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Model Selection in Survival Analysis of EXT2 Gene Polymorphisms with Age at Onset of Type 2 Diabetes

Research Article

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Abstract

Background: This study aimed to compare the Cox regression and parametric survival models in genetic association analysis of age at onset (AAO) of type 2 diabetes (T2D) and examine the effect of exostosin 2 (EXT2) gene on risk and AAO of T2D.

Methods: We tested 22 single nucleotide polymorphisms (SNPs) within the EXT2 gene in the Marshfield sample among 878 T2D cases and 2,686 non-diabetes controls. Multiple logistic and linear regression models in PLINK software were used to examine the association of each SNP with the risk of T2D and AAO of T2D, respectively. Cox regression in PROC PHREG and parametric survival models (including exponential, Weibull, log-normal, log-logistic and gamma models) in PROC LIFEREG in SAS 9.4 were used to perform survival analysis of AAO. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to compare the competing models.

Results: PLINK software initially identified 1 SNP associated with the risk of T2D (rs7111879 with $p=6.33 \times 10^{-3}$) and 3 SNPs associated with AAO (rs7111879, rs42376464 and rs4755230 with $p=3.26 \times 10^{-2}$, 5.79×10^{-5} and 2.76×10^{-5}, respectively). AIC values showed that the gamma distribution is the best model for above 3 SNPs and followed by the Weibull distribution; whereas BIC criteria showed that the gamma distribution is similar to the Weibull distribution.

Conclusion: This study reveals that the parametric gamma and Weibull models performed better than Cox regression in genetic association of AAO of T2D and provides evidence of several genetic variants within the EXT2 gene associated with the risk and AAO of T2D.

Keywords: Type 2 Diabetes; Age at onset; EXT2; Survival Analysis; Model Selection; Cox Regression; Parametric Models.

Introduction

Globally, there were 284.6 million of patients with diabetes in 2010 and it was predicted to be 438.4 million in 2025, including 90-95% type 2 diabetes (T2D) [1]. In the Unites States (US), the estimated prevalence of T2D is 8.3 % in adults (about 25.8 million) [2]. Individuals with T2D have higher risk for cardiovascular disease and complications [3]; while T2D is also associated with and/or has comorbidity with multiple cancers such as endometrial and prostate cancers [4-8]. T2D is a complex trait caused by a complex interplay between genetic and the environment factors. Previous twin study provided evidence that genetic factors contribute to the development of T2D [9] while the heritability of T2D is about 31–69% [10].

Cox model and Weibull proportional hazard regression model have been used to analyze incident diabetes [11-17]. To date, few studies have focused on survival analyses of genetic variants with age at onset (AAO) of T2D. One previous study examined the associations of genetic variants within alpha2B adrenoceptor gene with AAO of T2D using a multiple linear regression [18]. In another study, the Cox model was used to check the associations of transcription factor 7-like 2 (TCF7L2) gene and its upstream region with AAO of T2D in Mexican Americans [19]. In addition, the Mann-Whitney and the Kruskall-Wallis tests were used to test the associations of HNF1A gene with AAO of T2D in

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patients with maturity-onset diabetes of the young (MODY)-3 [20]. However, to the best of our knowledge, no study has used parametric survival models (including exponential, Weibull, log-normal, log-logistic, and gamma models) in genetic association analysis of AAO of T2D or compared Cox regression and parametric survival models.

The exostosin glycosyltransferase 2 (EXT2) gene (also known as SOTV; SSMS) is located at 11p11.2 [21-25]. A genome-wide association study (GWAS) in a French case-control cohort identified several novel loci for the risk of T2D including 3 single nucleotide polymorphisms (SNPs) (rs3740878, rs1113132 and rs11037909) within the EXT2 gene [26]. However, the associations were not replicated in the following cohorts including: Japanese [27], an African American case-control sample [28], the Diabetes Prevention Program (DPP) data [29], Northern European populations [30], Chinese [31-32], Mexican [33], or Lebanese Arabs [34]. Recently, a meta-analysis revealed that these three SNPs in the EXT2 were significantly associated with the risk of T2D [35]. Furthermore, another meta-analysis of 20 studies in Han Chinese confirmed the association of EXT2 gene with T2D [36]. However, no study has examined the effect of EXT2 gene on AAO of T2D.

