FOXO1A-209-by-Tea-Drinking Interaction

is Significantly Associated with Reduced Mortality Risk at Advanced Ages

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Acknowledgements: This study is supported by the National Natural Science Foundation of China (71233001) (YZ), National Key Basic Research Program of China (2013CB530700) (XLT), NIA/NIH (R01 AG023627) (YZ), NIH Pepper Center Grant (P30AG028716) (ERH), NIA/NIH (R01AG027060 and R01AG038707) (BW and CW). We thank Huiqing Cao, Wen-Jing Wang, Chong Wu, Fang-Yuan Hu, Jian Guo, Ling Zhao, Fan Yang, Yi-Xin Zhang, Wei Li, and Gu-Yan Zheng for their efforts in the lab work on DNA extractions and genotyping. We are grateful to Zhenyu Xiao, Yuzhi Liu, Chunyuan Zhang, Liqun Tao, Qin Xu, Ye Yuan, Jie Zhan, and Yun Zhou for their contributions in the Chinese Longitudinal Healthy Longevity Survey and the DNA samples collection, and we thank all interviewees and their families for their voluntary participation in this study.

Abstract

Proportional hazards model analysis based on data from 2,481 Han Chinese oldest-old aged 91+ demonstrated that interactions between carrying FOXO1A-209 genotype and tea-drinking are significantly associated with lower risk of mortality at advanced ages. The significant association is replicated in two independent CLHLS cohorts(p=0.018-0.048), and adjusted p-values for multiple comparisons by the Bonferroni method are 0.006-0.032 in combined dataset of two CLHLS cohorts. The results demonstrate that associations between tea-drinking and reduced mortality are much stronger among carriers of the genotypes of FOXO1A-209 compared to non-carriers. Based on previous research showing that intake of tea compounds activate FOXO1A gene expression and modulate its biological functions, we speculate that results in present study indicate that tea-drinking activates FOXO1A-209 gene expression which offers protection against mortality risk at oldest-old ages. Our empirical findings imply that health outcome of particular nutritional interventions, including tea drinking, may, in part, depend upon individual genetic profiles.

Key Words:

Aging, FOXO genotypes, tea-drinking, GxE interactions, mortality risk.

1. INTRODUCTION

Our prior study on which the current bio-demographic analysis is based, showed that two SNPs of the FOXO1A gene, located on chromosome 13, were

associated with longevity in the Han Chinese population (Li et al., 2009). FOXO1A genetic variants have also been linked to longevity in the Framingham cohort in the U.S. (Lunetta et al., 2007) and in the Leiden 85-plus study (Kuningas et al., 2007). Research also have found that the FOXO3A gene, located on chromosome 6, is associated with human longevity in Japanese-Americans from Hawaii (Willcox et al., 2008), Italians (Anselmi et al., 2009), Ashkenazi Jews, Californians and New Englanders (Pawlikowska et al., 2009), Germans (Flachsbart et al., 2009), and Han Chinese (Li et al., 2009).

Accumulating data from various studies indicates that tea drinking improves health and reduces the risk of mortality and age-associated chronic diseases, such as stroke (Arab et al., 2009), and effectively shuts down high mobility group box-1 (HMGB1)-induced inflammation in a life-saving fashion (Eagleton, 2014). Tea drinking also reduces depressive symptoms in old adults in rural China (Feng at al., 2013), and is associated with better cognitive function in Chinese elderly in Singapore (Feng et al., 2010) and in the oldest-old in China (Feng et al., 2012).

There is also accumulating evidence indicating that compounds in tea can increase lifespan in animal models (Kitani et al,, 2007; Abbas and Wink, 2009; Peng et al., 2009; Unno et al., 2011). Longitudinal or cohort studies from Japan reported that tea consumption was associated with reduced risk of mortality, death of cardiovascular diseases (Kuriyama et al., 2006; Suzuki et al., 2009), and death caused by cancer (Nakachi et al., 2003). Our recent study also showed that tea drinking was significantly associated with reduced mortality among the Chinese oldest-old aged 80+ (Ruan et al. 2013).

Various studies have shown that interactions between genotypes and environmental factors (GxE) play a crucial role in health outcomes (IOM, 2006; Guo et al., 2008), as environmental factors may activate or regulate gene expression, which

then influence health and longevity (e.g., Tsankova et al., 2007). Based on intensive evaluations and discussions, the Institute of Medicine (IOM) Committee recommends in their widely-cited report: "…to expand our knowledge of how to improve the health of individuals and populations, it becomes imperative to conduct research that explores the effects of interactions among social, behavioral and genetic factors on health" (IOM, 2006).

