Analysis of Gene-treatment Interaction for Age-related Macular Degeneration Patients Using Survival analysis – evidence from Age-Related Eye Disease Study 2

Qiaohui Zhou*[1]

†Biostatistics Department, Georgetown University
†3700 O St NW, Washington, DC 20057
†qz111@georgetown.edu

December 13, 2019

Abstract

Age-related macular degeneration (AMD) is a progressive retinal disease, which in the worst scenario, can lead to blindness and has become an enormous burden to the developed world. Previous researches showed that the progression of AMD can be slowed down through dietary modification. Several studies attempted to identify the interactive effect between genotype and dietary supplements. However, the evidence was later reversed by other research groups. In this study, we apply the frailty model to the AREDS2 (Age-Related Eye Disease Study 2) dataset to investigate the interactive effect between modified dietary supplements and the biomarkers rs3766405 and rs412852 of CFH. The results from our survival analysis suggest that the interactive effects are insignificant.

*The project is completed under the instruction of Professor Ruzong Fan.

1 Introduction

Age-related macular degeneration (AMD) is a progressive retinal disease, which in the worst scenario can lead to blindness and has become an enormous burden to the developed world. Previous researches showed that the progression of AMD can be slowed down through dietary modification. For example, according to findings from Age-Related Eye Disease Study (AREDS), the combination of high-does supplement of antioxidants and zinc helps to reduce progression to late AMD by 25% over a course of 5 years (Age-Related Eye Disease Study Research Group, 2001). However, whether differential response to the dietary supplements exists or not between different genetic subgroups remains controversial.

Several studies attempted to identify the interactive effect between genotype and dietary supplements. For example, Awh et al. (2013) found treatment of zinc and antioxidants was associated with 2 complement factor H (CFH) risk alleles with RR=1.83 (p=1.03*10-2) as compared to no CFH risk alleles. Treatment effect of antioxidants interacting with 1 age related maculopathy susceptibility 2 (ARMS2) risk alleles is 2.58, and is 3.96 if interacting with 2 ARMS2. Similarly, the protective effect was found among antioxidant and zinc supplement users if they carry high-risk ARMS2(TT) carriers (HR:0.52, 95%CI=(0.55,0.82)) (Seddon, Silver and Rosner, 2016). However, the evidence was latter reversed by other research groups. Three separate statistical teams reanalysed the data from AREDS and conclude that there were errors in previous studies in favour of the existence of interaction (Assel et al., 2018).

Whether the interactive effect exists or not has an important implication in practise. The significant statistical finding may serve as an excuse for profit-driven companies who may advise their patients to conduct gene test before recommending any dietary supplements. This study aims to add further evidence to the camp against the existence of interaction.

AREDS2 was a randomized control trail reassessing the original AREDS formula on AMD progression. In this study, we apply the bivariate frailty model to the AREDS2 dataset to investigate the interactive effect between modified dietary supplements involved in AREDS2 (adding lutein/zeaxanthin and/or omega-3 fatty acids, lowering the zinc dosage from 80mg to 25 mg, or eliminating beta-carotene) and the biomarkers rs3766405 and rs412852 of CFH.The chosen biomarkers were shown to have significant associations to the risk of developing the eye disease in previous literature.

2 Statistical model

Suppose the sample includes N patients. For patient i, let T_{i1} be the time to advanced AMD of the left eye and T_{i2} be the time to advanced AMD of the right eye. Assuming multiplicativity of the frailties and a proportional hazards model, the data for subject i's j eye ($i = 1, \ldots, N; j = 1, 2$) can has the form:

$$\lambda_{ij}(t|v_j) = \lambda_{0j}(t)e^{x_i\beta + s_i\gamma + g_i\mu + s_i g_i \tau + G_{ij}}. \quad (1)$$

where λ_{0j} is the baseline hazard functions for the j eye, x_i is the vector of the demographic characteristics of subject i: gender, age, educational level (high school or less, at least some college, post-graduate), smoking habits(never, past current), s_i is a is the indicator of whether the subject takes the dietary supplement of interest, g_i is the indicator of whether the subject possesses the biomarker of interest, and finally, $s_i g_i$ is the interactive term of dietary supplement and the biomarker, and the main interest of this study, and G_{ij} is a Gaussian random variable with mean ρ and variance σ^2. The random variable G_{ij} models the correlation between the times to advanced AMD of two eyes.

