Published in final edited form as:

Biometrics. 2020 June ; 76(2): 619–629. doi:10.1111/biom.13165.

Gene-based Association Analysis for Bivariate Time-to-event Data through Functional Regression with Copula Models

Yue Wei¹, Yi Liu², Tao Sun¹, Wei Chen³, Ying Ding^{1,*}

¹Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA, U.S.A

²Boehringer Ingelheim, Ridgefield, CT, U.S.A

³Department of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA, U.S.A

SUMMARY:

Several gene-based association tests for time-to-event traits have been proposed recently, to detect whether a gene region (containing multiple variants), as a set, is associated with the survival outcome. However, for bivariate survival outcomes, to the best of our knowledge, there is no statistical method that can be directly applied for gene-based association analysis. Motivated by a genetic study to discover gene regions associated with the progression of a bilateral eye disease, Age-related Macular Degeneration (AMD), we implement a novel functional regression method under the copula framework. Specifically, the effects of variants within a gene region are modeled through a functional linear model, which then contributes to the marginal survival functions within the copula. Generalized score test and likelihood ratio test statistics are derived to test for the association between bivariate survival traits and the genetic region. Extensive simulation studies are conducted to evaluate the type-I error control and power performance of the proposed approach, with comparisons to several existing methods for a single survival trait, as well as the marginal Cox functional regression model using the robust sandwich estimator for bivariate survival traits. Finally, we apply our method to a large AMD study, the Age-related Eye Disease Study (AREDS), to identify gene regions that are associated with AMD progression. The method has been implemented and added into a newly developed R package {CopulaCenR}.

Keywords

AMD progression; Bivariate time-to-event; Copula; Functional Regression; Gene-based association analysis

1. Introduction

In genome-wide association studies (GWAS), single variant test is useful in detecting significant SNPs across the whole genome. Typically, after top loci are detected and

*Corresponding author yingding@pitt.edu.

Supporting Information

Web Appendices, Tables, and Figures referenced in Section 2.4 and Section 4.1 are available with this paper at the Biometrics website on Wiley Online Library. The proposed method has been implemented in R. The key functions can be found in the GitHub <https://github.com/yingding99/CopulaFLM>. This approach has also been added to a newly developed R package {CopulaCenR}.

confirmed in replication studies, regions around top variants will then be fine-mapped to further evaluate the disease loci. However, there are limitations of single variant tests. First, with each SNP been tested individually, it may suffer from issues such as lack of power and multiple testing adjustment. Secondly, the true causal SNP may not be genotyped due to the technology or cost reason. Instead, a SNP that is close to the true causal variant is often captured. With partial linkage disequilibrium (LD), the observed effect size is likely to be smaller. Furthermore, most statistical approaches used for single variant test focus on common variants and can be too liberal for rare variants with low minor allele frequency (MAF). A threshold for MAF (e.g., $\geq 5\%$) is commonly applied when performing GWAS on single variants. To solve these problems, there has been increasing interests in developing gene-based tests in genetic association analysis, which can usually take the LD information within a region into account and are suitable for collapsing a set of variants with low MAF.

The statistical methods for gene-based association studies can be broadly classified as burden tests, kernel-based association tests and functional-regression-based methods. Burden test was first proposed by Li and Leal (2008) for binary traits to detect association with rare variants for common diseases. The general idea of burden test is based on collapsing rare variants in a genetic region to a summary variable, which is then used for testing the association with the phenotype. Han and Pan (2010) extended the burden test to the censored time-to-event traits under Cox proportional hazards (PH) framework and Chien et al. (2017) further developed burden test for survival traits in family-based designs. Traditional burden test suffers from the lack of power when heterogeneous genetic effects exist within a region, therefore several adaptive approaches have been proposed recently. For example, Wu et al. (2018) proposed to use mixed-model tests to make full use of genetic correlations across both samples and variants, and to gain power through “data-driven” weights which are adaptive to the direction of individual variant’s effect. Another popular approach is the sequence kernel association test (SKAT), proposed by Wu et al. (2011). It is considered as a computationally efficient score test on variance component parameter to test for association between genetic variants in a region and different types of traits. In 2014, Chen et al. (2014) proposed the SKAT tests for survival traits in a Cox PH regression framework. One advantage of SKAT is that it can quickly calculate p-values by fitting the null model containing only the non-genetic covariates. Luo et al. (2011, 2012a,b) first introduced the idea of functional regression (FR) for testing associations between genetic variants and quantitative traits. Unlike SKAT which assumes random effects for the genetic variants (within each region) and accounts for pairwise correlations among them by testing the corresponding variance-covariance components, the FR-based model treats the effect of genetic variants as an unknown fixed smooth function of the variant’s actual physical position through a functional linear model (FLM), which utilizes the LD information among close variants. Fan et al. (2013) extended the work and has shown that for continuous, binary and censored traits, the FLM approach can be more powerful than SKAT or burden test in various scenarios (Fan et al. 2013, 2014, 2016).

This work is motivated by a genetic study for Age-related Macular Degeneration (AMD) to identify risk regions associated with AMD progression. AMD, a leading cause of blindness in the developed world (Swaroop et al. 2009; The Eye Diseases Prevalence Research Group 2004), is a bilateral and progressive neurodegenerative disease. Recently, multiple studies

have been conducted to underpin the genetic causes for disease progression where the outcome of interest is the bivariate time-to-progression (e.g., time-to-late-AMD of the two affected eyes). For example, Sardell et al. (2016) and Ding et al. (2017) analyzed a small set of variants on AMD progression using a Cox PH model with the robust variance covariance estimate that takes the between-eye correlation into account. More recently, Yan et al. (2018) performed the first GWAS on AMD progression using a similar robust Cox PH model. Multiple risk loci have been identified to be associated with AMD progression. However, in all these studies, the focus is on common variants with $MAF \geq 5\%$. In this work, instead of evaluating the effect of single (common) variants on AMD progression, we are interested in identifying and assessing the gene regions associated with AMD progression. To our knowledge, there has been only one attempt so far on gene-based analysis for AMD progression, done by Fan et al. (2016). They applied the FR-based approach under the Cox PH model by using the left eye information only, due to the lack of methods for bivariate survival traits in gene-based analysis.