The aim of this study is twofold: (1) to examine the associations of EXT2 gene polymorphisms with AAO in a Caucasian sample and (2) to find the best model by comparing the semi-parametric Cox regression and parametric survival models in survival analysis of AAO of T2D.

Materials and Methods

Study subjects

The Marshfield sample is from the publicly available data from A Genome-Wide Association Study on Cataract and HDL in the Personalized Medicine Research Project Cohort-Study Accession: phs000170.v1.p1 (dbGaP). The primary goals of this project are to develop and validate electronic phenotyping algorithms, to accurately identify cases and controls while maintaining a positive predictive value (PPV) of > 95%, and to conduct a genome-wide association study that advances the understanding of two specific yet interrelated disease states, while simultaneously engaging the community in these research efforts. The details about these subjects were described elsewhere [37-38]. Social and behavioral factors used in this study were age, gender, alcohol use in the past month (yes or no), BMI, and smoking status (never smoking, current smoking or past smoking). Genotyping data using the ILLUMINA Human660W-Quad_v1_A are available. The genotypes of 22 SNPs within the EXT2 gene were available in this data.

Statistical methods

Descriptive statistics and genotype quality control

Descriptive statistics were used to characterize participants' sex, BMI, alcohol use, smoking, age and AAO of T2D stratified by T2D case and control status. Hardy-Weinberg equilibrium (HWE) was tested for all 22 SNPs using the controls; then, minor allele frequency (MAF) was determined for each SNP by using PLINK v1.07 [39]. To deal with population stratification, the principal-component analysis approach [40] in HelixTree software was used to identify and exclude outlier individuals [41]. Consequently, 3564 Caucasian individuals were included (878 individuals with T2D and 2686 non-T2D individuals).

Multiple logistic and linear regression models in PLINK software

Multiple logistic regression analysis (1) of each SNP with the risk of T2D as a binary outcome, adjusted for sex, age, alcohol use, smoking status and BMI, was performed using PLINK; while the asymptotic p-values were observed and the odds ratio (OR) and 95% confident interval (CI) were estimated.

$$\text{logit}(p(Y_1=1)) = \beta_0 + \beta_1 SNP_k + \beta_2 Sex + \beta_3 Age + \beta_4 Alcohol + \beta_5 Smoking + \beta_6 BMI \quad (1)$$

where Y_1 is T2D (Y_1=1 if T2D) and SNP_k is the genotype at the k^{th} SNP.

The similar procedure was performed for the multiple linear regression analysis (2) of each SNP with the AAO of T2D as a continuous outcome.

$$Y = \beta_0 + \beta_1 SNP_k + \beta_2 Sex + \beta_3 Alcohol + \beta_4 Smoking + \beta_5 BMI \quad (2)$$

where Y is AAO of T2D and SNP_k is the genotype at the k^{th} SNP.

Multiple testing

Bonferroni correction ($\alpha=0.05/22=2.27\times10^{-3}$) was used for statistical significance [42]. In addition to obtain nominal Type I error rate, empirical *p*-values were generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK software. The corrected values for multiple testing (corrected empirical *p*-values) were then calculated.

Cox proportional hazards model

The Cox proportional hazards model (3) or Cox regression model [43], is widely used in the analysis of time-to-event data [44-46].

$$h(t|x) = h_0(t)\exp(\beta_1 SNP_k + \beta_2 Sex + \beta_3 Alcohol + \beta_4 Smoking + \beta_5 BMI) \quad (3)$$

where $h(t|x)$ is the hazard at time t for a subject, $h_0(t)$ is the baseline hazard function. Then the hazard ratio (HR) is defined as the ratio of the predicated hazard function under two different values of a predictor variable. The PHREG procedure in SAS fits the Cox model by maximizing the partial likelihood function.

