau_{AB}, \sigma)}{\partial \tau_B}$$

$$= \frac{1}{2} [\mathbf{Y}'\mathbf{P}\mathbf{S}_B\mathbf{P}\mathbf{Y} - \text{tr}(\mathbf{P}\mathbf{S}_B)],$$

and

$$U_{\tau_{AB}}(\tau_A, \tau_B, \tau_{AB}, \sigma) = \frac{\partial L(\tau_A, \tau_B, \tau_{AB}, \sigma)}{\partial \tau_{AB}}$$

$$= \frac{1}{2} [\mathbf{Y}'\mathbf{P}\mathbf{S}_{AB}\mathbf{P}\mathbf{Y} - \text{tr}(\mathbf{P}\mathbf{S}_{AB})].$$

We construct the statistics based on the first terms of the score functions. Here after, we define matrices $\mathbf{P}_h$ and $\mathbf{V}_h$ as $\mathbf{P}$ and $\mathbf{V}$

evaluated under $H_{0,h}$. Then for the interaction test $H_{0,Int}$: $\tau_{AB} = 0$, we set the test statistic as

$$T_{Int} = 1/2 \mathbf{Y}' P_{Int} S_{AB} P_{Int} \mathbf{Y} \mid \tau_A = \hat{\tau}_A, \tau_B = \hat{\tau}_B, \sigma = \hat{\sigma}$$

where $P_{Int} = V^{-1}_{Int} - V^{-1}_{\hat{A}} \mathbf{X}(\mathbf{X}' V^{-1}_{\hat{A}} \mathbf{X})^{-1} \mathbf{X}' V^{-1}_{Int}$, $V_{Int} = \tau_A S_A + \tau_B S_B + \sigma I$ and $(\hat{\tau}_A, \hat{\tau}_B, \hat{\sigma})$ are the maximum REML estimates obtained under $H_{0,Int}$: $\tau_{AB} = 0$.

For the conditional main effect test $H_{0,A}$: $\tau_A = 0$ under the constrain of no interaction (i.e. $\tau_{AB} = 0$), we set the test statistic as

$$T_A = 1/2 \mathbf{Y}' P_A S_A P_A \mathbf{Y} \mid \tau_B = \hat{\tau}_B, \sigma = \hat{\sigma}$$

where $P_A = V^{-1}_{\hat{A}} - V^{-1}_{\hat{A}} \mathbf{X}(\mathbf{X}' V^{-1}_{\hat{A}} \mathbf{X})^{-1} \mathbf{X}' V^{-1}_{\hat{A}}$, $V_A = \tau_B S_B + \sigma I$ and $(\hat{\tau}_B, \hat{\sigma})$ are the maximum REML estimates obtained under $H_{0,A}$: $\tau_A = 0$. The test statistic T_B can be defined similarly for examining $H_{0,B}$: $\tau_B = 0$ under the constrain of $\tau_{AB} = 0$. We describe the EM algorithms that we use to obtain $(\hat{\tau}_A, \hat{\tau}_B, \hat{\sigma})$ and $(\hat{\tau}_B, \hat{\sigma})$ in Appendix B.

For the joint test $H_{0,Joint}$: $\tau_A = \tau_B = \tau_{AB} = 0$, because the values of τ are non-negative variance components, $\tau_A = \tau_B = \tau_{AB} = 0$ if and only if $\tau_A + \tau_B + \tau_{AB} = 0$. This motivates us to construct the test statistic based on the sum of the three score functions. That is

$$T_{Joint} = 1/2 \mathbf{Y}' P_{Joint} (S_A + S_B + S_{AB}) P_{Joint} \mathbf{Y} \mid \sigma = \hat{\sigma}$$

where the $P_{Joint} = V^{-1}_{Joint} - V^{-1}_{\hat{A}} \mathbf{X}(\mathbf{X}' V^{-1}_{\hat{A}} \mathbf{X})^{-1} \mathbf{X}' V^{-1}_{Joint}$, $V_{Joint} = \sigma I$ and $\hat{\sigma} = \mathbf{Y}' \{I - \mathbf{X}(\mathbf{X}' \mathbf{X})^{-1} \mathbf{X}'\} \mathbf{Y} / (n - K)$.

The distributions of the test statistics can be shown to follow a weighted χ^2 distribution via the fact that these statistics are a quadratic form of $\mathbf{Y}$. To illustrate this, consider $T_{Int} = 1/2 \mathbf{Y}' P_{Int} S_{AB} P_{Int} \mathbf{Y}$. As P_{Int} is a projection matrix, $P_{Int} \mathbf{X} \mathbf{Y} = 0$. Therefore,

$$\begin{aligned} T_{Int} &= \frac{1}{2} \mathbf{Y}' P_{Int} S_{AB} P_{Int} \mathbf{Y} = \frac{1}{2} (\mathbf{Y} - \mathbf{X} \mathbf{Y})' P_{Int} S_{AB} P_{Int} (\mathbf{Y} - \mathbf{X} \mathbf{Y}) \\ &= \frac{1}{2} (\mathbf{Y} - \mathbf{X} \mathbf{Y})' V^{-1/2}_{Int} V^{1/2}_{Int} P_{Int} S_{AB} P_{Int} V^{1/2}_{Int} V^{-1/2}_{Int} (\mathbf{Y} - \mathbf{X} \mathbf{Y}) \sim \sum_{i=1}^c \lambda_i \chi_i^2. \end{aligned}$$