Bivariate or multivariate survival analysis has been studied for decades. Thorough reviews with examples can be obtained from Joe (1997) and Hougaard (2000). The Copula family is one of the earliest approaches for modeling bivariate survival data (Clayton 1978). The idea is to model the joint distribution as a function of each marginal distribution together with a dependence parameter. Another popular approach for correlated survival data is the frailty model, of which the correlation is modeled through a common latent frailty variable (Oakes 1982). The third approach is a marginal method such as the robust Cox model used in Ding et al. (2017) and Yan et al. (2018), of which the variance-covariance matrix is estimated from a robust sandwich estimator (as introduced in Wei et al. (1989)).

In this work, we propose to develop and implement a novel gene-based association analysis method for bivariate survival traits based on functional regression under the copula framework. The paper is organized as follows. Section 2 introduces the proposed copula-based FLM method. Section 3 presents simulation studies for evaluating type-I error control and power performance under various settings. Section 4 demonstrates a real data analysis on AMD progression using the proposed method, and followed by the discussion of practical challenges and possible extensions in Section 5.

2. Method

2.1 Copula model for bivariate time-to-event data

One of the earliest distribution families for modeling correlated bivariate measurements is the copula family (Clayton 1978), originated from Sklar's Theorem (Sklar 1959), in which the joint distribution is modeled as a function of each marginal distribution together with a dependence parameter. Assume U and V are both uniformly distributed random variables, then a bivariate copula is a function defined as $\{C_\eta: [0, 1]^2 \rightarrow [0, 1] : (u, v) \rightarrow C_\eta(u, v), \eta \in R\}$. The parameter η in the copula function describes the dependence between U and V . By the Sklar's theorem (Sklar 1959), one can model the joint distribution by modeling the dependence parameter and the marginal distributions separately. This is the unique feature of copula functions that makes them attractive to use. The theorem is stated as: if marginal survival functions $S_1(t_1) = P(T_1 > t_1)$ and $S_2(t_2) = P(T_2 > t_2)$ for T_1 and T_2 are continuous,

then there exists a unique copula function C_η such that for all $t_1 \geq 0, t_2 \geq 0$, the joint survival function $S(t_1, t_2)$ can be written as

$$S(t_1, t_2) = C_\eta\{(S_1(t_1), S_2(t_2)), t_1, t_2 \geq 0\}. \quad (2.1)$$

Define the density function for C_η to be $c_\eta(u, v) = \frac{\partial^2 C_\eta(u, v)}{\partial u \partial v}$, then the joint density function of T_1 and T_2 can be expressed as $f(t_1, t_2) = c_\eta\{S_1(t_1), S_2(t_2)\} f_1(t_1) f_2(t_2), t_1, t_2 \geq 0$.

In this work, we choose to use the Archimedean copula family, which is one of the most popular copula families because of its flexibility and simplicity. For example, the rank-based dependence measure Kendall's τ can be directly obtained as a function of η in Archimedean copulas. Two most frequently used Archimedean copulas in survival analysis are: *Clayton copula* (Clayton 1978): $C_\eta(u, v) = (u^{-\eta} + v^{-\eta} - 1)^{-1/\eta}, \eta \in (0, \infty)$, which models the lower tail dependence in survival functions; and *Gumbel-Hougaard copula* (Gumbel 1960): $C_\eta(u, v) = \exp[-\{(-\log u)^\eta + (-\log v)^\eta\}^{1/\eta}], \eta \in [1, \infty)$ which models the upper tail dependence in survival functions.

We consider modeling the margins $S_1(\cdot)$ and $S_2(\cdot)$ inside the copula function using the Cox PH model, within which the effect of a gene region (captured by multiple single variants) is modeled through a FLM, as we describe below.

2.2 Functional Linear Model Specification

Assume n individuals with m variants being sequenced for a gene region. Physical positions for each variant within that region are denoted as $0 \leq u_1 < \dots < u_m$ (which are typically standardized into $[0, 1]$). Let $G_i = \{g_i(u_1), \dots, g_i(u_m)\}'$, $g_i(u_j) \in (0, 1, 2), i = 1, \dots, n, j = 1, \dots, m$, denote the genotype information for the m variants of subject i , indicating the number of copies of the minor allele for each of these m variants. Let $(X_{1i}, X_{2i}) = \{(x_{1i1}, \dots, x_{1ip}), (x_{2i1}, \dots, x_{2ip})\}$, denote a $p \times 2$ matrix of bivariate covariates for subject i . Then the hazard function for the k th margin ($k = 1, 2$) under the PH-FLM can be written as

$\lambda_{ki}(t) = \lambda_{k0}(t) \exp\{X'_{ki}\beta_k + \int_0^1 G_i(u)\gamma_k(u)du\}$, where $\lambda_{k0}(t)$ is the baseline hazard function for the k th margin, β_k is a $p \times 1$ vector of coefficients for the non-genetic covariates for the k th margin, and $\gamma_k(u)$ is the genetic effect function of the genetic variant function $G_i(u)$ at position u for the k th margin. We assume both $\gamma_k(u)$ and $G_i(u)$ are smooth functions. Then the corresponding marginal survival functions are

$$S_{ki}(t) = \exp(-\Lambda_{ki}(t)) = \exp\{-\int \lambda_{ki}(t)dt\} = \exp[-\int \lambda_{k0}(t) \exp\{X'_{ki}\beta_k + \int_0^1 G_i(u)\gamma_k(u)du\}dt], k = 1, 2.$$

Next, we describe how we handle the two functions $G_i(u)$ and $\gamma_k(u)$, respectively.

2.2.1 The genetic variant function (GVF) $G_i(u)$.—If the genotype data are of good quality with low missing rate, we can simply use the observed genetic information to represent the GVF directly, such as $\hat{G}_i(u) = G_i = \{g_i(u_1), \dots, g_i(u_m)\}$. In this case, the elements of $\hat{G}_i(u)$ take discrete values 0, 1, 2.

