Integrative eQTL-weighted hierarchical Cox models for SNP-set based time-to-event association studies

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Abstract

Background: Integrating functional annotations into SNP-set association studies has been proven a powerful analysis strategy. Statistical methods for such integration have been developed for continuous and binary phenotypes; however, the SNP-set integrative approaches for time-to-event or survival outcomes are lacking.

Methods: We here propose IEHC, an integrative eQTL (expression quantitative trait loci) hierarchical Cox regression, for SNP-set based survival association analysis by modeling effect sizes of genetic variants as a function of eQTL via a hierarchical manner. Three p-values combination tests are developed to examine the joint effects of eQTL and genetic variants after a novel decorrelated modification of statistics for the two components. An omnibus test (IEHC-ACAT) is further adapted to aggregate the strengths of all available tests.

Results: Simulations demonstrated that the IEHC joint tests were more powerful if both eQTL and genetic variants contributed to association signal, while IEHC-ACAT was robust and often outperformed other approaches across various simulation scenarios. When applying IEHC to ten TCGA cancers by incorporating eQTL from relevant tissues of GTEx, we revealed that substantial correlations existed between the two types of effect sizes of genetic variants from TCGA and GTEx, and identified 21 (9 unique) cancer-associated genes which would otherwise be missed by approaches not incorporating eQTL.

Conclusion: IEHC represents a flexible, robust, and powerful approach to integrate functional omics information to enhance the power of identifying association signals for the survival risk of complex human cancers.

Keywords: Integrative analysis, SNP-set association study, Joint effect test, Hierarchical modeling, Cox model, Expression quantitative trait loci, Aggregated Cauchy association test

Background

A wide range of recent genome-wide association studies (GWASs) have revealed that germline variants (i.e., single nucleotide polymorphisms [SNPs]) are also an important inherited component of cancer risk [1–3], although somatic mutations (e.g., copy number and DNA methylation alterations) play an essential role in the pathophysiology of many human cancers [4–6]. Conventionally, the association of SNPs is examined one at a time in cancer GWASs [1, 2]; however, the power for detecting such single SNP association signal remains limited because genetic variants generally have weak effect sizes [7–9], making the detection of cancer-associated SNPs difficult even with large samples. In addition, these identified genetic variants often explain only a very small fraction of cancer predisposition, leading to the so-called missing heritability [10–14], which also implies that a large...
amount of causal loci have yet been discovered and the endeavor to identify causative genes for cancers should continue.

As an effective alternative strategy, SNP-set analysis has been proposed in GWAS [15–21], where a set of SNPs defined a priori within a gene or other genetic units (e.g., pathway) are analyzed collectively to assess their joint influence on diseases or traits. Existing SNP-set approaches can be roughly grouped into two categories: (i) the burden test, in which the association with disease risk is evaluated for the overall effect of a weighted sum of variant alleles [22, 23]; and (ii) the variance component test, in which the association is examined for the variance of genetic variants under the framework of mixed-effects models [16, 24]. Due to the aggregation of multiple weak association signals and the reduced burden of multiple testing, SNP-set analysis is often more powerful than its counterpart of single SNP analysis. However, these SNP-set association approaches might be still underpowered when additional informative knowledge is available about the alternative. For example, if the association between a set of genetic variants and the survival risk of cancers is regulated through gene expression, the power improvement would be further achieved by integrating transcriptomic data into the test method. As it is widely demonstrated that disease-associated SNPs are more likely to be expression quantitative trait loci (eQTL) [25], it is thus conceivable that incorporating such knowledge would increase power for detecting association [26–28].

Several methods have been proposed for this goal within the mixed-effects model framework. For example, MiST was developed for continuous and binary phenotypes in rare variant association studies by modeling the effects of rare variants as a function of functional features while allowing for the heterogeneity of variant-specific effects [29]. This method was recently further generalized to integrate eQTL or other functional annotations [26, 30, 31]. Both simulations and real applications have exhibited the advantage of these integrative approaches compared with the general methods that do not incorporate functional characteristics of genetic variants. However, to our best knowledge, there is little relevant work on these two uncorrected statistics via various p-values combination strategies. To this aim, we consider three data-driven approaches (e.g., the Fisher’s combination, the optimally weighted combination, and the adaptively weighted combination) for combining them to capture the association signals from both sources. To further enhance power, we exploit the recently developed aggregated Cauchy association test (ACAT) to integrate the strengths of all the five types of test methods (i.e., three combination tests as well as the burden test and the variance component test; the latter was also called the kernel machine [KM] test) [34, 35]. We refer to our proposed approach and test framework described above as integrative eQTL hierarchical Cox model (IEHC). Extensive simulations demonstrate that the three combination tests have comparable power or are better than both the burden and variance component tests under some specific scenarios, while IEHC-ACAT enjoys consistently higher power across all simulation scenarios. We finally apply IEHC to ten TCGA cancers which have one explicit relevant tissue in The Genotype-Tissue Expression (GTEx) project and integrate eQTL into our method [36]. We identified a total of 21 (9 unique) cancer-associated genes which would otherwise be missed by the general SNP-set based survival association methods that do not consider eQTL.