Parametric survival models

Several commonly used parametric distributions in survival models include exponential, Weibull, gamma, log-normal, and log-logistic [44-47].

$$\ln(T) = \beta_0 + \beta_1 SNP_k + \beta_2 Sex + \beta_3 Alcohol + \beta_4 Smoking + \beta_5 BMI + ln(\varepsilon) \quad (4)$$

where is *T* the time to event; $ln(\varepsilon)$ is the natural log of the error term. The exponentials of the β coefficients may be interpreted as the time ratio (TR) [45-46,48]. If TR >1, the event is less likely to occur; whereas if TR <1, the event is more likely to happen.

The LIFEREG procedure in SAS fits parametric survival models, where the link function can be taken from a class of distributions that include exponential, Weibull, log-normal, log-logistic, and gamma distributions.

Supremum test for proportional hazards assumption

Both the graphical and numerical methods [49] were used to check the proportional hazards assumption in the ASSESS option of PROC PHREG. These methods are based on cumulative sums of martingale residuals over follow-up times or covariate values. The ASSESS option plots the cumulative score residuals against time for each independent variable and the RESAMPLE option computes the *p*-value of a Kolmogorov-type supremum test based on a sample of 1,000 simulated residual patterns. A significant p-value indicates a poor fit. The parametric Weibull and the exponential regression models share the assumption of proportional hazards with the Cox regression model [50].

Evaluation criteria for goodness of fit

The Akaike information criterion (AIC) statistic [51-52] and the Bayesian information criterion (BIC) statistic [53] were used to measure the goodness of model fit and compare survival models.

$$AIC = -2\ln\{p(x|\hat{\theta})\}+2k \qquad (5)$$

and

$$BIC = -2\ln\{p(x|\hat{\theta})\}+k\ln n \qquad (6)$$

where x is the random variable, $\hat{\theta}$ is the maximum likelihood estimate, k is the number of parameters, and n is the sample size. Smaller AIC and/or BIC indicate a better model fit.

Survival analysis of AAO of T2D

The PHREG procedure in SAS was used to fit the Cox model; while the LIFEREG procedure was used to fit parametric survival models including the exponential, Weibull, log-normal, log-logistic, and gamma distributions. Multivariate Cox regression analysis and parametric survival analyses were conducted to detect associations of each SNP with AAO adjusting for gender, alcohol use in the past month, BMI and smoking status, respectively. The AIC and BIC values were used to compare the Cox regression and parametric survival models [45, 54-57]. Descriptive statistics, Cox regression, and parametric models analyses were conducted with SAS v.9.4 (SAS Institute, Cary, NC, USA). SAS codes are listed in Appendix.

Linkage disequilibrium and Haplotype block

To examine the relationships among the SNPs within the EXT2 gene, the pairwise linkage disequilibrium (LD) statistics (r^2) based on the HapMap data were calculated in the HAPLOVIEW software [58]. Haplotype blocks were built with stringent criteria, that SNPs within each block have strong LD with each other, sometimes resulting in splitting of visually recognized blocks.

Results

Descriptive statistics and genotype quality control

The demographic characteristics of the subjects are presented in Table 1. There were slightly more females than males in both cases and controls. The age ranged from 46 to 90 years and AAO of T2D ranged from 26 to 90 years. Two SNPs with MAF<5% were removed and all the left 20 SNPs were in Hardy-Weinberg equilibrium in the controls (p>0.05).

Table 1. Descriptive characteristics of cases and controls.

	Non-Diabetes	Type 2 Diabetes
Number Sex, N (%)	2686	878
Males	1051(39%)	424(48%)
Females	1635(61%)	454(52%)
BMI, kg/m^2		
Mean ± SD	28.8±5.2	32.4±6.7
Range	16.1-61.3	16.8-64.4
Alcohol, N (%)		
No	930(35%)	418(48%)
Yes	1752(65%)	456(52%)
Smoking, N (%)		
Never	1405(52%)	327(47%)
Current	245(9%)	54(7%)
Past	1032(39%)	331(46%)
Age, years		
Mean ± SD	65.4±11.4	69.2±10.6
Range	46-90	46-90
AAO, years		
Mean ± SD	-	62.6±11.8
Range	-	26-90

Table 2. SNPs associated with risk and/or AAO of T2D(p<0.05).