If the genotype data have a fairly high missing rate or equivalent, the region has only a small number of variants being genotyped, one may consider applying an ordinary linear square smoother (Ramsay et al. 2009) to obtain a continuous realization of G_i . Let $\phi(u) = \{\phi_1(u), \dots, \phi_{B_G}(u)\}'$ be a series of basis functions (e.g., B-spline or Fourier spline basis). Denote by Φ the $m \times B_G$ matrix with elements $\phi_b(u_j)$, $b = 1, \dots, B_G$, $j = 1, \dots, m$. Then through a linear square smoother, an estimate of the GVF can be written as

$$\hat{G}_i(u) = \{g_i(u_1), \dots, g_i(u_m)\} \Phi [\Phi' \Phi]^{-1} \phi(u). \quad (2.2)$$

Under the situation with missing data, we have $\hat{G}_i(u) = \{g_i(u_1), \dots, g_i(u_m)\} \tilde{\Phi} [\tilde{\Phi}' \tilde{\Phi}]^{-1} \phi(u)$, where $\{g_i(u_1), \dots, g_i(u_m)\}$ are the observed non-missing genotypes and $\tilde{\Phi}$ is the corresponding basis matrix evaluated at the non-missing genotypes.

2.2.2 The genetic effect function (GEF) $\gamma_k(u)$.—The GEF $\gamma_k(u)$ is an unknown smooth function with an arbitrary form that we need to estimate. To do this, one can approximate it using a sieve approach with a linear combination of basis functions and coefficients. For the k th margin, define a series of $B_{k\gamma}$ basis function by $\psi_k(u) = \{\psi_{k1}(u), \dots, \psi_{kB_{k\gamma}}(u)\}'$ and a $B_{k\gamma} \times 1$ vector $\gamma_k = (\gamma_{k1}, \dots, \gamma_{kB_{k\gamma}})'$, then $\gamma_k(u)$ can be approximated by

$$\hat{\gamma}_k(u) = \{\psi_{k1}(u), \dots, \psi_{kB_{k\gamma}}(u)\} (\gamma_{k1}, \dots, \gamma_{kB_{k\gamma}})', k = 1, 2. \quad (2.3)$$

To test whether the variants in a region, as a set, is associated with the k th outcomes, it is to test whether the GEF is a zero function $\gamma_k(u) = 0$, which is equivalent to test the null hypothesis: $H_0: \gamma_{k1} = \dots = \gamma_{kB_{k\gamma}} = 0$. Typically, the number of basis $B_{k\gamma}$ is much smaller than the number of variants m in the region. For “symmetric” bilateral diseases (e.g., AMD), it is plausible to assume that the genetic effects are identical for each of the bivariate outcomes, and in that case, the model can be simplified by setting $\gamma_1(u) = \gamma_2(u)$.

2.2.3 The hazard function $\lambda(t)$ under the FLM.—Depending on whether or not to smooth $G(u)$, we propose two types of functional regression models for the hazard function. The first option is to smooth both $G(u)$ and $\gamma(u)$. By replacing $G(u)$ and $\gamma(u)$ with their approximated values in (2.2) and (2.3), the hazard function for each margin can be written as

$$\begin{aligned} \lambda_{ki}(t) &= \lambda_{k0}(t) \exp[X'_{ki} \beta_k + \{g_i(u_1), \dots, g_i(u_m)\} \Phi [\Phi' \Phi]^{-1} \gamma_k \int_0^1 \phi(u) \psi_k(u) du] \\ &= \lambda_{k0}(t) \exp(X'_i \beta_k + M'_{ki} \gamma_k), \end{aligned} \quad (2.4)$$

where $M'_{ki} = \{g_i(u_1), \dots, g_i(u_m)\} \Phi [\Phi' \Phi]^{-1} \int_0^1 \phi(u) \psi_k(u) du$. The integral $\int_0^1 \phi(u) \psi_k(u) du$ can be calculated using the R package `{fda}` (Ramsay et al. 2009) once basis functions are chosen.

Another option is to smooth $\gamma_k(u)$ only. In this case, we directly replace $G_i(u)$ by the observed $G_i = (g_i(u_1), \dots, g_i(u_m))'$ and replace $\gamma_k(u)$ by (2.3), which yields

$$\begin{aligned}
 \lambda_{ki}(t) &= \lambda_{k0}(t) \exp \left[X'_{ki} \beta_k + \left\{ \sum_{j=1}^m (g_i(u_j) \times (\psi_{k1}(u_j), \dots, \psi_{kB_{k\gamma}}(u_j))) \right\} (\gamma_{k1}, \dots, \right. \\
 &\quad \left. \gamma_{kB_{k\gamma}})' \right] \\
 &= \lambda_{k0}(t) \exp(X'_{ki} \beta_k + M'_{ki} \gamma_k),
 \end{aligned} \tag{2.5}$$

where $M'_{ki} = \sum_{j=1}^m g_i(u_j) \times \{\psi_{k1}(u_j), \dots, \psi_{kB_{k\gamma}}(u_j)\}$ is a fully observed term.

Two frequently used basis functions are B-splines and Fourier splines. The B-spline basis (de Boor 2011) is a series of non-periodic functions with polynomial segments joint at values called knots. The segments have specifiable smoothness across every breaks. Advantages of applying the B-spline basis include its fast computation and great flexibility in structure. The Fourier splines consist of a set of periodic functions, with the basis function being $\Phi_1(u) = 1$, $\Phi_{2f}(u) = \cos(2\pi ru)$ and $\Phi_{2r+1}(u) = \sin(2\pi ru)$ for a pre-specified r (de Boor 2011).