**Methods**

An overview of the IEHC model and the joint test

First, consider that there are $S$ genotypes (denoted by $G_i$ and coded as 0, 1 or 2 in terms of the number of effect allele) of SNPs located within a given gene and $p$ covariates $X_i$ (e.g., age, gender, and cancer stage) for $n$ individuals, and $S$ in general varies gene by gene. In addition, denote the observed survival time by $t_i$ and
the true survival time by $T_i$ with $d_i$ indicating the censored status; that is, $d_i = 1$ if $T_i = t_{i+}$ whereas $d_i = 0$ if $T_i < t_{i+}$. Under the proportional hazards condition, we assume the hazard function $\lambda(t)$ of the survival time $t_i$ is related to $G_i$ and $X_i$ through the classical Cox model [37]

$$\log \left( \frac{\lambda(t_i)}{\lambda_0(t_i)} \right) = G_i^T \beta + X_i^T c$$

where $\lambda_0$ is an arbitrary baseline hazard function, $\beta = (\alpha_1, ..., \alpha_p)$ is an $S$-vector of effect sizes for SNPs and $c = (c_1, c_2, ..., c_p)$ is a $p$-vector of fixed effect sizes for clinical covariates.

We here only provide an overview of IEHC, with technical details demonstrated in the Additional file 1. In brief, IEHC examines a group of SNPs in one gene at each time and integrates eQTL information by extending the Cox model above in a hierarchical manner

$$\log \left( \frac{\lambda(t_i)}{\lambda_0(t_i)} \right) = \sum_{j=1}^{S} G_{ij} \alpha_j + \sum_{k=1}^{p} X_{ik}^T c_k = \eta = G^T \beta + X^T c$$

$$\alpha_j = \beta_j \times \theta + b_j$$

$$b_j \sim N(0, \tau)$$

Of note, plugging $\alpha_j$ into the first line leads to $\eta = (G_i^T \beta) \theta + G_i^T b + X_i^T c$. In the above, $\beta_j$ is the known eQTL effect size of the $j$th SNP and directly obtained in terms of summary statistics from the GTEx project [36, 38], $\theta$ is a scale of coefficient for eQTL and quantifies the association between the survival risk and the weighted burden score $G^T \beta$, and $b_i$ is the normal residual variant-specific effect size that is not interpreted by eQTL alone. Then, the hypothesis of no association between a set of SNPs and the survival outcome is

$$H_0 : \theta = 0 \text{ and } b = 0 \Leftrightarrow H_0 : \theta = 0 \text{ and } \tau = 0$$

This is a joint test including both fixed effect and random effects: the first component examines the influence of genetic variants on the survival risk explained by eQTL (i.e., $\theta = 0$); while the second component examines the impact of genetic variants beyond the effects of eQTL (i.e., $\tau = 0$).

To implement the hypothesis testing while circumventing the potential correlation between statistics and improving the statistical computation, we propose the following two-stage strategy. Briefly, we derive the test statistic for $\theta$ under $H_0 : \theta = 0$ and $\tau = 0$ as usual, while derive the score statistic for $\tau$ under $\tau = 0$ but without the constraint of $\theta = 0$. By doing this, we ensure that these two statistics are independent (see simulation results in Additional file 1). This strategy substantially eases the construction of test statistics for the joint test and two asymptotically independent statistics are eventually derived: one for $\theta$ in the general Cox model (say $U_\theta$) [37] and the other for the variance component parameter $\tau$ in the kernel machine (KM) Cox model (say $U_\tau$) [32, 33]. We combine the two uncorrelated statistics via several aggregation approaches, including the Fisher’s combination (IEHC-Fisher) [39, 40], the optimally weighted linear combination (IEHC-optim) as well as the adaptively weighted linear combination (IEHC-adapt). For IEHC-optim we establish $T_{\rho} = \rho U_\theta + (1-\rho) U_\tau$ with $\rho \in [0, 1]$ controlling the contribution of the fixed-effect component. The final $\rho$ in IEHC-optim is selected by optimizing $T_{\rho}$. On the other hand, IEHC-adapt is a data-adaptive generalization of the Fisher’s combination [39, 40], for which the test statistic takes the form $T = \rho_0 Z_0 + \rho_1 Z_1$, where $Z_0 = -2 \log(p_0)$ and $Z_1 = -2 \log(p_1)$, based on which $\rho_0$ and $\rho_1$ are determined via an adaptive manner.