SNP	Position	Allele[a]	MAF[b]	HWE[c]	OR-Diabetes[d]	p-Diabetes[e]	EMP2[f]	β-AAO[g]	p-AAO[h]	EMP2[i]
rs7111879	44090717	G	0.45	0.698	0.85(0.75,0.95)	6.33E-3	0.124	1.22(0.10, 2.33)	3.26E-2	0.515
rs4237646	44093796	G	0.26	0.352	0.87(0.74,1.04)	0.12	0.931	3.18(1.64,4.72)	5.79E-5	3.00E-3
rs4755230	44140920	G	0.27	0.312	0.91(0.77,1.07)	0.238	0.995	3.19(1.71,4.67)	2.76E-5	1.00E-3

[a] Minor allele; [b] Minor allele frequency; [c] Hardy-Weinberg equilibrium test p-value; [d] Odds ratio for diabetes based on multiple logistic regression; [e] p-value based on logistic regression; [f] Corrected empirical p-value generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK; [g] Regression coefficient for AAO based on multiple linear regression; [h] p-value based on linear regression; [i] Corrected empirical p-value generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK.

Table 3. Results of the Cox regression and parametric models in survival analysis of AAO of T2D.

Models	AIC[a]	Rank	BIC[b]	Rank	AIC[c]	Rank	BIC[d]	Rank	AIC[e]	Rank	BIC[f]	Rank
Cox	100336.1	6	10067	6	10009.3	6	10056.7	6	10010.7	6	10044.1	6
Weibull	6659.8	2	6702.7	2	6648.5	2	6691.5	2	6641.5	2	6684.4	2
Exponential	8969.3	5	9007.5	5	8968.9	5	9007.2	5	8959.2	5	8997.3	5
Log-logistic	6723.2	4	6766.1	4	6711.5	3	6754.4	3	6703.9	3	6746.9	3
Log-normal	6722.9	3	6765.8	3	6712.9	4	6755.8	4	6705.9	4	6748.8	4
Gamma	6653.8	1	6701.3	1	66642.6	1	6690.3	1	6635.5	1	6683.2	1

[a] AIC for rs7111879 adjusted for sex, alcohol use, smoking status, and BMI; [b] BIC for rs7111879 adjusted for sex, alcohol use, smoking status, and BMI; [c] AIC for rs4237646 adjusted for sex, alcohol use, smoking status, and BMI; [d] BIC for rs4237646 adjusted for sex, alcohol use, smoking status, and BMI; [e] AIC for rs4755230 adjusted for sex, alcohol use, smoking status, and BMI; [f] BIC for rs4755230 adjusted for sex, alcohol use, smoking status, and BMI.

Multiple linear and logistic regression analyses using PLINK

We found that 1 SNP associated with risk of T2D (rs7111879 with p=6.33 x 10^{-3}) and 3 SNPs associated with AAO (rs7111879, rs42376464 and rs4755230 with p=3.26 x 10^{-2}, 5.79 x 10^{-5} and 2.76 x 10^{-5}, respectively) (Table 2). Interestingly, the same SNP rs7111879 showed associations with both the risk and AAO of T2D. However, the associations of rs7111879 with risk and AAO were not significant after a Bonferroni correction (p>2.27x10^{-3}) or multiple testing correction using a permutation test (corrected p>0.05). The results of other 2 AAO associated SNPs (rs42376464 and rs4755230) remained significant after a Bonferroni correction (p<2.27x10^{-3}) and multiple testing correction using a permutation test (corrected p=3.0x10^{-3} and 1.0x10^{-3}, respectively).