3 Population, measure of progression, and genotyping

4203 participants were enrolled in the randomized clinical trial whom had either bilateral large drusen or late AMD in one eye. The study spans for up to 6 years from 2006 to 2016. In contrary to AREDS, AREDS2 modifies the treatment formula by adding lutein/zeaxanthin and/or omega-3 fatty acids, lowering zinc dosage from 80mg to 25mg or eliminating beta-carotene. If not receiving the modified version of supplement, participants were still offered with original supplement. For the sake of study, only white AREDS2 participants (n=1684) who had eyes without AMD at baseline were analysed (n=2775).

In tracking the progression of the disease, participants received eye examinations and stereoscopic fundus annually from the time they entered the study. 6-month interim telephone calls were also made to keep the history of treatment for AMD up-to-date. Late AMD is determined when neovas-

cular AMD or of any GA occurs. The participants were genotyped using a custom Illymina HumanCoreEzome arrays as part of the International AMD Genomics Consortium. In resonant to the previous study, risk rs3766405 and rs412852 in CFH gene were selected to test for the supplement interaction in this study. Additivity of the risk alleles were assumed.

4 Data analysis

Assuming a Gaussian distribution for frailty, we can use the survival package **coxph()** function, specifying a gaussian distribution in the argument to perform the model fit, then run chi-square test to test for the significance of interaction.

By including the random effect frailty term, we are able to assess the progression to all types of late AMD on eye-level, accounting for the correlation between eyes within the same individual. The model not only includes the treatment factor, the genotype indicators and the interaction term, it also adjusted for demographic factor related to AMD progression, as mentioned above.

The event occurs if late AMD is diagnosed. Since two different symptoms draws the same conclusion (neovascular AMD and GA), in addition to assess the event of late AMD, we also consider the event to be neovascular AMD and GA and conducted separate analysis.

The effect size is measured by the hazard ratio with 95% CI, the statistical significance is subjected to Bonferroni correction – the p-value is 0.05/30=0.0017, accounted for the multiple testing.

5 Results

The interactive effect is shown in table 1. There was no interaction between either genotypes with any kinds of dietary supplement at 5% significance level, with respect to developing late AMD, neovascular AMD or any geographic atrophy.

The interactive effects across different genotype subgroups are also statistically insignificant (shown in table 2). The only exception exists for the CFH rs412852, where beta-carotene exhibits significant protective effect if one risk

Table 1: Interaction results for genotype and treatment arm

	Late AMD			Neovascular AMD			Any geographic		
	HR	95%-CI	p-value for interaction	HR	95%-CI	p-value for interaction	atrophy	95%-CI	p-value for interaction
CFH_rs3766405									
Lutein/zeaxanthin	0.821	(0.604-1.115)	0.206	0.835	(0.585-1.191)	0.319	0.805	(0.544-1.191)	0.278
Omega-3 fatty acids	1.052	(0.772-1.433)	0.748	1.015	(0.710-1.452)	0.933	0.892	(0.602-1.322)	0.569
Low vs. high zinc	1.213	(0.855-1.720)	0.280	1.154	(0.769-1.731)	0.489	1.189	(0.753-1.876)	0.457
Beta-carotene	1.073	(0.742-1.552)	0.709	1.297	(0.846-1.989)	0.233	0.821	(0.503-1.340)	0.430
Lutein vs. beta-carotene	0.941	(0.566-1.563)	0.814	0.840	(0.469-1.505)	0.558	0.994	(0.505-1.959)	0.987
CFH_rs412852									
Lutein/zeaxanthin	0.854	(0.665-1.096)	0.215	0.865	(0.646-1.160)	0.333	0.886	(0.647-1.214)	0.453
Omega-3 fatty acids	0.953	(0.742-1.224)	0.708	0.841	(0.628-1.128)	0.248	1.040	(0.759-1.425)	0.809
Low vs. high zinc	1.154	(0.868-1.534)	0.323	1.025	(0.732-1.435)	0.886	1.292	(0.900-1.855)	0.165
Beta-carotene	1.117	(0.828-1.507)	0.467	1.247	(0.874-1.780)	0.223	0.892	(0.610-1.304)	0.554
Lutein vs. beta-carotene	0.972	(0.647-1.459)	0.890	0.827	(0.513-1.335)	0.437	1.235	(0.737-2.072)	0.423