Previous studies have shown that the effects of tea consumption on diseases vary by genotypes (Bonner et al., 2005; Yuan et al., 2005; Xu et al., 2007; Lin et al., 2012). Anton et al. (2007) discovered that the tea Epigallocatechin gallate (EGCG) mimics insulin action on the transcription factor FOXO1A and elicits cellular responses in the presence and absence of insulin, namely, the intake of tea compounds activates FOXO1A gene expression and modulates its biological functions. Recent prior research based on data from 822 Han Chinese oldest-old aged 91+ demonstrated that GxE interactions between carrying one of the single-nucleotide polymorphisms (SNPs) of FOXO1A-rs17630266, FOXO3A-rs2253310 or FOXO3A-rs2802292 and tea drinking were significantly associated with lower risks of cognitive disability at advanced ages (Zeng et al., 2014). Other research based on these data discovered that the GxE interaction between FOXO1A-rs2755209 genotype and regular exercise significantly reduces mortality risk at very advanced ages by 31-32 percent ($P<0.05$), adjusted for various covariates (Zeng et al., 2010). However, we are not aware of any studies of the effects of GxE interactions between the FOXO1A genotype and tea drinking on mortality in humans.

Given the important roles of the FOXO1A gene and tea polyphenols in the health outcomes of humans, the FOXO1A-by-tea interaction effects in animal models and human cells reported in the literature, the association of the two FOXO1A SNPs with longevity, and the effects of FOXO1A-209-by-regular exercise interaction on mortality

at advanced ages in China as reviewed above, we posed the research question to be explored in present study: Are the GxE interactions between carrying the FOXO1A genotype and tea drinking significantly associated with reduced mortality risk in the Chinese oldest-old?

Previous studies have indicated that, in general, genetic and GxE impacts on health and mortality are more profound at advanced ages (Hjelmborg et al., 2006; Jylhävä & Hurme, 2010; Tan et al., 2013). Also, the oldest-old population, which is more likely to need care assistance, has been increasing much more rapidly than any younger age groups in many countries, including China (Zeng et al., 2014). These facts imply that focusing on the oldest-old is a useful way to investigate GxE effects on healthy longevity. However, almost all previous studies in this field have focused on young-old and middle-aged adults and few studies have had large enough numbers of oldest-old subjects. The objective of the present study is to make contributions to this field based on reasonably large samples of oldest-old.

2. DATA SOURCES, MEASUREMENTS, AND METHODS

Data sources

Our analyses are based on genotypic and phenotypic data from two independent cohorts: (1) Cohort 1998 consisting of 810 oldest-old aged 91+ interviewed in the 1998 initial baseline survey of the Chinese Longitudinal Healthy Longevity Survey (CLHLS) and its subsequent follow-up surveys conducted in 2000, 2002, 2005, 2008-2009, 2011-2012; and 99.7 percent of them had died before the CLHLS 2011-2012 wave; data on their dates of death were collected; (2) Cohort 2008-2009 consisting of 1,671 oldest-old aged 91+ interviewed in the CLHLS 2008-2009

wave and its subsequent follow-up surveys conducted in 2011-2012, and 55.7 percent of them died before the CLHLS 2011-2012 wave; data on their dates of death were collected. The CLHLS longitudinal surveys have been conducted in a randomly selected half of the counties and cities in 23 out of 31 provinces in China, with replacement for deceased elders, namely, new participants were recruited in each of the waves from 1998 to 2008-2009 (Zeng et al., 2008). The 23 provinces where CLHLS was conducted cover about 85% of the total population of China. There is no overlap between the CLHLS participants of the 1998 cohort and the 2008-2009 cohort, and there is no familial-kinship relations among the participants within and across the cohorts. Thus, these two cohorts are totally independent study samples, and the Cohort 1998 and Cohort 2008-2009 will be used as discovery and replication dataset, respectively. All of the participants of these two cohorts belong to the same ethnic group of Han Chinese living in one country with the same culture, and thus we also performed a combined analysis including all members of Cohort 1998 and Cohort 2008-2009.

Extensive data were collected in CLHLS using internationally standardized questionnaires adapted to the Chinese cultural and social context. Careful evaluations, including reliability coefficients, factor analysis, and age reporting at the oldest-old ages, have shown that the data (including mortality) quality of CLHLS surveys is of reasonably high quality (Gu, 2008; Goodkind, 2009). All of the genotypic and phenotypic data used in this study are from participants who belong to the same ethnic group of Han Chinese in China.

The FOXO1A genotype data of Cohort 1998 were produced by Xiaoli Tian's lab at Peking University, and analyses of the genotypic data, including quality control procedures, single SNP association analysis, genotype association analysis, linkage disequilibrium and haplotype association analysis etc., were presented in Li et al. (Li et

al., 2009); thus we will not repeat them here. Li et al. (2009) found that the two SNPs rs2755209 and rs2755213 of the FOXO1A gene (abbreviated as FOXO1A-209 and FOXO1A-213 hereafter) were significantly associated with longevity. In this work we explore the effects of GxE interactions between one of these two FOXO1A SNPs and tea drinking on mortality at advanced ages.