The IEHC test described above includes two special cases: the burden test for examining the fixed effect $\theta$ (with $\tau = 0$) in the general Cox model and the KM test examining the variance component parameter $\tau$ (with $\theta = 0$) in the KM Cox model. To further boost the power, we employ the recently developed aggregated Cauchy association test (ACAT) to combine the strengths of these five methods (i.e., the burden test, the KM test and three joint tests including IEHC-Fisher, IEHC-optim and IEHC-adapt) [34, 35]. The advantage of IEHC-ACAT is that it allows us to aggregate correlated p-values obtained from multiple various tests into a single well-calibrated p-value while maintaining the type I error control correctly. The detailed procedures for these approaches are relegated to Additional file 1. The code for IEHC is freely available at https://github.com/biostatpzeng/IEHC.

**Simulations for type I error control and power evaluation**

We now perform simulations to evaluate the type I error control and power for IEHC. To mimic the truth, we undertook simulations based on realistic genotypes available from the Geuvadis program because the sample size in the real-life applications used in this paper matched closely that of Geuvadis [41]. First, we obtained 550 a group of correlated SNPs in a local genetic region from 465 individuals in Geuvadis. During the simulation we randomly selected $S$ nearby SNPs (denoted by $G_i$), with $S$ varying according to a uniform distribution ranging from 20 to 50 (i.e., $S$ was on average equal to 35); among these selected genetic variants we further randomly set $0\%$, $30\%$ or $50\%$ of SNPs having zero effect sizes. We generated the gene expression level with the first 165
Table 1  Summary information of 10 TCGA cancers and the number of genes, sample sizes and SNPs of these cancers after combining the tissue in GTEx and quality control

| Cancer | $N_0$ | $N_1$ | $m$ | Age | Female/male | Censored rate (%) | Stage or grade (1/2/3/4/5) | Tissue in GTEx | $N_2$ | $k_0$ | $k_1$ |
|--------|-------|-------|-----|-----|-------------|-------------------|-----------------------------|---------------|-------|-------|-------|
| ACC    | 97    | 75    | 4,473,001 | 47.6±16.5 | 50/25 | 60.0 | 8/33/16/18/0 | Adrenal gland | 175   | 11,822 | 11,165 |
| BRCA   | 1283  | 736   | 2,281,892 | 58.8±13.0 | 736/0 | 85.9 | 138/408/174/11/5 | Breast mammary tissue | 251   | 13,068 | 7,793 |
| COAD   | 570   | 201   | 4,216,239 | 66.0±13.0 | 97/104 | 75.1 | 34/78/62/27/0 | Colon transverse | 246   | 12,779 | 11,586 |
| LIHC   | 469   | 166   | 3,369,784 | 62.7±14.1 | 73/93 | 60.8 | 79/44/39/4/0 | Liver | 153   | 11,073 | 8,595 |
| LUAD   | 877   | 384   | 3,831,580 | 65.9±9.9  | 213/171 | 63.0 | 216/90/62/16/0 | Lung | 383   | 13,300 | 10,617 |
| LUSC   | 765   | 344   | 3,279,532 | 67.1±8.8  | 94/250 | 57.3 | 176/117/48/3/0 | Lung | 383   | 13,300 | 9,450 |
| OV     | 758   | 455   | 1,527,607 | 60.2±11.4 | 455/0 | 37.4 | 9/19/353/74/0 | Ovary | 122   | 12,623 | 7,851 |
| PAAD   | 223   | 159   | 4,679,901 | 65.6±10.8 | 69/90 | 45.9 | 20/130/4/5/0 | Pancreas | 220   | 11,173 | 10,728 |
| STAD   | 544   | 249   | 3,384,736 | 64.8±10.2 | 97/152 | 60.2 | 33/75/128/13/0 | Stomach | 237   | 12,045 | 9,247 |
| UCEC   | 605   | 368   | 4,008,620 | 64.4±10.8 | 368/0 | 83.2 | 239/33/79/17/0 | Uterus | 101   | 12,592 | 10,948 |

$N_0$: the initial sample size in TCGA; $N_1$: the sample size after quality control; $m$: the number of SNPs after combination; $N_2$: the sample size in GTEx; $k_0$: the number of genes after combination; $k_1$: the number of genes after quality control
individuals and sampled the effect sizes $\beta$ from a normal distribution with a special variance so that the proportion of the explained variation (PVE) of the expression level would be 30% or 50%.