Comparison of Cox Regression and Parametric Models using PROC PHREG and PROC LIFEREG

Table 3 shows the comparisons through AIC and BIC for the 6 types of models of the 3 SNPs associated with AAO (p<0.05). Overall, gamma distribution demonstrated the best model fit for all 20 SNPs, followed by the Weibull distribution. For example, rs4755230, the gamma distribution has the smallest AIC (AIC=6635.5), and the AIC for Weibull distribution is slightly larger (AIC=6641.5). BIC also indicated that Gamma (BIC=6683.2) and Weibull (BIC=6684.4) distribution had a similar fit and outperformed the rest models.

Supremum test for proportional hazards assumption

Figures 1 and 2 display the observed standardized score process with 20 simulated realizations from the null distribution for rs4755230 AA and AG genotypes, respectively. The plots showed that the observed process is atypical compared to the simulated realizations and revealed proportional hazards for the two genotypes compared with GG. The Kolmogorov-type supremum test results based on 1,000 simulations for all the covariates are shown in Table 4. The proportional hazards assumption was valid for all the variables (p>0.05).

Survival analysis of AAO using Cox regression, gamma and Weibull models

The results based on the Cox regression and parametric survival analyses using gamma and Weibull models are presented in Table 5. All the HR values for 3 SNPs are larger than 1 while all the TR values are smaller than 1. For example, the genotype AA of rs4755230 has HR=1.63, which indicates that the participant with AA has a 63% higher hazard rate of AAO than participant with GG. The TR using Weibull model is 0.93, which indicates that the participant with AA has a shortened AAO by 7% compared to participant with GG. In addition, the mean AAO was approximately 5.7 years earlier in the individuals who had two major allele (AA) of rs4755230 (mean AAO=61.6 years) compared with those who were homozygous for the minor allele (GG) (mean AAO = 67.3 years.

Figure 1. Explore plot for checking proportional hazards assumption for rs4755230AA compared with rs4755230GG.