The FOXO1A genotype data of Cohort 2008-2009 were taken from the CLHLS genome-wide association analysis (GWAS) dataset which was most recently produced by the Beijing Genomics Institute (BGI); quality control procedures of the CLHLS GWAS are presented in Appendix A of this article.

We briefly discuss the dependent variable, main independent variables and covariates below. The statistical frequency distributions of these variables are presented in Table 1.

--Table 1 about here--

Dependent variable: mortality risk

Mortality information on date of death was collected in the CLHLS follow-up surveys for participants who were interviewed in the CLHLS wave(s) but died afterwards, by interviewing a close family member of the deceased participants. Survival time of the subjects analyzed in this study was entered as days counted from the date of the initial interview in 1998 or 2008-2009 to the date of death or censored at the time of the 2010-2011 interview for those who were still alive, while we control for respondent's exact age in 1998 or 2008-2009 survey.

Main explanatory variable: FOX01A and tea drinking

We explore GxE interactions between tea drinking and the *FOXO1A genotype* (carrying the SNP of FOXO1A-209 or the SNP of FOXO1A-213) following the additive, recessive, and dominant models. In an additive model, a genotype that contains 0, 1 or 2 copies of the minor allele is coded as 0, 1 or 2. In a recessive model, a genotype that contains 2 copies of the minor allele is coded as 1; otherwise the genotype that does not contain or contain one copy of the minor allele is coded as 0. In a dominant model, any genotype that contains 1 or 2 copies of the minor allele is coded as 1; and otherwise the genotype that does not contain any copy of the minor allele is coded as 0.

Drinking tea. CLHLS respondents were asked questions: Do you drink tea regularly? Response categories were “almost every day”; “sometimes”; and “rarely or never”. We defined the “tea drinking” as a binary variable and as an ordered variable. The binary tea drinking variable is coded as 1 if the answer is “almost every day” or “sometimes”, and coded as 0 otherwise. The ordered tea drinking variable is coded as 0, 1 or 2 if the answer is “rarely or never”, “sometimes” or “almost every day”.

Other covariates

Other covariates controlled in our multiple statistical models include gender, age at time of the DNA sample collection, residence (rural vs. urban), education (<1 year of schooling vs. ≥1 year of schooling), marital status (currently married vs. unmarried including never-married, divorced or widowed), regular exercise (yes vs. no), smoking (yes vs. no), and alcohol drinking (yes vs. no).

Statistical analysis

We employ multiple proportional hazards regression model analysis with survival time and mortality as the dependent variable. Following the standard Aiken and West procedure, we conducted blocked multiple proportional hazards model analysis and Chi-square tests to examine whether the difference in likelihood ratios between the full models including the interaction block and the model without the interaction block are statistically significant. Such tests also inform whether the interaction terms included in the regressions are statistically significant (Aiken and West, 1991). The results of these additional tests are listed in the last three rows of Tables 2a and 2b, and they are consistent with the p value estimates of the interaction terms. The significant results of these additional tests also imply that the likelihood of a type I error in our estimates of the interaction terms is small (Helm and Mark, 2012).The analyses were performed using Stata/SE 12.0.

Note that the analyses reported in this paper are not based on genome-wide association studies (GWAS) in which tests of the statistical significance of the associations of an outcome phenotype/trait with millions of SNPs are evaluated and the $p < 5 \times 10^{-8}$ is recommended as the ideal GWAS statistical significance level, to adjust for the multiple comparisons. Rather, we seek to assess whether the associations of the outcome phenotype of mortality at advanced ages with two candidate SNPs that have been identified in prior research, including our own prior research, are statistically significant and replicated in the discovery CLHLS Cohort 1998 (N = 810) and replication Cohort 2008-2009 (N = 1,671). Accordingly, there is no need to use the $p < 5 \times 10^{-8}$ of GWAS studies as the ideal threshold for statistical significance. Rather, for studies of this size, we use $p < 0.05$ as a statistical threshold for associations estimated in each of the discovery and replication cohort and multiply the p value by 2 (number of the SNPs tested) for the analysis using data of the two

cohorts combined, adjusted for the multiple comparisons by the Bonferroni step-down procedure (Holm, 1979).