Then, we calculated $\alpha = \beta \times \theta + b$, with $b$ following a normal distribution with variance $\tau$. Two independent covariates (i.e., $X_1$ was binary and $X_2$ was continuous) were also generated with each having an effect size of 0.50. We employed the inverse probability method to generate the survival time which followed a Weibull distribution with the shape parameter equal to 1 and the scale parameter equal to 0.01 [42]. The location parameter (denoted by $\mu$) of this Weibull distribution was determined by $\alpha$ and the two covariates: $\mu = \exp(\eta)$ and $\eta = G_2 \alpha + 0.5X_1 + 0.5X_2$, with $G_2$ representing the remaining genotypes of 300 samples in Geuvadis. The censored rate was fixed to be 50% in a random manner. Note that, this relatively high censored rate corresponded to the similar situation observed in the TCGA cancer patients from primary tumor tissues and excluded patients with genotype call rate < 95%, MAF < 0.01 and imputation score < 0.30.

TCGA genotypes, imputation, and quality control
For each cancer we first filtered out SNPs that had missingness rate > 0.95 across patients, genotype calling rate < 0.95, minor allele frequency (MAF) > 0.01, or Hardy–Weinberg equilibrium (HWE) $p$-value < $10^{-4}$. Then, we undertook imputation by first phasing genotypes with SHAPEIT [45], then imputed SNPs based on the Haplotype Reference Consortium panel [46] on the Michigan Imputation Server using minimap3 [47]. The filtering procedure for imputed genotypes included HWE $p$-value $< 10^{-4}$, genotype call rate $< 95\%$, MAF $< 0.01$ and imputation score $< 0.30$.

GTex eQTL summary statistics and the combination with TCGA
At the same time, for these kept cancers we obtained eQTL summary statistics of the related tissue from GTEx [36] and performed a stringent quality control (Table 1): (i) reserved SNPs with MAF > 0.05; (ii) excluded non-biallelic SNPs and SNPs with strand-ambiguous alleles; (iii) excluded SNPs that had no rs labels as well as duplicated ones; (iv) kept only SNPs which were included within TCGA; (v) removed SNPs whose alleles did not match those in TCGA; (vi) aligned the effect allele of SNP between TCGA and GTEx.

For comparison we implemented the following six methods in both simulations and real-life applications within the context of Cox modeling $\log[\lambda(t)/\lambda_0(t)] = \eta$: (i) the burden test: to examine $H_0: \eta = 0$ in $\eta = (G\beta) \times \theta + Xc$ using the Wald test in the general Cox model; (ii) the KM test: to assess $H_0: \tau = 0$ in $\eta = Gb + Xc$ and $b \sim N(0, \tau)$ using the kernel-machine based approach; (iii) IEHC-Fisher: to jointly test $H_0: \tau = 0$ and $\theta = 0$ in $\eta = (G\beta) \times \theta + Gb + Xc$ and $b \sim N(0, \tau)$ using the Fisher’s combination method, or (iv) IEHC-adapt using the adaptive combination method, or (v) IEHC-optim using the optimal combination method; (vi) IEHC-ACAT: to aggregate the first five tests using the Cauchy combination method.

Results
Independence of the two statistics in the joint test and type I error control
First, in order to validate the independence of the two statistics (denoted by $U_a$ and $U_j$) constructed in the joint test of IEHC, we computed the Pearson’s correlation coefficient between them under the null of our simulation and find little evidence supporting the dependence of the two statistics (Additional file 1: Figure S1). For instance, across the $10^5$ replications, the overall correlation between $U_a$ and $U_j$ is $1.75 \times 10^{-3}$ (95% confidence interval: $-4.44 \times 10^{-3}$–$7.95 \times 10^{-3}$, $P = 0.580$), confirming the validity of our proposed joint test framework within which we can combine two uncorrelated statistics in a statistically straightforward fashion. Next, the Q-Q
plots demonstrate all the tests, including the burden test, the KM test, IEHC-Fisher, IEHC-adapt, IEHC-optim as well as IEHC-ACAT, effectively control the type I error (Fig. 1). Particularly, we find IEHC-ACAT correctly maintains the type I error control even if the aggregated test methods (i.e., the first five) are highly correlated (Additional file 1: Figure S2). Furthermore, IEHC-Fisher is more powerful when the fixed effect explained by eQTL and random effects beyond eQTL exist simultaneously, but is less powerful when only one of the two types of effects is true or under the null that both $\theta$ and $\tau$ are zero, where the deflated $p$-values are observed (Fig. 1).

Simulation results for power evaluation

We now compare the power of these tests under the alternative. To save the space, here we only present the results under three scenarios: the PVE of the gene expression level explained by $\beta$ (the effect sizes of eQTLs) was equal to 0.3 or 0.5, the effect size $\theta$ (the effect size of the eQTL-based genetic score) was set to 0 or 0.4, and $\tau$ (the variance of the direct effect sizes of genetic variants) was set to 0 or 0.04. The results for other scenarios are displayed in Additional file 1: Figures S3-S9. As for the results shown in Fig. 2, we find the burden test is in general powerful when the association signal comes only from eQTL (i.e., $\theta=0.4$ and $\tau=0$), while is underpowered when the association signal comes only from SNPs (i.e., $\theta=0$ and $\tau=0.04$). The opposite results are observed for the KM test. Compared to the burden test and the KM test, the three joint tests (i.e., IEHC-Fisher, IEHC-adapt, and IEHC-optim) are often better when the association signal is contributed by both eQTL and SNPs (i.e., $\theta=0.4$ and $\tau=0.04$).