Define $\mathbf{z} = V^{-1/2}_{Int} (\mathbf{Y} - \mathbf{X} \mathbf{Y})$ and $C_{Int} = 1/2 V^{-1/2}_{Int} P_{Int} S_{AB} P_{Int} V^{-1/2}_{Int}$, we have

$$T_{Int} = \mathbf{z}' C_{Int} \mathbf{z} \sim \sum_{j=1}^c \lambda_{j,Int} \chi_1^2,$$

where $\lambda_{j,Int}$ is the ordered nonzero eigenvalue of matrix C_{Int} . By the same manner, one can obtain that

$$T_A \sim \sum_{j=1}^c \lambda_{j,A} \chi_1^2,$$

with $\lambda_{j,A}$ being the ordered nonzero eigenvalue of $C_A = 1/2 V^{1/2}_{\hat{A}} P_A S_A P_A V^{1/2}_{\hat{A}}$, and

$$T_{Joint} \sim \sum_{j=1}^c \lambda_{j,Joint} \chi_1^2,$$

with $\lambda_{j,Joint}$ being the ordered nonzero eigenvalue of $C_{Joint} = 1/2 V^{-1/2}_{Joint} P_{Joint} (S_A + S_B + S_{AB}) P_{Joint} V^{-1/2}_{Joint}$.

Appendix B

The EM Algorithm to Obtain the Maximum REML Estimates

We first describe the EM algorithm for $(\hat{\tau}_A, \hat{\tau}_B, \hat{\sigma})$, i.e. the maximum REML estimates under $H_{0,Int}$: $\tau_{AB} = 0$. Under $H_{0,Int}$, the LMM is

$$\mathbf{Y} = \mathbf{X} \mathbf{y} + \mathbf{g}_A + \mathbf{g}_B + \mathbf{e},$$

where $\mathbf{e} \sim N(0, \sigma I)$, $\mathbf{g}_A \sim N(0, \tau_A S_A)$ and $\mathbf{g}_B \sim N(0, \tau_B S_B)$ as $\tau_A = \nu_A$ and $\tau_B = \nu_B$. Define $\mathbf{U} = \mathbf{A}' \mathbf{Y}$ with the restriction that $\mathbf{A}' \mathbf{A} = I_{n-K}$ and $\mathbf{A} \mathbf{A}' = I - \mathbf{X}(\mathbf{X}' \mathbf{X})^{-1} \mathbf{X}'$. Then $\mathbf{U} \mid \mathbf{g}_A, \mathbf{g}_B \sim N(\mathbf{A}' \mathbf{g}_A + \mathbf{A}' \mathbf{g}_B, \sigma I_{n-K})$, which is independent of the fixed effect $\hat{\mathbf{y}} = (\mathbf{X}' \mathbf{X})^{-1} \mathbf{X}' \mathbf{Y}$. Therefore the maximum REML estimates can be obtained by maximizing the marginal distribution of $\mathbf{U}$, i.e. $f(\mathbf{U}) = \int f(\mathbf{U} \mid \mathbf{g}_A, \mathbf{g}_B) f(\mathbf{g}_A) f(\mathbf{g}_B) d\mathbf{g}_A d\mathbf{g}_B$. This motivated an EM algorithm based on $\mathbf{U}$ (i.e. the observed data) and $(\mathbf{g}_A, \mathbf{g}_B)$ (i.e. the missing data). The complete data log-likelihood, based on $f(\mathbf{U}, \mathbf{g}_A, \mathbf{g}_B)$, is

$$\begin{aligned} \log f(\mathbf{U}, \mathbf{g}_A, \mathbf{g}_B; \tau_A, \tau_B, \sigma) &= \log f(\mathbf{U} \mid \mathbf{g}_A, \mathbf{g}_B; \tau_A, \tau_B, \sigma) + \log f(\mathbf{g}_A; \tau_A, \tau_B, \sigma) + \log f(\mathbf{g}_B; \tau_A, \tau_B, \sigma) \\ &= -\frac{n-K}{2} \log \sigma - \frac{1}{2\sigma} (\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B)' (\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B) \\ &\quad - \frac{q_A}{2} \log \tau_A - \frac{1}{2} \log(|S_A|_+) - \frac{1}{2\tau_A} \mathbf{g}_A^T S_A^- \mathbf{g}_A \\ &\quad - \frac{q_B}{2} \log \tau_B - \frac{1}{2} \log(|S_B|_+) - \frac{1}{2\tau_B} \mathbf{g}_B^T S_B^- \mathbf{g}_B, \end{aligned}$$

where q_A and q_B are the rank for matrix S_A and S_B , respectively, $|S_A|_+$ is the pseudodeterminant and S_A^- is the generalized inverse (as S_A and S_B may be singular).