3. RESULTS

We found that the GxE interaction term between carrying the FOXO1A-209 minor allele (recessive model) and tea drinking is significantly associated with lower risk of mortality at advanced ages in the Cohort 1998 (P = 0.037 or P = 0.048 for tea drinking as binary or ordinal variable, see models A-I and A-II in Table 2). This significant association was replicated in the Cohort 2008-2009 (p = 0.028 or P = 0.042 for tea drinking binary or ordered variable, see models B-I and B-II in Table 2). The association of the FOXO1A-209-by-tea-drinking interaction terms with substantially reduced mortality risk (hazard ratio = 0. 465 to 0.666) is highly significant in the integrated analysis of combined dataset of Cohort 1998 and Cohort 2008-2009 (p = 0.003 or P = 0.006 for tea drinking binary or ordered variable, see models C-I and C-II in Table 2). The GxE interaction term between carrying the FOXO1A-209 minor allele following the additive model and drinking tea (ordered variable) is significantly associated with lower mortality risk at advanced ages in the Cohort 2008-2009 (p = 0.018, see model B-III in Table 2), but the association is not significant in the Cohort 1998, although the effect direction of reducing mortality risk is consistent between the two cohorts; the associations is significant in the combined analysis (P = 0.019, see model C-III in Table 2).

-- Table 2 about here ---

Note that the significant estimates of the GxE interaction terms (as shown in Table) represent synergistic associations, but may not exactly reflect the true effects of

GxE interactions on the mortality risk because the estimates may be confounded by correlations between the genotype and environmental factor (abbreviated as rGE) (Rothman, 2002). Therefore, we use the two-sample t-test or the Pearson's chi-squared tests to explore whether the rGE exists. More specifically, we test whether the differences in the percentages carrying the FOXO1A-209 genotype (recessive model) between the tea drinkers and non-drinkers (binary variable) or among those who never, sometime or often drink tea (ordered variable), are statistically significant. In the additive model of the genotypes, we test whether the differences in the average number of copies of the minor allele between the tea drinkers and non-drinkers (binary variable) or among those who never, sometime or often drink tea (ordered variable), are statistically significant. If rGE is not statistically significant, the estimates of the interaction terms represent the true GxE interaction effects. Otherwise, we need to conduct path analysis employing structural equation models, adjusted for various confounders, to further explore the direct, indirect, and interactive associations of the genotype and the environmental factor with the health outcome indicator (Zeng et al. 2013).

The results of the statistical tests shown in Table 3 were not significant, ruling out the rGE correlation as the explanation for the interacton between carrying the FOXO1A-209 minor alleles and tea drinking. Thus, the estimates of the GxE interaction terms between carrying the FOXO1A-209 minor alleles and tea drinking presented in Tables 2a and 2b represent true associations between the GxE interactions and mortality risk at advanced ages and they are not confounded by a rGE correlation.

-- Table 3 about here –

An interaction between an environmental factor and a genotype is present if the association between the environmental factor and a health outcome indicator differs

among individuals with different genotypes, or if the association between the genotype and a health outcome indicator differs among individuals with different environmental factors (IOM, 2006). Consequently, in addition to looking at the hazard ratios of the GxE interaction terms presented in Tables 2a and 2b, another more intuitive way to understand the effects of the FOXO1A-by-tea-drinking interactions is to assess differences in the hazard ratio of mortality risk between those who have different combination of the statuses of tea drinking and carrying the FOXO1A-209 genotype (see the Appendix B for a technical note).

The estimates presented in Figure 1 and middle panel of Table A2 (in Appendix B) show that, among the non-carriers of FOXO1A-209 minor alleles (recessive model), drinking tea (binary variable) did not affect the mortality risk; but the mortality risk reduction effects of tea drinking among the FOXO1A-209 carriers was -53.3%. As shown in Figure 2 and the right panel of Table A2, the effects of sometimes or often drinking tea (compared to not-drinking tea) on reduced mortality risk were very minor (-1.4% or -2.8%) among non-carriers of FOXO1A-209 minor alleles (recessive model). In contrast, however, the effects of sometimes or often drinking tea on reduced mortality risk among the carriers of FOXO1A-209 genotype were -34.39% or -56.9%. The estimates presented in Figure 3 and Table A3 (in Appendix B) indicate that, among those who do not carry any copy of the FOXO1A-209 minor allele, sometimes or often drinking tea was associated with slightly increased mortality risk (+2.4% or +4.9%); among those who carry 1 copy of the FOXO1A-209 minor allele, sometime or often drinking tea was associated with mortality that was reduced by -9.8% or -18.7%;at the same time, sometimes or often drinking tea reduced mortality risk by about -20.6% or -37.0% among those who carry two copies of FOXO1A-209 minor alleles.

--Figures 1, 2 and 3 about here—

The Cox proportional hazard model analysis using the FOXO1A-209 genotype, following the dominant model, produced estimates of a general reduction of mortality risk that was similar to those following the additive and recessive model, but the estimates were mostly not statistically significant. We also tried to explore the effects of GxE interactions between tea drinking and carrying the minor alleles of the FOXO1A-213 SNP on mortality risk at advanced ages, but the estimates are not statistically significant (hazard ratios were between 0.742 to 0.952 and the p values were between 0.066 to 0.578). These not-significant results are not presented in this article due to space limitations.