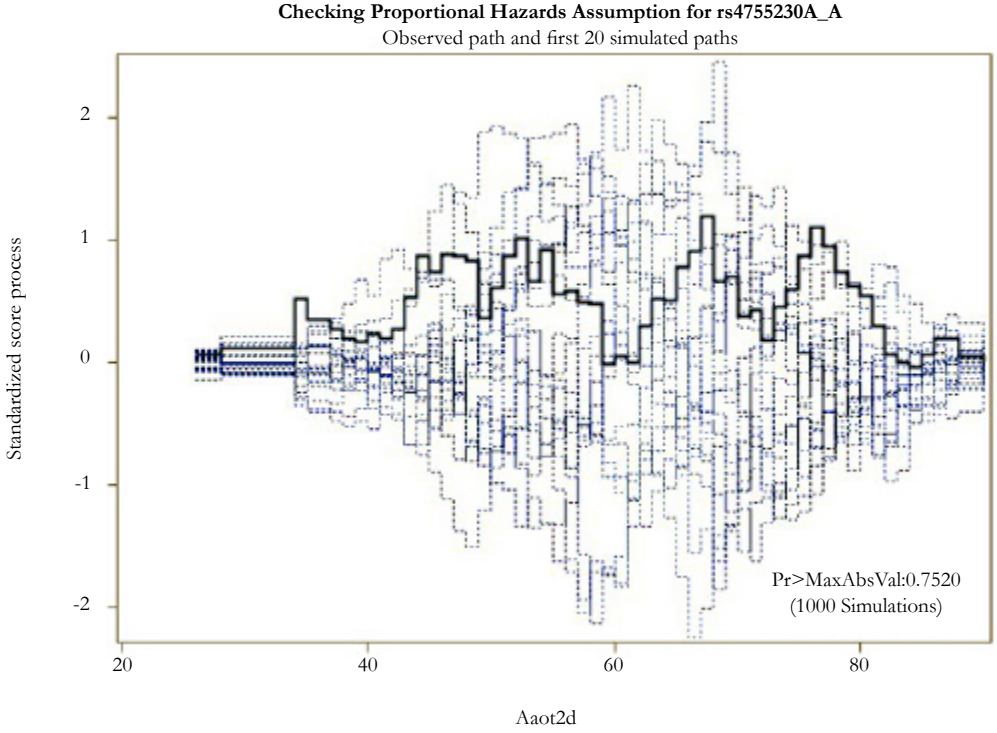
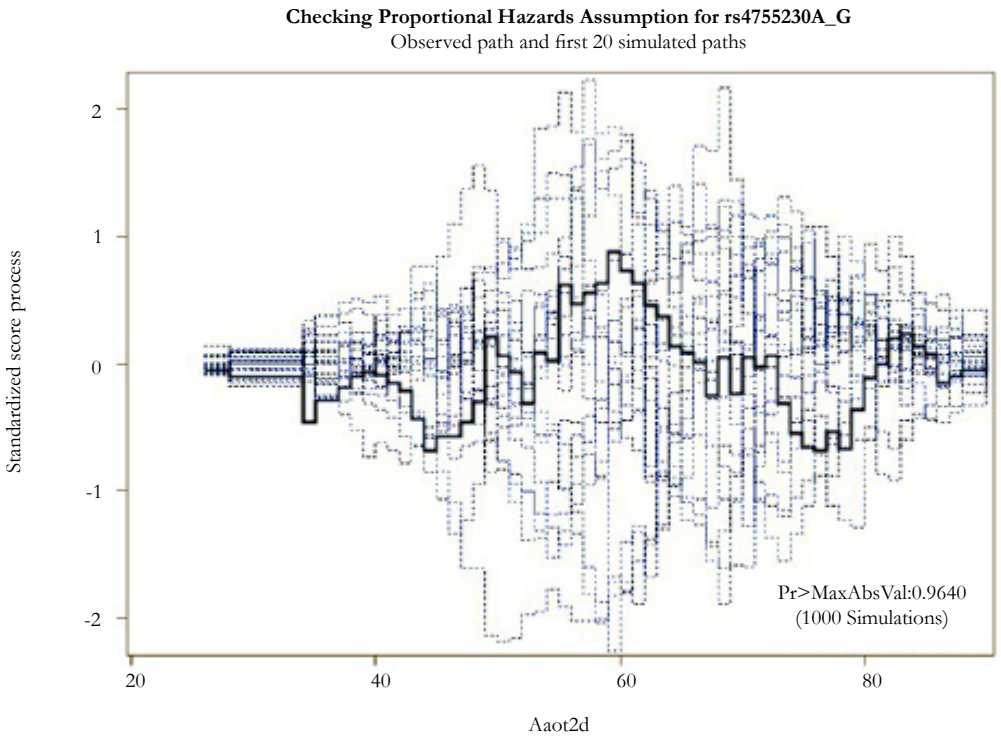


Figure 2. Explore plot for checking proportional hazards assumption for rs4755230AG compared with rs4755230GG.



Linkage disequilibrium and haplotype block

All SNPs including three previous identified T2D associated SNPs (rs3740878, rs1113132 and rs11037909) were within a haplotype block (Figure 3).

Discussion

To our knowledge, this is the first application to evaluate the Cox regression and 5 parametric survival models in genetic association analysis of the AAO of T2D. It is also the first candidate gene study to examine the associations of EXT2 gene polymorphisms with the AAO of T2D. In the procedure of model selection, the parametric gamma model outperformed the other models in the genetic association of the AAO of T2D, meanwhile we identified 1 SNP (rs7111879) associated with the risk of T2D and 3 SNPs (rs7111879, rs42376464 and rs4755230) associated with the AAO of T2D.

Table 4. Supremum Test for Proportional Hazards Assumption.