In addition, we find the relative performance of power between the joint tests (i.e., IEHC-Fisher, IEHC-adapt, and IEHC-optim) and the burden test as well as the KM test depends on the magnitude of $\theta$ and $\tau$. More specifically, when $\theta$ and $\tau$ are not large enough, the burden test or (and) the KM test may behave better than the joint tests even the association signal is contributed by both the two components. For instance, the KM test is more powerful compared to the joint tests when $\theta=0.1$ and $\tau=0.04$ (Additional file 1: Figures S4); whereas the burden test has a higher power when $\theta=0.2$ and $\tau=0.02$ (Additional file 1: Figures S5). Finally, IEHC-ACAT, which integrates the five tests, consistently behaves better
across various simulation scenarios (Fig. 2 and Additional file 1: Figures S3-S9).

**Correlation between cis-SNP marginal effect sizes of each gene in TCGA and GTEx**

In the real application, we first quantify the association of cis-SNP effect sizes between TCGA and GTEx. To do so, for each SNP in TCGA we generated its marginal effect size using the general Cox model while adjusting for available cancer-specific covariates (e.g., age and tumor stage), and then conducted a simple linear regression with the two sets of estimated SNP effect sizes for each gene of these cancers. Note that, the SNP effect sizes of GTEx can be directly accessed through public portal (https://www.gtexportal.org/). Such regression analysis renders us a rough insight to interrogate the relationship of the two types of SNP effect sizes.

We discover that these two types of SNP effect sizes are substantially correlated for a great deal of genes for each cancer (Table 2). For example, we find that on average ~72.8% (ranging from 67.6% for BRCA to 76.4% for ACC) of regression coefficients are significant (false discovery rate [FDR] < 0.05). Notably, for a given cancer the regression coefficients may be positive for some genes while negative for others (Fig. 3A). Particularly, among a total of 118 genes whose regression coefficients are significant across all the ten cancers, we still find the regression coefficients are either positive or negative across diverse cancers (Fig. 3B), indicating distinct genetic influences of SNPs on the regulation of gene expression and the survival risk of cancers. More importantly, a small fraction of (~3.4% on average) determination coefficients ($R^2$) are larger than 10%, implying that the cis-SNP effect sizes of some certain genes in TCGA cancers can be indeed explained by eQTL of relevant tissue in the GTEx (Table 2).

Taken together, although the average strength of the relationship between the two types of SNP effect sizes across genes may be relatively moderate, it nevertheless suggests potential genetic overlap especially at some certain genes. It is therefore worthy of integrating the eQTL of GTEx into the SNP-set based survival association analysis of TCGA cancers to boost the power.

**Associated genes identified with the IEHC method**

We here demonstrate that incorporating the eQTL information of GTEx into the SNP-set association analysis has the potential to enhance the power. We also exhibit that integrating all available tests by IEHC-ACAT can further increase the power. For each cancer and each type of joint tests (i.e., IEHC-Fisher, IEHC-optim, IEHC-adapt, and IEHC-ACAT), we classify all the genes into four various groups in terms of the regression coefficients (i.e., FDR < 0.05) and the results of joint tests ($P$ < 0.05) (Additional file 1: Tables S1–S4 and Figures S10, S11). Taking ACC for example, there are a total of 8533 ($= 259 + 8274$) genes whose regression coefficients are significant (FDR < 0.05) and 2,630 ($= 19 + 2611$) genes whose regression coefficients are non-significant (FDR > 0.05); among these genes, 259 (~3.04% = 259/8533) and 19 (~0.72% = 19/2630) genes have a p-value less than 0.05 in terms of IEHC-Fisher, indicating that IEHC-Fisher has a fourfold higher likelihood (~4.22 = 3.04/0.72) to discover association signals ($p = 4.61 \times 10^{-11}$; Additional file 1: Table S1). The basic logic is that a smaller p-value would be generated in the joint tests if the eQTL of GTEx is predictive to the effect size of SNP in TCGA. Therefore, we expect that the detection rate of potentially associated genes
### Table 2  Summary information of cis-SNPs for the 10 cancers and the association of cis-SNP effect sizes for each gene in TCGA and GTEx