In the present study, we tested the effects of GxE interactions between one of the two SNPs (FOXO1A-209 and FOXO1A-213) and one environmental factor of tea drinking on mortality risk at advanced ages. The two SNPs of FOXO1A-209 and FOXO1A-213 are highly correlated (with a correlation coefficient of 0.631) and in moderate linkage disequilibrium with R-square between 0.481 and 0.518 (HapMap data from phases I+II+III; HapMap rel #27, NCBI B36). Based on statistical methodological guidelines which indicate that if the null hypotheses are not independent, then the p values may not need to be corrected by the Bonferroni method or other similar method (McDonald, 2009; Motulsky, 2010), it may not be necessary for us to correct the p values of the GxE interactions in this study as the two tested SNPs of FOXO1A-209 and FOXO1A-213 are not fully independent. Nevertheless, we multiplied the p value of the GxE terms by 2 (the number of SNPs tested in present study) following the Bonferroni step-down procedure (Holm, 1979) in the integrated analysis using the combined dataset of the two CLHLS cohorts, in order to strictly adjust for any potential multiple comparison bias; and the adjusted p values of the GxE terms vary from 0.006 to 0.032 (see Table 2).

DISCUSSION

The foregoing analyses have shown that GxE interactions between carrying the FOXO1A-209 genotype and regularly drinking tea were significantly associated with lower risk of mortality at advanced ages in the Chinese Han population, and potential confounding effects of correlations between carrying the FOXO1A-209 minor alleles and tea drinking were ruled out. The significant association between the FOXO1A-209-by-tea-drinking GxE interaction and reduced mortality risk at advanced ages is replicated in the two independent CLHLS cohorts (with P from 0.018 to 0.048; Table 2), and the adjusted *p* value for multiple comparison by the Bonferroni step-down procedure (Holm, 1979) varied from 0.006 to 0.032 in the integrated analysis using the combined dataset of the two CLHLS cohorts (Table 2). Our estimates clearly showed that the associations between tea drinking (either binary or ordered variable) and reduced mortality at advanced ages were much stronger among carriers of the genotypes of FOXO1A-209 compared to non-carriers, following either recessive or additive model (Figures 1, 2 and 3).

Based on previous research showing that intake of tea compounds activate FOXO1A gene expression and modulate its biological functions (Anton et al., 2007), we speculate that results in our present study indicate that tea drinking may activate FOXO1A-209 gene expression which offers protection against mortality risk at oldest-old ages.

Our empirical findings imply that health outcome benefits of certain nutritional interventions, including tea drinking, may, in part, depend upon individual genetic profiles. This suggests that, if the exploratory findings in this study are further replicated in other populations and verified by biological functional studies in humans, health care professionals may advise elders who carry the FOXO1A-209 genotypes to drink tea frequently and continuously to reduce mortality risk. At the same time, such advice on tea

drinking may not be as applicable to non-carriers of the FOXO1A-209 genotype, and it may be more beneficial to suggest that they may frequently drink other beverages that are scientifically proven being more appropriate for promoting their health.

We note that our statistical association study did not establish the causal effects of FOXO1A gene on aging and longevity and their biological mechanisms. However, analyses of biological functions of this gene indicates that such casual connection is likely to take place. FOXO1A is a central regulator of metabolism in several cell types. It is involved in many biological processes relevant to aging and longevity including cellular response to starvation, apoptosis, blood vessel development, stress response, regulation of protein catabolic process, innate immune response, and many others.

Because the FOXO1A genotype data were available for 2,481 oldest-old aged 91+ only, we restricted the present study to advanced ages, and we were not able to explore age, period and cohort effects on the GxE interactions. Furthermore, we were not able to distinguish what type of teas the participants used to drink, as no such information was collected in CLHLS 1998 and 2008-2009 cohorts. We were also not able to quantify the intake of EGCG and other tea catechins or measure their concentration in peripheral blood or urine. As the sub-sample size of the male oldest-old was not large enough, we included sex as a covariate in the hazard models to control for the potential confounding effects of gender, but we were not able to conduct more detailed analysis for the male oldest-old separately. These limitations will need to be addressed in the future when the new genotypic/phenotypic datasets covering all elderly age groups with much larger sample sizes for both genders are available.

Finally, we emphasize caution in interpreting our results as exploratory findings and look forward to further replication studies and functional analysis.

Appendix A: A Note on the Quality Control Procedures of the CLHLS GWAS dataset

The FOXO1A genotypes data of the CLHLS Cohort 2008-2009 were taken from the CLHLS genome-wide association analysis (GWAS) dataset which was recently produced by the Beijing Genomics Institute (BGI). Quality control procedures of this GWAS are as follows.