Variables	Maximum Absolute Value[a]	Replications	Seed	Pr > MaxAbsVal[b]
Sex	1.139	1000	1000	0.141
BMI	1.313	1000	1000	0.053
Alcohol	0.367	1000	1000	0.989
Smoking1	0.445	1000	1000	0.967
Smoking2	0.56	1000	1000	0.776
rs4755230AA	1.187	1000	1000	0.752
rs4755230AG	0.88	1000	1000	0.964

[a] Maximum absolute value based on the supremum test for proportional hazards assumption; [b]The p-value for the supremum test for proportional hazards assumption.

Table 5. Survival Analysis of 3 SNPs Associated with AAO using the Cox regression, gamma and Weibull models.

SNP	GT[a]	β(SE)[b]	p[c]	HR(95%CI)[d]	β(SE)[e]	p[f]	TR(95%CI)[g]	β(SE)[h]	p[i]	TR(95%CI)[j]
rs7111879										
	AA	0.206(0.103)	0.045	1.23(1.01,1.50)	-0.033(0.016)	0.038	0.97(0.94,0.99)	-0.036(0.017)	0.029	0.96(0.93,0.99)
	AG	0.118(0.095)	0.217	1.13(0.93,1.36)	-0.018(0.015)	0.225	0.98(0.95,1.01)	-0.018(0.015)	0.246	0.98(0.95,1.01)
	GG			1			1			1
rs42376464										
	TT	0.498(0.143)	0.0005	1.64(1.24,2.18)	-0.079(0.022)	0.0003	0.92(0.89,0.96)	-0.085(0.023)	0.0002	0.92(0.88,0.96)
	GT	0.374(0.146)	0.011	1.45(1.09,1.94)	-0.059(0.022)	0.008	0.94(0.90,0.98)	-0.064(0.024)	0.007	0.94(0.90,0.98)
	GG			1			1			1
rs4755230										
	AA	0.487(0.137)	0.0004	1.63(1.24,2.13)	-0.078(0.021)	0.0002	0.93(0.89,0.96)	-0.084(0.022)	0.0001	0.92(0.88,0.96)
	AG	0.386(0.140)	0.006	1.47(1.12,1.94)	-0.061(0.022)	0.005	0.94(0.90,0.98)	-0.066(0.023)	0.004	0.94(0.90,0.98)
	GG			1			1			1

[a] Genotype; [b] Regression coefficient and standard error (SE) based on the Cox regression; [c] p-value based on the Cox regression; [d] Hazard ratio (HR) and 95% confident interval (CI) based on the Cox regression; [e] Regression coefficient and SE based on the Weibull model; [f] p-value based on the Weibull model; [g]Time ratio (TR) and 95%CI based on the Weibull model; [h] Regression coefficient and SE based on the gamma distribution; [i] p-value based on the gamma model; [j] Time ratio (TR) and 95%CI based on the gamma model.

Cox regression and Weibull model have been extensively used to analyze incident diabetes [11-17] while non-parametric methods such as the Mann-Whitney and the Kruskall-Wallis tests [20], multiple linear regression [18] and Cox model [19] have been used to examine the associations of genetic variants with the AAO of T2D. However, no study was found to compare the Cox regression and parametric survival models in genetic association analysis of the AAO of T2D. Our results provided the first empirical evidence of the model comparisons in genetic studies of the AAO of T2D and showed that the parametric gamma and Weibull models performed better compared to Cox regression. It has been reported that the Weibull model shares the assumption of proportional hazards with the Cox regression model [50]. Particularly, if the assumption is met, then Weibull distribution provides an alternative, fully parametric approach to the Cox model, while if violated, other parametric models can be used with distributions rather than Weibull distribution [45]. In the present study, both the graphic and numeric methods in ASSESS statement in PROC PHREG showed that the assumption of proportional hazards is met. Therefore, Weibull model will be the first choice for genetic association study of AAO of T2D. In addition, the Weibull distribution provides similar HR estimates to Cox model; whereas a key strength of Weibull model allows the simultaneous estimates of treatment effects in terms of both HR and TR, which may lead to increase or decrease in survival time [59]. The survival function could be assumed in a certain form such as exponential, Weibull, and so on, with one or more parameters whose values are unknown, to be estimated from the real data [44]. Furthermore, if the shape of the survival distribution is known, parametric regression models may produce more efficient estimates than Cox model [60].