| Cancer | Mean | Median | Max    | Min | \(R^2 > 0.10\) (%) | FDR < 0.05 (%) |
|--------|------|--------|--------|-----|---------------------|---------------|
| ACC    | 3,521| 3,597  | 13,279 | 8   | 325 (2.9)           | 8533 (76.4)   |
| BRCA   | 3,089| 3,337  | 13,086 | 9   | 309 (4.0)           | 5265 (67.6)   |
| COAD   | 3,655| 3,737  | 12,881 | 4   | 297 (2.6)           | 8504 (73.7)   |
| LIHC   | 3,492| 3,597  | 8,479  | 3   | 202 (2.4)           | 6396 (74.6)   |
| LUAD   | 3,732| 3,733  | 12,717 | 20  | 170 (1.6)           | 7917 (74.6)   |
| LUSC   | 3,525| 3,611  | 12,726 | 6   | 282 (3.0)           | 7004 (74.2)   |
| OV     | 1,935| 1,750  | 6,752  | 3   | 977 (12.7)          | 5329 (69.0)   |
| PAAD   | 3,801| 3,784  | 13,382 | 9   | 167 (1.6)           | 7905 (73.7)   |
| STAD   | 3,500| 3,687  | 13,091 | 3   | 168 (1.8)           | 6496 (70.3)   |
| UCEC   | 3,721| 3,727  | 13,063 | 7   | 186 (1.7)           | 8086 (73.9)   |
| Average| 3,397| 3,456  | 11,946 | 7   | 308 (3.4)           | 7145 (72.8)   |

Mean: the average number of cis-SNPs across genes; Max or Min: the maximal or minimal number of cis-SNPs across genes. \(R^2\) denotes the determination coefficient of the cis-SNP effect sizes for each gene in the linear regression with the effect sizes of a gene in TCGA as response and the effect sizes of that gene in GTEx as the covariate. The last column denotes the number of genes whose regression coefficient is significant (FDR < 0.05).

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**Fig. 3**  A Distribution of estimated regression coefficients for each gene across all the 10 TCGA cancers; B Heatmap of estimated regression coefficients of 47 of 118 genes that are simultaneously significant (FDR < 0.05) across all the 10 TCGA cancers; the density and the size of the color represent the magnitude of the regression coefficients.
genes (determined by \( p < 0.05 \)) would be greater among genes with significant regression coefficients compared to those with non-significant regression coefficients. Formally, we employ the chi-square test to examine the difference in the detection rates (e.g., 3.04% vs. 0.72%) and observe a pronounced improvement of the detection rate for the four joint tests across nearly all cancers except OV (Fig. 4), in line with our expectation and suggesting the improvement of power when integrating the eQTL information of GTEx.

Finally, the number of associated genes identified with various test approaches is summarized in Table 3. Note that, the KM test cannot identify any associations, whereas a total of 21 (9 unique) genes are discovered for four cancers after incorporating the eQTL information of GTEx (Table 4). Specifically, IEHC-ACAT and the burden test detect 5 genes, followed by IEHC-adapt (4 genes), IEHC-optim (4 genes) and IEHC-Fisher (3 genes). We find that some genes are specifically discovered by some methods (e.g., COL9A1, MSANTD2, and LMBRD1 by the burden test), although several genes are simultaneously identified by multiple tests (e.g., RP11-1391J7.1 by the burden test, IEHC-adapt, IEHC-optim, and IEHC-ACAT), suggesting the various power of these test approaches across diverse genes, in line with the observation found in the simulations. Among the nine unique genes, the SNP effect sizes have a moderate correlation between TCGA and GTEx (Table 4 and Additional file 1: Figure S12).

With regards to these discovered genes, there are previous studies which provided evidence supporting their associations with the cancers. For instance, it was discovered the methylation level of COL9A1 reduced more evidently in tumors compared to that in the blood or healthy breast tissue, suggesting the association between COL9A1 and the risk of breast cancer [48]. Dysregulation of EGFR expression and signaling was previously well documented to contribute to the progression and metastasis of breast cancer while MSANTD2 played a crucial role in decreased epidermal growth factor endocytosis [49]. It was recently shown LMBRD1 was significantly over-expressed in BRCA1 mutated cell line compared to BRCA1 wild-type cell line [50]. As another example, COMMD1 was under-expressed in ovarian cancer, and the lack of detectable COMMD1 protein expression was more frequent in ovarian cancer; COMMD1 was also shown to be related to the cisplatin sensitivity in ovarian cancer [51]. In addition, we observe that four genes (i.e., COL9A1, MSANTD2, LMBRD1 and RP11-1391J7.1) were differentially expressed between normal samples and tumor samples (Additional file 1: Figure S13), and that COL9A1 was differentially expressed among different tumor stages (Additional file 1: Figure S14). In summary, these identified genes may represent potentially promising candidate biomarkers for cancer prediction, clinical treatment, and survival prognosis evaluation.