Our overall GWAS samples consist of 2,578 long-lived cases and 2,387 middle age controls. After DNA extraction, all of these cases and controls were genotyped using the Illumina HumanOmniZhongHua-8 (LHOZ-8) BeadChips according to the Illumina Infinium HD protocol, with a starting number of 900,015 SNPs. The LHOZ-8 BeadChip was created by strategically selecting optimized tag SNP content from all three HapMap phases and the 1000 Genomes Project (1kGP). The LHOZ-8 BeadChip allows profiling of 900,000 SNPs per sample, including 600k SNPs of common variants (MAF≥5%), 290k SNPs of the rare variants (MAF<5%) and 10k SNPs existing only among Chinese and other Asian populations. In other words, 98.9% of the 900k SNPs of the LHOZ-8 BeadChip are internationally compatible, with 1.1% specific to Chinese and Asian populations; this provides coverage of about 81% of common variation at r^2 >0.8 with MAF ≥5%, and about 60% of rare variants at r^2 >0.8 with MAF<5%. Our selection of this represents a state-of-the-art choice for GWAS in Asian populations with full international compatibility.

For the sample filtering, individuals with generated genotypes of a call rate less than 95% were excluded; consequently, 339 individuals were excluded. We also conducted identity-by-state probabilities for all subjects to search for any possible duplicates and kinship-related individuals among the samples, using PLINK 1.06 software. After the sample filtering, 2,178 long-lived cases (mean age 101.5 ± 3.45 (SD)) and 2,299 middle-age controls (mean age 48.4 ± 7.44(SD)) were enrolled in the subsequent GWAS dataset. We also conducted quality-control filtering of the GWAS data from these in total 4,477 individuals. SNPs with call rates of less than 90% were

removed from our GWAS dataset. SNPs were also excluded if they had a MAF less than 5% or if there was significant deviation from Hardy-Weinberg equilibrium in the samples defined as
$P < 10^{-5}$. SNPs on the X and Y chromosomes and mitochondria were removed from further GWAS analysis, in keeping with the recent GWAS practices. After quality filtering and cleaning, 818,084 SNPs remained for association analysis in our final GWAS dataset.

To further increase genome coverage, we performed imputation analysis to infer the genotypes of all SNPs (MAF≥0.05) using IMPUTE software (version 2) and the 1000 Genomes Project integrated phase 1 release as reference panel. SNPs with a quality score (Rsq) of <0.9 were discarded before analysis. After standard GWAS quality-control filtering for subjects and SNPs as described above (excluding those SNPs with MAF<0.05, Hardy-Weinberg $P < 10^{-5}$, call rate<90%, on the X and Y chromosomes and mitochondria), we obtained data for 4,595,614 genotyped or imputed SNPs in 2,178 long-lived cases and 2,299 middle-age controls for the subsequent GWAS dataset. Principal component analysis (PCA) showed that the cases and controls in our GWAS dataset were of truly Han Chinese ancestry and were well matched. We performed the GWAS analysis using logistic regression, adjusted for the top two principal components C1 and C2 (the first two eigenvectors) to minimize the effects of population stratification (Price et al., 2008).

Note that we used the data on the two SNPs of the Cohort 2008-2009 from the CLHLS GWAS dataset of the long-lived cases in present study, and SNP FOXO1A-213 was initially genotyped using the LHOZ-8 BeadChip, and the SNPs FOXO1A-209 was imputed, as described above.

Appendix B: A technical note on how to estimate the hazard ratios of mortality risk for those who have different combinations of the statuses of genotype and environmental factor

Regressions for genotype carriers and non-carriers (dominant or recessive models) may be estimated separately, or for those who carry 0, 1, or 2 copies of the minor alleles (additive model) separately, to assess the differences in effects of an environmental factor on a health outcome indicator among those who have different genotypes, if the sub-sample size is sufficiently large for each of groups of the participants with different genotypes. However, this may very likely not be the case in most circumstances including our present study. Thus, we apply a simple procedure to assess the differences in effects of an environmental factor on a health outcome indicator among those who have different genotypes, without further dividing the samples. Note that this procedure was applied to the case of defining the genotype by either the dominant or recessive model and employing binary environmental variables in previous publications (Zeng et al., 2013; Zeng et al., 2014), and we extend here the procedure to the genotypes with the additive model and ordered environmental factors.

In general, the Cox proportional hazards model is expressed as:

$$\log h_i(t) = \log h_0(t) + [\beta_1 G_i + \beta_2 E_i + \beta_3 G_i * \mathrm{E}_i + \sum_j \alpha_j X_{j_i}]$$

(1)

where $h_i(t)$ is the hazard at time t of the i^{th} individual and $h_0(t)$ is the baseline hazard at time t; G_i represents the genotype and E_i represents environmental factor status of the i^{th} individual; G_ixE_i is the interaction variable of the genotype and environmental factor; X_{ji} is a vector of covariate values corresponding to the i^{th} individual. Coefficients β_1, β_2, β_3 and α_j measure the hazards of mortality risk for the corresponding variables.