In the expectation step, we compute

$$\begin{aligned} Q(\tau_A, \tau_B, \sigma; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}) &\equiv E\{\log f(\mathbf{U}, \mathbf{g}_A, \mathbf{g}_B; \tau_A, \tau_B, \sigma) \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} \\ &= -\frac{n-K}{2} \log \sigma - \frac{1}{2\sigma} E\{(\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B)' (\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B) \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} \\ &\quad - \frac{q_A}{2} \log \tau_A - \frac{1}{2} \log(|S_A|_+) - \frac{1}{2\tau_A} E\{\mathbf{g}_A^T S_A^- \mathbf{g}_A \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} \\ &\quad - \frac{q_B}{2} \log \tau_B - \frac{1}{2} \log(|S_B|_+) - \frac{1}{2\tau_B} E\{\mathbf{g}_B^T S_B^- \mathbf{g}_B \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\}. \end{aligned}$$

In the maximization step, we solve for $\partial Q / \partial \tau_A = 0$, $\partial Q / \partial \tau_B = 0$ and $\partial Q / \partial \sigma = 0$, and obtain

$$\hat{\tau}_A = \frac{1}{q_A} E\{\mathbf{g}_A^T S_A^- \mathbf{g}_A \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} = \frac{1}{q_A} \left\{ \hat{\mathbf{g}}_A^{(t)'} S_A^- \hat{\mathbf{g}}_A^{(t)} + tr(S_A^- \hat{\mathbf{v}}_A^{(t)}) \right\},$$

where

$$\hat{\mathbf{g}}_A^{(t)} \equiv E(\mathbf{g}_A \mid \mathbf{g}_B, \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}) = \tau_A S_A P_{Int} \mathbf{Y} \mid \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)},$$

and

$$\hat{\mathbf{v}}_A^{(t)} \equiv Var(\mathbf{g}_A \mid \mathbf{g}_B, \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}) = \tau_A S_A - \tau_A^2 S_A P_{Int} S_A \mid \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}.$$

Similarly, we have

$$\hat{\tau}_B = \frac{1}{q_B} E\{\mathbf{g}_B^T S_B^- \mathbf{g}_B \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} = \frac{1}{q_B} \left\{ \hat{\mathbf{g}}_B^{(t)'} S_B^- \hat{\mathbf{g}}_B^{(t)} + tr(S_B^- \hat{\mathbf{v}}_B^{(t)}) \right\}.$$

Finally,

$$\begin{aligned} \hat{\sigma}^{(t)} &= \frac{1}{n-K} E\{(\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B)' (\mathbf{U} - \mathbf{A}' \mathbf{g}_A - \mathbf{A}' \mathbf{g}_B) \mid \mathbf{U}; \hat{\tau}_A^{(t)}, \hat{\tau}_B^{(t)}, \hat{\sigma}^{(t)}\} \\ &= \mathbf{Y}'^T \mathbf{A} \mathbf{A}' \mathbf{Y}^* + tr \left[\mathbf{A} \mathbf{A}' \begin{pmatrix} \hat{\tau}_A^{(t)} S_A - (\hat{\tau}_A^{(t)})^2 S_A P_{Int} S_A + \hat{\tau}_B^{(t)} S_B - (\hat{\tau}_B^{(t)})^2 S_B P_{Int} S_B \\ - 2 \hat{\tau}_A^{(t)} \hat{\tau}_B^{(t)} S_A P_{Int} S_B \end{pmatrix} \right]. \end{aligned}$$

The EM algorithm for obtaining $(\hat{\tau}_B, \hat{\sigma})$ under $H_{0,A}$: $\tau_A = 0$ is similar to the above algorithm except that τ_A is set to be 0.

When applying the above EM algorithm, we add an additional testing step as detailed below. As the EM algorithm provides non-

negative estimates, when τ_A and τ_B are 0 or close to 0, the EM estimates obtained from the above algorithm can be biased as

$$E(\hat{\tau}_A) > \tau_A$$

and

$$E(\hat{\tau}_B) > \tau_B.$$

To solve this problem, we first apply conditional main effect tests for $H_0: \tau_A = 0$ and $H_0: \tau_B = 0$ to examine if these nuisance variance components are significantly different from 0. If we fail to reject the null hypotheses, then we set the corresponding $\hat{\tau}$ values as 0. If τ for a gene is significantly different from 0, then we obtain its estimate using the EM algorithm described above. By applying this

additional step, $E(\hat{\tau})$ would be closer to 0 when τ is 0 or close to 0. When τ is relatively large, the conditional main effect test would reject $H_0: \tau = 0$ and the final estimate is the same as the estimate from the original EM algorithm.

Acknowledgements

The authors thank the International Warfarin Pharmacogenetics Consortium and the PharmGKB resources for supplying the Warfarin data. They also thank Dr. Daowen Zhang for his helpful discussions and suggestions on the work. This work was supported by NIH grants R01 HG007508, R01 MH084022, and P01 CA142538.

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