**Table 3** The number of significant genes identified by different test approaches in the 10 TCGA cancers (FDR < 0.1)

| Cancer | Burden | KM | IEHC-Fisher | IEHC-adapt | IEHC-optim | IEHC-ACAT |
|--------|--------|----|-------------|------------|------------|-----------|
| BRCA   | 4      | 0  | 0           | 1          | 1          | 1         |
| COAD   | 1      | 0  | 0           | 2          | 3          | 3         |
| OV     | 0      | 0  | 2           | 3          | 0          | 1         |
| STAD   | 0      | 0  | 1           | 4          | 4          | 5         |
| Total  | 5      | 0  | 3           | 4          | 4          | 5         |

We here ignore those cancers for which non-associated genes are discovered by any methods.
### Table 4
Summary information for associated genes for the four cancers identified by different tests

| Cancer | Gene     | chr (pos) | S       | r       | Burden   | KM        | IEHC-Fisher | IEHC-adapt | IEHC-optim | IEHC-ACAT |
|--------|----------|-----------|---------|---------|----------|-----------|-------------|-------------|------------|-----------|
| BRCA   | COL9A1   | 6 (70,924,764–71,012,786) | 4,121   | −0.32   | 8.94 × 10^{-2} | 8.43 × 10^{-1} | 1.41 × 10^{-1} | 1.54 × 10^{-1} | 2.18 × 10^{-1} | 1.56 × 10^{-1} |
| BRCA   | MSANTD2  | 11 (124,636,394–124,670,569) | 4,563   | −0.16   | 8.94 × 10^{-2} | 8.43 × 10^{-1} | 4.64 × 10^{-1} | 2.03 × 10^{-1} | 2.20 × 10^{-1} | 2.33 × 10^{-1} |
| BRCA   | LMBRD1   | 6 (70,385,694–70,507,003) | 4,324   | 0.26    | 8.94 × 10^{-2} | 8.43 × 10^{-1} | 1.67 × 10^{-1} | 1.54 × 10^{-1} | 2.18 × 10^{-1} | 1.56 × 10^{-1} |
| BRCA   | RP11-1391171 | 11 (856,880–85,795) | 4,090   | 0.34    | 1.77 × 10^{-2} | 8.43 × 10^{-1} | 1.25 × 10^{-1} | 3.85 × 10^{-2} | 5.91 × 10^{-2} | 4.65 × 10^{-2} |
| COAD   | CTA-407F11.6 | 22 (26,043,228–26,045,199) | 4,290   | 0.23    | 9.42 × 10^{-2} | 8.41 × 10^{-1} | 6.67 × 10^{-1} | 2.09 × 10^{-1} | 3.04 × 10^{-1} | 2.48 × 10^{-1} |
| OV     | KIAA1841  | 2 (61,293,006–61,391,960) | 3,162   | 0.01    | 9.52 × 10^{-1} | 262 × 10^{-1} | 2.28 × 10^{-1} | 4.15 × 10^{-1} | 5.75 × 10^{-2} | 1.75 × 10^{-1} |
| OV     | FAM161A   | 2 (62,051,989–62,081,278) | 3,064   | 0.17    | 8.63 × 10^{-1} | 262 × 10^{-1} | 1.70 × 10^{-1} | 4.15 × 10^{-1} | 1.68 × 10^{-1} | 1.75 × 10^{-1} |
| OV     | COMMD1    | 2 (62,115,859–62,374,382) | 3,047   | 0.16    | 9.93 × 10^{-1} | 226 × 10^{-1} | 6.75 × 10^{-1} | 5.38 × 10^{-1} | 1.32 × 10^{-1} | 4.18 × 10^{-2} |
| STAD   | CTC-338M12.5 | 5 (180,618,924–180,621,429) | 1,051   | 0.06    | 9.96 × 10^{-1} | 588 × 10^{-1} | 6.55 × 10^{-4} | 9.82 × 10^{-5} | 4.63 × 10^{-1} | 4.27 × 10^{-4} |

The italic values represent genes identified by different tests (FDR < 0.1 is marked as italic).

S: the number of SNPs for each gene; r: estimated correlation coefficients for SNP effect sizes between the TCGA and GTEx datasets. The FDR level is set to 0.1.
human cancers [1–3]. However, how to effectively leverage the useful omics information is still an open problem. Therefore, there is a great demand for powerful analysis tools to fully harness the utility of these datasets. To fill such knowledge gap in the literature, herein we have proposed a novel genetic integrative Cox approach, called IEHC, to undertake the association analysis particularly for survival (time-to-event) phenotypes.