2.3 Bivariate FLM under the copula framework

Let (T_{1i}, T_{2i}) and (C_{1i}, C_{2i}) denote the bivariate survival times and censoring times for subject i , respectively. Denote by $\delta_i = (\delta_{1i}, \delta_{2i})$ the corresponding censoring indicator. We consider right censoring and assume that given covariates, (T_1, T_2) and (C_1, C_2) are independent. Then for each subject, we observe $D_i = \{(Y_{1i}, Y_{2i}, \delta_{1i}, \delta_{2i}, X_{1i}, X_{2i}, G_i) : Y_{ki} = \min(T_{ki}, C_{ki}), \delta_{ki} = I(T_{ki} \leq C_{ki}), k = 1, 2\}$.

Denote by θ all the parameters in $S(t_1, t_2)$, then the joint likelihood for the observed data $\{D_i\}_{i=1}^n$ can be written as

$$\begin{aligned}
 L\{\theta; D = (Y_1, Y_2, \Delta_1, \Delta_2, X_1, X_2, G)\} \\
 &= \prod_{i=1}^n f(y_{1i}, y_{2i})^{\delta_{1i}\delta_{2i}} \times \left\{ -\frac{\partial S(y_{1i}, y_{2i})}{\partial y_{1i}} \right\}^{\delta_{1i}(1-\delta_{2i})} \\
 &\quad \times \left\{ -\frac{\partial S(y_{1i}, y_{2i})}{\partial y_{2i}} \right\}^{(1-\delta_{1i})\delta_{2i}} \times S(y_{1i}, y_{2i})^{(1-\delta_{1i})(1-\delta_{2i})},
 \end{aligned} \tag{2.6}$$

where $(\delta_{1i}, \delta_{2i}) \in \{(0, 0), (0, 1), (1, 0), (1, 1)\}$. Then under the copula framework (2.1), with a FLM for the genetic effect, the joint survival function can be further written as

$S(y_{1i}, y_{2i}) = C_\eta[\exp\{-\int_0^{y_{1i}} \lambda_{1i}(t)dt\}, \exp\{-\int_0^{y_{2i}} \lambda_{2i}(t)dt\}]$, where $\lambda_{ki}(t)$ can be modeled by either (2.4) or (2.5), depending whether to smooth the GVF $G_f(u)$ or not.

2.4 Generalized score test

We are interested in testing whether a gene region is associated with the disease progression, after adjusting for other risk factors. To accomplish this, we separate the entire parameter θ into two parts: θ_1 , the parameter of interest; and θ_2 , the nuisance parameter. For the simplicity of notation, we now assume $\gamma_1 = \gamma_2$ and denote as γ . Under the bivariate FLM-based copula model, assuming we are interested in testing the genetic effects, then we have

$\theta_1 = \gamma$ and $\theta_2 = (\beta_1, \beta_2, \eta, \lambda_{10}, \lambda_{20})$ contains the rest of parameters in the likelihood. The null hypothesis can be formulated as

$$H_0: \theta_1 = (\gamma_1, \dots, \gamma_{B_\gamma})' = 0 \text{ and } \theta_2 \text{ is arbitrary.} \quad (2.7)$$

In GWAS, the score test is typically preferred than other likelihood-based tests due to its computational advantage. This is because only one null model (without any SNP) needs to be fitted for the score test. Details for constructing the generalized score test for testing (2.7) under the FLM-based copula framework can be found in our Supporting Information.

3. Simulation Study

In this section, we performed simulation studies to evaluate the performance of the proposed method in terms of type-I error control and power. Two scenarios were considered: (1) a mixture of common (MAF > 5%) and rare causal variants (MAF $\in$ [1%, 5%]); (2) all causal variants are rare (MAF $\in$ [1%, 5%]). To mimic the motivating eye disease AMD, we assumed the genetic effects are the same for both margins (by setting $\gamma_1 = \gamma_2 = \gamma$).

3.1 Data generation and simulation settings

Genotype datasets were generated from European ancestry of 10,000 haplotypes covering 1Mb regions, simulated by Yun Li at the University of North Carolina (Fan et al. 2016), where calibrated coalescent model as programmed in COSI was used to generate the LD information (Schaffner et al. 2005). With 10,000 haplotypes, we chose a genetic region of length 6 Kb and 30 Kb for all and rare only variants scenarios, respectively. For both scenarios, the regions contain a mean of 20 variants. A random mating technique was then applied to generate genetic information for the offsprings.

The bivariate time-to-event phenotypes were generated from a Clayton Weibull model as follows. Recall that under a copula model $S(t_1, t_2) = C_\eta(S_1(t_1), S_2(t_2))$, $U = S_1(T_1)$, $V = S_2(T_2)$ each follows a uniform distribution $U[0, 1]$. Define $W_\eta(u) = h(u, v) = P(U \leq u | V = v) = C_\eta(u, v)/v$. First, we generated v_i and w_i from two independent standard uniform distributions. Then let $w_i = h(u_i, v_i) = C_\eta(u_i, v_i)/v_i$ and solve for u_i from the inverse function h^{-1} . Finally, we obtained two survival times t_{1i} and t_{2i} from $S_1^{-1}(u_i)$ and $S_2^{-1}(v_i)$ respectively. The scale and shape parameters in the baseline Weibull distribution were set to be $\lambda = 0.1$ and $k = 2$, same for both margins. For Clayton copula, the dependence strength is determined by Kendall's τ . Here we chose $\tau = 0.2, 0.4$ and 0.8 to represent weak to strong dependence between two marginal survivals. Censoring times c_{1i} and c_{2i} were generated from uniform distribution $U(a, b)$ with a, b chosen to yield a 50% censoring rate for each margin. The non-genetic covariate $X_{ng,k}$ ($k = 1, 2$) was generated from $N(6, 2^2)$, and the effect was set to 0.1 ($\beta_1 = \beta_2 = 0.1$).