Let HR_{GE} represent the hazard ratio of mortality risk of those with a combination of the genotype status of carrying the minor allele(s) (G) such as the FOXO1A-266 genotype and an environmental factor (E) such as tea drinking. G may be a binary variable (dominant or recessive model) or an ordered variable (additive model); E may be a binary variable (E = 1 or 0 refers to exposure or not exposure to the environmental factor, such as drinking tea or not-drinking tea) or an ordered variable (E=0, 1, 2,…; such as E=0, 1, 2 refers to never, sometimes and often drinking tea). Following standard statistical methodology (e.g. Li & Chambless, 2007; Cohen et al., 2003, chapter 9), we estimated the Hazard ratios (HR_{GE}) of the mortality risk of those who have different combinations of the genotype (G, additive model, G=0,1,2) and environmental factor (E, ordered variable, E=0,1,2), compared to the reference group with G=0 and E=0, using the following general formula:

$$HR_{GE} = \frac{h_i(t)|_{G_i,E_i}}{h_i(t)|_{G_i(=0),E_i(=0)}} = \frac{h_0(t)\exp(\beta_1 * G_i + \beta_2 * E_i + \beta_3 * G_i * E_i + \sum_j \alpha_j X_{j_i})}{h_0(t)\exp(\beta_1 * 0 + \beta_2 * 0 + \beta_3 * 0 * 0 + \sum_j \alpha_j X_{j_i})} = \exp(\beta_1 * G_i + \beta_2 * E_i + \beta_3 * G_i * E_i)$$

Thus, the HR_{GE} of those who have different combinations of G and E can be estimated based on the coefficients β_1, β_2, and β_3 obtained in the Cox model regression (as shown in Table A1)

Table A1. Hazard ratios (HR_{GE}) of the mortality risk of those who have different combinations of the genotype (G, additive model, G=0,1,2) and environmental factor (E, ordered variable, E=0,1,2), estimated based on the coefficients β_1, β_2, and β_3 obtained in the Cox model regression

Genotype (G; additive model)	Environmental factor (E; ordered variable)		
	E=0	E=1	E=2
G=0	1.00 (ref.)	$\exp(\beta_2)$	$\exp(2\beta_2)$
G=1	$\exp(\beta_1)$	$\exp(\beta_1+\beta_2+\beta_3)$	$\exp(\beta_1+2\beta_2+2\beta_3)$
G=2	$\exp(2\beta_1)$	$\exp(2\beta_1+\beta_2+2\beta_3$	$\exp(2\beta_1+2\beta_2+4\beta_3)$

Table A2 and Table A3 present the estimates of the hazard ratios of mortality risk (HR_{GE}) by combinations of statuses of tea drinking (E) and carrying the FOXO1A-209 minor allele (G), and their differences, estimated using the formulas presented in Table A1.

Table A2. The hazards ratios of mortality risk (HR_{GE}) and their differences by tea drinking status (E) and the status of carrying the minor allele of FOXO1A-209 following the *recessive* model (G), based on the data of the two cohorts combined

G: genotypic status *Recessive genetic model*	Binary tea drinking variable			Ordered tea drinking variable				
	E=0	E=1	% diff. E=1 vs.0	E=0	E=1	E=2	% diff. E=1 vs.0	% diff. E=2 vs.0
0 or 1 copy minor allele: G=0	1.00(ref.)	1.004	+0.4%	1.00(ref.)	0.986	0.972	-1.4%	-2.8%
2 copies of minor allele: G=1	1.259	0.587	-53.3%	1.232	0.809	0.531	-34.3%	-56.9%
% difference G=1 vs.0	+25.9%	-41.5%		+23.2%	-17.9%	-45.3%		
HRIT: GxE interaction	0.465 =0.003; b*P*=0.006; CI:0.28,0.7			0.666 P=0.006; b*P*=0.012; CI: 0.50, 0.89				

Notes: (1) diff.: difference; (2) b*P* is the adjusted p value for multiple comparison by the Bonferroni step-down procedure; (3) *HRIT* (hazards ratio of the GxE interaction term) are taken from the proportional hazards model estimates.

Table A3. The hazards ratios of mortality risk (HR_{GE}) and their differences by tea drinking status (E) and the status of carrying the minor allele of FOXO1A-209 following the *additive* model (G), based on the data of the two cohorts combined

G: genotypic status *Additive genetic model*	Ordered tea drinking variable				
	E=0	E=1	E=2	% diff. E=1 vs.0	% diff. E=2 vs.0
0 copy minor allele: G=0	1.00(ref.)	1.024	1.049	+2.4%	+4.9%
1 copy minor allele: G=1	1.034	0.933	0.841	-9.8%	-18.7%
2 copies of minor allele: G=2	1.070	0.849	0.674	-20.6%	-37.0%
% difference G=1 vs.0	+3.4%	-8.9%	-19.8%		
% difference G=2 vs.0	+7.0%	-17.1%	-35.7%		
HRIT: GxE interaction	0.880 P=0.016; b*P*=0.032; CI: 0.79, 0.98				

Notes: the same as in Table A2.