T2D is caused from the insulin resistance and β-cell dysfunction in the pancreas [61-63]. EXT2 gene is involved in the synthesis of heparin sulphate, abnormal bone growths (exostoses) [64] and in neural development [65]. The associations between three SNPs (rs3740878, rs1113132, rs11037909) in this gene and the risk of T2D have been previously reported in several studies [26, 35-36] but was not replicated in other studies [27-34]. Recently, the rs3740878 risk T allele was found to be nominally associated with reduced insulin secretion in carriers of the high-risk genotype compared with those with the low-risk genotype [29]; while two SNPs (rs3740878 and rs1113132) were associated with several measures of insulin resistance such as glucose and insulin levels in Pima Indians [66]. Furthermore, the EXT2 was found to have increased expression in brain, which suggested a possible site of action as to where this gene could affect diabetes risk [63]. A more

Figure 3. Linkage disequilibrium structure (r^2) within the EXT2 gene using the HapMap data.

recent study provided the first evidence on the relation between genetic defects in heparan sulfate synthesis and decreased pancreas anatomic volume with ensuing impaired beta-cell reserve capacity in carriers of loss-of-function mutations in EXT [67]. In the present study, we showed the first evidence of rs7111879 associated with the risk of T2D and 3 SNPs strongly associated with the AAO of T2D. Furthermore, the T2D associated SNP rs1113132 identified in the Bernelot Moens et al. study [67] also showed borderline association with the risk of T2D (p=0.0903) (data not shown). Additionally, three SNPs (rs3814767, rs7935138 and rs4379834) revealed nominal associations with the risk of T2D (p=0.0552, 0.0836 and 0.0835, respectively) (data not shown). However, the other two previously associated SNPs (rs3740878 and rs11037909) were not available in the Marshfield dataset. To examine the relationship among the SNPs within the EXT2 gene, we identified a haplotype block for 22 SNPs including rs3740878 and rs11037909 using the Hapmap data (Figure 3). We found that the three previously T2D risk associated SNPs (rs3740878, rs1113132, rs11037909) also had strong LD with the three nominal associated SNPs (rs3814767, rs7935138 and rs4379834 with r^2=0.78, 0.72, and 0.89, respectively) in the present study. Our results support a role of EXT2 in the development of T2D.

EXT2 is considered as a putative tumor suppressor gene and is associated with hereditary multiple exostoses, which is an autosomal dominant condition characterized by growth of multiple benign cartilage-capped tumors [22-23, 25, 68]. Recently, a gene expression study showed that the EXT2 gene activity in benign prostatic hyperplasia and prostate tumors was lower than that in normal prostate tissue [69]. Considering the comorbidity of T2D with multiple cancers such as endometrial and prostate cancers [4-8], it may be hypothesized that EXT2 gene may be involved in the pathogenesis of T2D and several cancers.

There are several strengths in this study. First, this study simultaneously demonstrated the performance of the semi-parametric Cox regression and five different parametric survival models in genetic association of the AAO of T2D with real data. Second, we examined 22 SNPs within the EXT2 gene and especially identified several genetic variants associated with the risk and AAO of T2D. Several limitations also need to be acknowledged. First, only one sample was used to examine the association of EXT2 gene with the risk of T2D due to limited data resources. Second, our current findings might be subject to type I error and need to be replicated in future studies.

Conclusions

The present study reveals that parametric gamma and Weibull models performed better than semi-parametric Cox proportional hazards model and other parametric models (including exponential, log-normal, and log-logistic) in the genetic association of the AAO of T2D. Furthermore, this is the first candidate gene study which investigates the associations between EXT2 SNPs and the AAO of T2D. These findings may serve as a resource for replication in other populations for future research on target genetic variation and the risk of T2D. Further functional study of the EXT2 gene will also help to better characterize the genetic basis of the risk and AAO of T2D.

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