For type-I error control simulations, we assumed there is no genetic effect ($\gamma(u) = 0$). We simulated 500 genotype datasets, each with a sample size of 1,000. For each genotype set, 2,000 phenotype datasets of bivariate survival times and one non-genetic covariate were generated, which led to a total of 10^6 datasets. The type-I error was evaluated at various α

levels: 0.05, 0.01, 10^{-3} and 10^{-4} , respectively. We reported the results of our copula-based functional regression model using the correct copula function (i.e., Clayton), and compare with the following methods: (a) a misspecified Gumbel copula model, (b) FLM under Cox PH assuming the bivariate traits are independent (i.e., univariate survival outcome with doubled sample size) (Cox-Ind), (c) FLM under Cox PH with “robust sandwich estimator” for the variance-covariance to account for the between margin correlation (Cox-Rst), (d) univariate FLM under Cox PH (Cox-FLM), (e) Burden test, and (f) SKAT test. The last two methods are from R package {seqMeta} (Voorman et al. 2016) and the default weights were used (1 for Burden test and values sampled from distribution $\text{beta}(\text{MAF}, 1, 25)$ for SKAT, indicating that variants with smaller MAF are given larger weights). Please note that methods (d) - (f) are for univariate outcomes, and thus one margin from simulated data was used. For all FLM based methods, B-splines with order = 4 and number of basis = 5 were used.

For power analysis, we generated data to evaluate both homogeneous genetic effects (genes with effects in the same direction) and heterogeneous genetic effects (genes with effects in opposite directions). Similar as in Fan et al. (2016), the effect size (i.e., $\chi(u)$) for each causal variant was chosen to be a constant that depends on MAF: $c \frac{|\log_{10} \text{MAF}|}{2}$, where $c = 0.4, 0.3, 0.25$ for scenarios with 10%, 20% and 30% of causal variants in a given region, respectively. We performed 10,000 simulations (100 genotype datasets $\times$ 100 phenotype datasets). We evaluated power at significance level of 0.05.

3.2 Type-I error

Table 1 presents the type-I error results for the scenario with both common and rare variants. As expected, the independent Cox FLM (Cox-Ind) yields inflated type-I errors and the inflation becomes more severe when data are more dependent. With robust variance-covariance estimates, Cox-Rst still shows some inflation in type-I error, especially under small α tail. Similar observations were found in testing single variants with bivariate survival outcomes (Sun et al. 2019). Within the copula-based FLM, the correct Clayton model produces satisfactory-controlled type-I errors while the Gumbel model has shown some inflation due to the misspecified correlation structure.

Table 2 presents the type-I error results for testing regions that contain only rare variants ($\text{MAF} \in [1\%, 5\%]$). The overall performance is similar to the all variants scenario. The inflation in Cox-Rst is slightly more compared to the all variants scenario, which is also consistent with the observation in Sun et al. (2019).

3.3 Empirical power

Given the independent Cox FLM (on bivariate data) cannot control type-I error, we did not include it in the power analysis. Although the robust Cox FLM has shown some inflation in type-I error also, the magnitude is very small under $\alpha = 0.05$. In addition, it is the only method that utilizes bivariate data from both margins besides our copula FLM method. Thus we included it in the power comparison. Figure 1 illustrates the power bars over different dependence levels and genetic effect sizes for the mixture of common and rare variants scenario. Overall, the proposed copula-based FLM model (ClaytonFLM) produces the

highest power among all univariate and bivariate models with both homogeneous and heterogeneous scenarios.

Figure 2 shows the power analysis for the rare variants only scenario. Our ClaytonFLM produces the highest power in homogenous effect scenarios, but not in heterogenous effect scenarios. With heterogeneous effect, SKAT (on univariate data) is even more powerful than ClaytonFLM (on bivariate data). Similar to the observations when both common and rare variants are included, the power is low for burden test when very few variants are causal (e.g., 10 %) or the genetic effects are heterogeneous.

From our power analysis, we recommend using the copula-based FLM method for bivariate survival traits in the situation with both common and rare variants. If only rare variants (in a region) are tested, one may consider developing a copula-based SKAT model, which is expected to have the power advantage (compared to copula-based FLM method).

4. Application on AREDS data

4.1 Examination on the Reported Four Regions from Previous Publications

Age-Related Eye Disease Study (AREDS) is a large clinical trial sponsored by National Eye Institute to study the clinical course of, and risk factors for the development and progression of AMD (Age-Related Eye Disease Study Research Group 1999). We implemented our proposed method to analyze the bivariate time-to-progression data from AREDS for discovering gene regions that are associated with AMD progression.

Over the past few years, many case-control GWAS studies have identified multiple SNPs associated with AMD susceptibility (Chen et al. 2010; Fritsche et al. 2013, 2016) and recent genetic studies on AMD progression identified SNPs that are associated with disease progression (Ding et al. 2017; Yan et al. 2018). Web Table 1 presents the top four genetic regions which contain SNPs associated with AMD risk identified by the traditional single-marker GWAS in Fritsche et al. (2016). These four regions were also reported to be top gene regions containing variants associated with AMD progression in Yan et al. (2018). Therefore, we first examined these four candidate regions specifically. Location of gene regions were extracted based on GRCh37/hg19 assembly from UCSC Genome Browser. Variants within ± 5 Kb of the region boundary were included in the analysis.

We included all Caucasian participants with neither eye progressed at the time of enrollment into the study. For bivariate approaches, time-to-progression was calculated for each eye of each patient. For univariate approaches, only left-eye data were used. A total of 2,296 subjects were included in the analysis. The baseline age and disease severity score (on a continuous scale ranging from 1 to 8) were included in the regression part of the model as the non-genetic risk factors.

To decide which copula function and marginal distribution to choose, we compared the AICs from models (with non-genetic covariates only) using Clayton or Gumbel copula with Weibull or Loglogistic margins. The Clayton copula with Weibull margin yielded the smallest AIC and thus was selected. B-spline was used to smooth the genetic effect function.

The number of basis is usually decided based upon the sample size (or equivalently, the total number of events in survival analysis) or through cross validations. In our analysis, we examined three different number of bases: 5, 6 and 7. A mixture of common ($MAF > 5\%$) and rare causal variants ($MAF \in [1\%, 5\%]$) were tested. Similar to the simulation studies, we compared our proposed copula-based FLM score test to the robust Cox FLM, the univariate Cox FLM, SKAT and burden test.