A hazard ratio for interaction lower than 1 means that the effect of the treatment becomes more beneficial with higher genetic risk.

allele is presented (1.306 (1.037-1.643), p=0.023). However, it doesn't remain significant after Bonferroni correction.

Table 2: The effect of dietary supplements in strata

		Late AMD			Neovascular AMD			Any geographic		
	CFH_rs412852	HR	95%-CI	p-value	HR	95%-CI	p-value	HR	95%-CI	p-value
Beta-carotene	0	1.168	(0.870-1.569)	0.302	1.032	(0.721-1.477)	0.862	1.180	(0.820-1.700)	0.373
	1	1.306	(1.037-1.643)	0.023	1.288	(0.983-1.686)	0.066	1.052	(0.784-1.413)	0.734
	2	1.459	(0.935-2.276)	0.096	1.606	(0.955-2.701)	0.074	0.938	(0.528-1.666)	0.828

A hazard ratio for interaction lower than 1 means that the effect of the treatment becomes more beneficial with higher genetic risk.
AMD = age-related macular degeneration; HR = Hazard Ratio; CI = confidence interval.

6 Discussion and conclusion

This study collects data from AREDS2 to assess whether interactive effects exist between genotype and dietary supplements, using frailty model. The dietary supplements include lutein/zeaxanthin omega-3 fatty acid, low vs. high zinc, beta-carotene and lutein vs. beta-carotene. We consider the interaction between these supplements and the rs3766405 and rs412852 genotypes. Except for beta-carotene with rs412852, where statistical significance presents with one risk allele. This statistical significance does not hold when we conduct the Bonferroni correction.

Overall, we conclude that the interaction between different dietary supplements and the genotype doesn't exist. This fact remains when we conduct the analysis according to genotype subgroups.

7 Acknowledgement

The project is completed under the guidance of Professor Ruzong Fan from the Biostatistics Department of Georgetown University. The data is collected from the Age-Related Eye Disease Study 2, a major clinical trail sponsored by the National Eye Institute, one of the federal government's National Institutes of Health.

References

1. Age-Related Eye Disease Study Research Group (2001). A Randomized, Placebo-Controlled, Clinical Trial of High-Dose Supplementation With Vitamins C and E and Beta Carotene for Age-Related Cataract and Vision Loss. Archives of Ophthalmology, 119(10), p.1439.

2. Assel, M., Li, F., Wang, Y., Allen, A., Baggerly, K. and Vickers, A. (2018). Genetic Polymorphisms of CFH and ARMS2 Do Not Predict Response to Antioxidants and Zinc in Patients with Age-Related Macular Degeneration. Ophthalmology, 125(3), pp.391-397.

3. Awh, C., Lane, A., Hawken, S., Zanke, B. and Kim, I. (2013). CFH and ARMS2 Genetic Polymorphisms Predict Response to Antioxidants and Zinc in Patients with Age-related Macular Degeneration. Ophthalmology, 120(11), pp.2317-2323.

4. Seddon, J., Silver, R. and Rosner, B. (2016). Response to AREDS supplements according to genetic factors: survival analysis approach using the eye as the unit of analysis. British Journal of Ophthalmology, 100(12), pp.1731-1737.