By characterizing effect sizes of SNPs between GTEx and TCGA, we found that there existed a substantial correlation across genes between the two types of effect sizes, indicating that we had the potential to improve the power if incorporating the GTEx eQTL into the survival SNP-set association studies. Methodologically, under the hierarchical model framework, IEHC has an appealing property that it models the effect sizes of SNPs as a function of variant characteristics (i.e., eQTL) to leverage information across loci while allowing for individual heterogeneous variant effects [26, 29]. Moreover, IEHC can be further interpreted within the framework of transcriptome-wide association studies (TWAS) [30, 31, 52]. In brief, the weighted burden score in IEHC (i.e., $G_i^T \beta$) is viewed as an imputed expression level with the weights of SNPs estimated from external tissue-related transcriptome reference datasets (i.e., GTEx), and the association between imputed expressions and cancers is examined for that gene while controlling for the direct effects of SNPs (i.e., $G_i^T b$). Because TWAS is effectively viewed as performing a two-sample causal inference [53, 54]; consequently, in this sense, IEHC has the ability to identify putative causal genes for cancers under certain regularity conditions [53–55].

Compared to the permutation test which is often computationally intensive, the proposed joint tests in IEHC are much more efficient because only two independent statistics are involved, both of which can be implemented with existing software and can be further combined via three kinds of p-values combination strategies. In addition, two previously used tests, including the burden test and the KM test, can be special cases of the joint test. Furthermore, in IEHC we utilized ACAT to combine all these test methods. IEHC-ACAT enjoys the attractive strength that it takes the summary of a set of p-values as the test statistic and evaluates the significance analytically without the knowledge of correlation structure [34, 35]; thus, it is extraordinarily flexible and computationally fast. As a result, IEHC-ACAT allows us to aggregate dependent p-values obtained from these tests into a single well-calibrated p-value that can achieve the maximal power while maintaining the type I error correctly [34, 35, 56].

Extensive simulations revealed the relative performance of these joint test methods in IEHC and highlighted the strength of IEHC-ACAT. In agreement with the results of simulation, in the real application to ten TCGA cancers, we found that integrating eQTL can in general enhance the power and discover more genes that might be related to the survival risk of cancer. Particularly, IEHC-ACAT identified the highest number of associated genes among these competitive methods. In contrast, the KM test, which did not consider the eQTL of GTEx, cannot identify any association signals, suggesting the usefulness of integrating external informative variant annotations. Besides the attractive property in methodology, IEHC is also biologically interpretable when integrating transcriptomic information. First, it has been revealed that molecular features measured at the transcription level generally affect clinical outcomes more directly than those measured at other omic levels. Thus, the gene expression level would have the best predictive power for cancer prognostic evaluation compared to other genomic measurements [44]. Second, as it is widely demonstrated that SNPs associated with complex phenotypes are more likely to be eQTL [25], implying that gene expression may mediate the influence of genetic variants on the cancer risk. Therefore, eQTL can bridge the gap between cancers and many identified causal SNPs which have unknown function roles.

It needs to be emphasized that for the current IEHC model we only considered one type of variant characteristics (i.e., eQTL) but ignored other relevant information (e.g., protein quantitative trait loci). Therefore, the power of IEHC-Fisher, IEHC-adapt, IEHC-optim, and IEHC-ACAT may be further improved if more useful variant annotations would be employed in IEHC. The hierarchical modeling in IEHC offers an effective and general manner to incorporate more functional annotations as they become available. However, when many functional annotations can be applied, some of them may not be useful for determining associated genes. Therefore, the selection of informative annotations during the association analysis is necessary, which may be an interesting avenue for future investigations. Furthermore, although there include many cancer types in TCGA, their effective sample sizes are still relatively small, and the censored proportions are high [43, 44], which inevitably undermines the power of any methods and may lead to the failure of identifying some associated genes with survival. In addition, we only considered the linear kernel in these joint tests of IEHC when assessing the direct effects of SNPs (i.e., $H_0: \tau = 0$). The linear kernel may be sub-optimal if the relationship between SNPs and the survival risk is non-linear. Intuitively, the power of IEHC would depend on how well the chosen kernel captures the true relationship between SNPs and the survival risk, which
can differ in the numbers, effect sizes, and effect directions of causal variants across diverse genes. For example, if only a very small fraction of SNPs may be causal, then the sparse kernel should be a better choice; if SNPs have mutual interaction effect sizes, then the product kernel consisting of main effects and interaction terms is preferred. However, in practice the relation is rarely known in advance, selecting an optimal kernel may be very challenging [20, 57–61]. Therefore, adaptive IEHC model and test methods for multiple candidate kernel functions are warranted to study in the future [20].

Conclusion
Overall, IEHC represents a flexible, robust, and powerful approach to integrate functionally omic datasets to improve the power of identifying associated genes for the survival risk of complex human cancers.

Supplementary Information
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Authors’ contributions
PZ conceived the idea for the study. PZ, SH and YW obtained the data, PZ and HL performed the data analyses. PZ, JZ, TZ, TW, and HL interpreted the results of the data analyses. PZ and HL drafted the manuscript, and all authors approved the manuscript and provided relevant suggestions. All authors read and approved the final manuscript.

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Availability of data and materials
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Competing interests
The authors declare that they have no competing interests.

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