Table 3 presents p -values of testing the four candidate gene regions that contain both common and rare variants. The bivariate approaches, i.e., Clayton FLM and robust Cox FLM identify *CFH*, *C2-CFB-SKIV2L* and *ARMS2* regions at the significance level of 0.05, with smaller p -values produced from Clayton FLM. For the *C3* region, both bivariate methods have shown marginal significance. Due to ignoring data from the right eye, none of the univariate approaches identifies all four gene regions, with the best case scenario, SKAT, identifying three gene regions except the *ARMS2* region. The univariate Cox FLM identifies two gene regions: *CFH* and *ARMS2*, while the burden test only shows that *CFH* is significant. Note that for the three approaches that involve FLM, varying the number of basis from 5 to 7 produces similar results in this data set.

Web Figure 1 presents the estimated genetic effect functions for the four gene regions (when the number of B-spline basis was chosen as 6). The *CFH* shows the largest effect size with most of the positions having a negative effect, and it explains the smallest p -values obtained from the FLM based methods for this region. Since most of the effects seem to be homogeneous in the *CFH* region, the burden test maintains the power by providing a p -value as small as 7.11×10^{-4} . For other regions, they all show heterogeneous effects with some positions having positive effects while others having negative effects, so the burden test loses power under these situations. The *ARMS2* and *C2* regions both show reasonable amount of effects and thus Clayton FLM detected these two regions to be significant. The relative flat curve presented by the *C3* region also explains the marginal significance from Clayton FLM.

4.2 Genome-wide Association Study

We then applied the copula-based model on the whole genome to search for top gene regions that are associated with the AMD progression. Again, all gene regions were extracted based on GRCh37/hg19 assembly from UCSC Genome Browser and those with less than 10 SNPs were excluded from the analysis. In total we analyzed 22,747 gene regions.

Table 4 presents p -values of top gene regions identified by the proposed copula-FLM approach, at the α level of 1.0×10^{-5} . All methods have generated consistent p -values for these regions, with copula-FLM producing the smallest p -values. The last column presents the number of significant SNPs identified by the traditional single-SNP-based Clayton copula model under the nominal α level of 0.05. In addition to the *CFH* and *ARMS2* regions, *DLGAP2-AS1*, *GABRA5*, *CLEC4GPI* and *HAL-C* regions all have more than 30% of the SNPs to be associated with the AMD progression in a less stringent sense, which explains the reason why they were not among the top genes list in the single-SNP-based GWAS studies but were identified in a gene-based analysis. Among these gene regions, gene *PCDH9* and *DLGAP2* are reported to have significant SNP associated with AMD, although not the top ones, in a GWAS study (Yan et al. 2018). Gene *HLA-C* has been shown to have

association with AMD in combination with inhibitory Killer-cell immunoglobulin-like receptors on natural killer (NK) cells (Goverdhan et al. 2005).

5. Conclusion and Discussion

In this work, we have developed a new gene-based association analysis method for bivariate time-to-event data using the functional linear model under the copula framework. On one hand, the genetic effect can be viewed as a function of the physical positions of variants under the functional linear model. On the other hand, the copula model can effectively handle the correlation between two margins. Combining the FLM with copula framework takes the advantage of both methods for detecting gene regions that are significantly associated with the progression of bilateral diseases.

Extensive simulation studies were performed to evaluate the type-I error rates and power performance of our method. The score test from our copula FLM model controls type-I error well. However, when the copula structure is misspecified, inflated type-I error rates are observed. Similar observations were found in Sun et al. (2019) where they consider fitting copula models to test a single covariate (e.g., SNP) with bivariate censored outcomes. Therefore, as in all parametric analysis, the model needs to be correctly specified. In real data analysis, one may consider fitting several different copula models and use AIC to select a copula model (with the smallest AIC). A rigorous goodness-of-fit test would be more desirable. We are in the process of developing such a test for copula-based regression models.

For the power analysis, our copula-based FLM shows great advantage by utilizing all available data with correlation between two margins being accounted, especially under the situation where both common and rare variants (in a region) are tested. If only rare variants are tested, one may consider developing a copula-based SKAT model, which may have better power advantage compared to copula FLM.

We successfully applied our method on AREDS data at four known AMD risk gene regions and obtained consistent findings. Through the genome-wide study, nine gene regions were found to be significantly associated with the disease progression, where *PCDH9-AS4*, *DLGAP2-AS1*, *LINC00476*, *GABRA5*, *CLEC4GPI*, *HLA-C* and *SULF1* are novel regions or less-reported regions. The findings from this research provide new perspectives on the genetic underpinning of AMD progression, which will be valuable to establish novel and reliable predictive models for AMD progression. The proposed method is useful for genome-wide association studies of any bilateral disease with survival traits to identify disease susceptible gene regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors Yue Wei and Yi Liu contributed equally to this paper. This research is supported by NIH (grant EY024226). We would like to thank the participants in the AREDS study, who made this research possible, and International AMD Genomics Consortium for generating the genetic data and performing quality check.

References

- Age-Related Eye Disease Study Research Group (1999). The age-related eye disease study (areds): design implications. areds report no. 1. *Control Clinical Trials* 20, 573–600.
- Chen H, Lumley T, Brody J, Heard-Costa NL, Fox CS, Cupples LA, et al. (2014). Sequence kernel association test for survival traits. *Genet Epidemiol* 38, 191–197. [PubMed: 24464521]
- Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, et al. (2010). Genetic variants near *TIMP3* and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. *PNAS* 107, 7401–7406. [PubMed: 20385819]
- Chien L-C, Bowden D, and Chiu Y-F (2017). Region-based association tests for sequencing data on survival traits. *Genetic epidemiology* 41, 511–522. [PubMed: 28580640]
- Clayton DG (1978). A model for association in bivariate life tables and its application in epidemiological studies of familial tendency in chronic disease incidence. *Biometrika* 65, 141–151.
- de Boor C (2011). *A Practical Guide to Splines (Applied Mathematical Sciences)*. Springer.
- Ding Y, Liu Y, Yan Q, Fritsche LG, Cook RJ, Clemons T, et al. (2017). Bivariate analysis of age-related macular degeneration progression using genetic risk scores. *Genetics* 206, 119–133. [PubMed: 28341650]
- Fan R, Wang Y, Mills JL, Carter TC, Lobach I, Wilson AF, et al. (2014). Generalized functional linear models for case-control association studies. *Genet Epidemiol* 38, 622–637. [PubMed: 25203683]
- Fan R, Wang Y, Mills JL, Wilson AF, Bailey-Wilson JE, and Xiong M (2013). Functional linear models for association analysis of quantitative traits. *Genet Epidemiol* 37, 726–742. [PubMed: 24130119]
- Fan R, Wang Y, Yan Q, Ding Y, Weeks D, Lu Z, et al. (2016). Gene-based association analysis for censored traits via fixed effect functional regressions. *Genet Epidemiol* 40, 133–143. [PubMed: 26782979]
- Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G, et al. (2013). Seven new loci associated with age-related macular degeneration. *Nature Genetics* 45, 433–439. [PubMed: 23455636]
- Fritsche LG, Igl W, Bailey JNC, Grassmann F, Sengupta S, Bragg-Gresham JL, et al. (2016). A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nature Genetics* 48, 134–143. [PubMed: 26691988]
- Goverdhan SV, Howell MW, Mullins RF, Osmond C, Hodgkins PR, Self J, et al. (2005). Association of hla class i and class ii polymorphisms with age-related macular degeneration. *Investigative Ophthalmology & Visual Science* 46, 1726–1734. [PubMed: 15851575]
- Gumbel EJ (1960). Bivariate exponential distributions. *Journal of the American Statistical Association* 55, 698–707.
- Han F and Pan W (2010). A data-adaptive sum test for disease association with multiple common or rare variants. *Human Heredity* 70, 537–545.
- Hougaard P (2000). *Analysis of Multivariate Survival Data*. Springer New York.
- Joe H (1997). *Multivariate models and dependence concepts*. Chapman & Hall.
- Li B and Leal SM (2008). Methods for detecting associations with rare variants for common diseases: Application to analysis of sequence data. *American Journal of Human Genetics* 83, 311–321. [PubMed: 18691683]
- Luo L, Boerwinkle E, and Xiong M (2011). Association studies for next-generation sequencing. *Genome Research* 21, 1099–1108. [PubMed: 21521787]
- Luo L, Zhu Y, and Xiong M (2012a). Quantitative trait locus (qtl) analysis for next-generation sequencing with the functional linear models. *Journal of Medical Genetics* 49, 513–524. [PubMed: 22889854]

- Luo L, Zhu Y, and Xiong M (2012b). Smoothed functional principal component analysis for testing association of the entire allelic spectrum of genetic variation. *European Journal of Human Genetics* 21, 217–224. [PubMed: 22781089]
- Oakes D (1982). A model for association in bivariate survival data. *Journal of the Royal Statistical Society. Series B* 44, 414–422.
- Ramsay JO, Hooker G, and Graves S (2009). *Functional Data Analysis With R and Matlab*. Springer New York.
- Sardell RJ, Persad PJ, Pan SS, Whitehead P, Adams LD, Laux RA, et al. (2016). Progression rate from intermediate to advanced Age-Related Macular Degeneration is correlated with the number of risk alleles at the CFH locus. *Invest Ophthalmol Vis Sci* 57, 6107–6115. [PubMed: 27832277]
- Schaffner SF, Foo C, Gabriel S, Reich D, Daly MJ, and Altshuler D (2005). Calibrating a coalescent simulation of human genome sequence variation. *Genome Res* 15, 1576–1583. [PubMed: 16251467]
- Sklar A (1959). Fonctions de repartition a n dimensions et leurs marges. *Publications de L Institut de Statistique de L Universite de Paris* 8, 229–231.
- Sun T, Liu Y, Cook RJ, Chen W, and Ding Y (2019). Copula-based score test for bivariate time-to-event data, with application to a genetic study of amd progression. *Lifetime Data Analysis* 25, 546–568. [PubMed: 30560439]
- Swaroop A, Chew EY, Rickman CB, and Abecasis GR (2009). Unraveling a multifactorial late-onset disease: from genetic susceptibility to disease mechanisms for age-related macular degeneration. *Annual review of genomics and human genetics* 10, 19–43.
- The Eye Diseases Prevalence Research Group (2004). Causes and Prevalence of Visual Impairment Among Adults in the United States. *Archives of Ophthalmology* 122, 477–485. [PubMed: 15078664]
- Voorman A, Brody J, Chen H, Lumley T, and Davis B (2016). Meta-Analysis of Region-Based Tests of Rare DNA Variants. R package version 1.6.7.
- Wei LJ, Lin D, and Weissfeld L (1989). Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. *Journal of the American Statistical Association* 84, 1065–1073.
- Wu MC, Lee S, Cai T, Li Y, Boehnke M, and Lin X (2011). Rare-variant association testing for sequencing data with the sequence kernel association test. *Am J Hum Genet* 89, 82–93. [PubMed: 21737059]
- Wu X, Guan T, Liu DJ, Leon Novelo LG, and Bandyopadhyay D (2018). Adaptive-weight burden test for associations between quantitative traits and genotype data with complex correlations. *Annals of Applied Statistics* 12, 1558–1582. [PubMed: 30214655]
- Yan Q, Ding Y, Liu Y, Sun T, Fritsche LG, Clemons T, et al. (2018). Genome-wide analysis of disease progression in age-related macular degeneration. *Human Molecular Genetics* 27, 929–940. [PubMed: 29346644]

Figure 1. Power analysis for the situation when both common and rare variants (in a region) are tested, with bivariate data generated from various dependence strength levels.

Figure 2. Power analysis for the situation when only rare variants (in a region) are tested, with bivariate data generated from various dependence strength levels.

Table 1

Type-I error of seven methods for testing gene regions that contain both common and rare variants. The data are simulated from Clayton copula with Weibull margins (at various dependence levels).

τ	α level	Bivariate FLM				Univariate		
		Clayton	Gumbel	Cox-Ind	Cox-Rst	Burden	SKAT	CoxFLM
0.2	0.05	0.05235	0.07970	0.11135	0.05784	0.05073	0.05122	0.05139
	0.01	0.01104	0.02035	0.03238	0.01333	0.01037	0.01025	0.01032
	0.001	0.00119	0.00296	0.00546	0.00178	0.00106	0.00098	0.00110
	0.0001	0.00014	0.00043	0.00088	0.00028	0.00013	0.00009	0.00011
0.4	0.05	0.05170	0.09241	0.18160	0.05769	0.05079	0.05170	0.05130
	0.01	0.01059	0.02484	0.06681	0.01302	0.01028	0.01034	0.01046
	0.001	0.00112	0.00388	0.01534	0.00175	0.00108	0.00103	0.00108
	0.0001	0.00013	0.00064	0.00344	0.00030	0.00012	0.00011	0.00013
0.8	0.05	0.05219	0.07650	0.29056	0.05552	0.05077	0.05170	0.05128
	0.01	0.01090	0.01901	0.13585	0.01219	0.01026	0.01035	0.01045
	0.001	0.00122	0.00268	0.04387	0.00154	0.00108	0.00103	0.00108
	0.0001	0.00016	0.00042	0.01364	0.00024	0.00012	0.00011	0.00013

Table 2

Type-I error of seven methods for testing gene regions that contain rare variants only. The data are simulated from Clayton copula with Weibull margins (at various dependence levels).

τ	α level	Bivariate FLM				Univariate		
		Clayton	Gumbel	Cox-Ind	Cox-Rst	Burden	SKAT	CoxFLM
0.2	0.05	0.05280	0.0806	0.11148	0.06118	0.05127	0.05107	0.05170
	0.01	0.01106	0.02082	0.03237	0.01462	0.01000	0.01040	0.01051
	0.001	0.00121	0.00304	0.00530	0.00207	0.00098	0.00108	0.00113
	0.0001	0.00013	0.00044	0.00090	0.00033	0.00010	0.00012	0.00012
0.4	0.05	0.05257	0.09323	0.18244	0.06041	0.05169	0.05127	0.05106
	0.01	0.01098	0.02575	0.06721	0.01439	0.01051	0.00999	0.01040
	0.001	0.00117	0.00424	0.01552	0.00211	0.00113	0.00098	0.00108
	0.0001	0.00013	0.00073	0.00364	0.00036	0.00012	0.00010	0.00012
0.8	0.05	0.05392	0.07810	0.29159	0.05781	0.05170	0.05127	0.05106
	0.01	0.01158	0.01983	0.13627	0.01333	0.01052	0.00999	0.01040
	0.001	0.00139	0.00294	0.04402	0.00180	0.00113	0.00098	0.00108
	0.0001	0.00018	0.00047	0.01376	0.00029	0.00012	0.00010	0.00012

Table 3

Results from different gene-based tests on four candidate regions (including both common and rare variants) using the AREDS data.

Gene	Chr	basis #	Bivariate FLM		Univariate		
			Clayton	Cox-Rst	Burden	SKAT	CoxFLM
<i>CFH</i>	1	5	6.79×10^{-10}	1.05×10^{-7}			3.82×10^{-4}
		6	9.93×10^{-10}	1.43×10^{-7}	7.11×10^{-4}	0.01	4.14×10^{-4}
		7	3.24×10^{-9}	3.56×10^{-7}			8.14×10^{-4}
<i>C2-CFB-SKIV2L</i>	6	5	2.02×10^{-3}	8.17×10^{-3}			0.13
		6	3.74×10^{-3}	1.40×10^{-2}	0.43	0.02	0.19
		7	6.81×10^{-3}	2.50×10^{-2}			0.23
<i>ARMS2</i> regions	10	5	5.40×10^{-4}	1.32×10^{-2}			0.03
		6	8.06×10^{-4}	1.46×10^{-2}	0.88	0.19	0.03
		7	4.81×10^{-5}	5.91×10^{-3}			0.01
<i>C3</i>	19	5	0.05	0.03			0.14
		6	0.05	0.04	0.77	1.37×10^{-3}	0.15
		7	0.10	0.06			0.24

Table 4

Top gene regions identified by applying Clayton FLM approach on all gene regions from AREDS data.
Results from two additional methods (Cox-R

Gene	Chr	# of SNPs	Clayton	Cox-Rst	CoxFLM	# of Sig. SNP (%) [*]
<i>CFH</i>	1	281	9.93×10^{-10}	1.43×10^{-7}	4.14×10^{-4}	186 (66.2)
<i>PCDH9-AS4</i>	13	55	6.64×10^{-8}	4.21×10^{-4}	3.85×10^{-3}	9 (16.4)
<i>DLGAP2-AS1</i>	8	336	8.40×10^{-7}	7.53×10^{-6}	2.14×10^{-5}	229 (68.2)
<i>ARMS2</i>	10	51	2.07×10^{-6}	1.37×10^{-3}	1.91×10^{-2}	33 (64.7)
<i>LINC00476</i>	9	201	2.23×10^{-6}	3.41×10^{-5}	2.52×10^{-2}	57 (28.4)
<i>GABRA5</i>	15	168	2.59×10^{-6}	7.75×10^{-6}	6.18×10^{-4}	68 (40.5)
<i>CLEC4GPI</i>	19	75	3.23×10^{-6}	2.60×10^{-4}	4.61×10^{-4}	30 (40.0)
<i>HLA-C</i>	6	574	3.12×10^{-6}	3.85×10^{-4}	8.06×10^{-4}	187 (32.6)
<i>SULF1</i>	8	563	8.72×10^{-6}	4.91×10^{-5}	1.40×10^{-3}	75 (13.3)

^{*} based on 0.05 alpha level