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Table 1. Descriptive statistics for the main independent variables and covariates

Variables	% or mean		
	Combined	Cohort 1998	Cohort 2008-2009
Main independent variables			
Carrying FOXO1A-209 minor allele status			
0 copy	58.4%	58.9%	58.2%
1 copy	35.4%	34.6%	35.7%
2 copies	6.3%	6.5%	6.1%
Drink tea at present time			
Almost everyday	21.6%	19.6%	20.3%
Sometimes	13.5%	17.1%	15.6%
Rarely or never	64.9%	63.3%	64.1%
Covariates			
Males	25.0%	22.7%	26.1%
Average age	100.0	100.9	99.6
Urban residents	34.5%	28.3%	37.5%
1+ year of schooling	18.1%	16.5%	18.9%
Regular exercise	23.3%	23.6%	23.1%
Smoking in recent 5 years	11.1%	11.7%	10.8%
Currently drink alcohol	17.3%	23.0%	14.6%
Number of participants	2481	810	1671

Table 2. Hazard ratios of mortality risk at advanced ages: exploring the effects of GxE interactions between FOXO1A-209 genotype and tea drinking, Cohort 1998, Cohort 2008-2009, and the two cohorts combined

Cohort	Cohort 1998			Cohort 2008-2009			Two cohorts Combined		
Model	A-I	A-II	A-III	B-I	B-II	B-III	C-I	C-II	C-III
FOXO1A-209,recessive=1(0)	1.159	1.137		1.315*	1.288		1.259*	1.232*	
FOXO1A-209,additive			1.022			1.048			1.034
Drink tea, Yes (no)	1.060			0.972			1.004		
Drink tea, ordered variable		1.006	1.023		0.977	1.032		0.986	1.024
GxE interactions									
(FOXO1A-209,recessive) x (Drink tea, binary variable)	0.456** (0.037)			0.452** (0.028)			0.465*** (P=0.003)		
95% Confidence Interval	0.22,0.95			0.22,0.92			0.28, 0.77		
(FOXO1A-209,recessive) x (Drink tea, ordered variable)		0.662** (0.048)			0.653** (0.042)			0.666*** (P=0.006)	
95% Confidence Interval		0.44,1.00			0.43,0.98			0.50, 0.89	
(FOXO1A-209,additive) x (Drink tea, ordered variable)			0.918 (0.265)			0.841** (0.018)			0.880** (P=0.016)
95% Confidence Interval			0.79,1.07			0.73,0.97			0.79, 0.98
Covariates									
Male (female)	1.141	1.136	1.128	1.337***	1.340***	1.346***	1.247***	1.249***	1.250***
Age	1.078***	1.078***	1.076***	1.101***	1.101***	1.100***	1.097***	1.097***	1.096***
Urban (Rural)	1.151	1.146	1.124	0.824**	0.827**	0.830**	0.917	0.919	0.916
1+ year of schooling (0)	0.967	0.966	0.974	1.073	1.071	1.071	1.034	1.032	1.034
Regular exercise (no)	0.771***	0.777***	0.784**	0.798**	0.798**	0.790***	0.791***	0.792***	0.789***
Smoke in recent 5 years (no)	1.089	1.099	1.080	0.957	0.956	0.954	0.992	0.994	0.982
Currently drink alcohol (no)	1.011	1.017	1.018	1.044	1.044	1.040	1.051	1.055	1.053
-2LL (-2 log Likelihood)	7304.4 (7309.1)	7304.7 (7309.0)	7308.0 (7309.3)	11355.9 (11361.4)	11356.4 (11361.2)	11355.2 (11360.9)	20417.6 (20427.5)	20417.9 (20426.6)	20420.0 (20425.8)
LR chi2	4.7	4.3	1.3	5.5	4.8	5.7	9.8	8.7	5.9
Prob > chi2 (P)	0.030	0.038	0.262	0.019	0.028	0.017	0.002	0.003	0.015

Notes: (1) *: p<0.1, **: p<0.05; ***: p<0.01; (2) The categories “no” and “0” in the parentheses after the variables in the first column are reference groups; (3) The figures in parentheses below the significant estimate of GxE terms are P-values; (4) The figures in parentheses below the estimate of “-2LL” are the estimates of “-2LL” in the models without the interaction blocks whose other estimates are not presented due to space limitation (available upon request). The last line “Prob > chi2 (P)” presents the P values of the Chi-square tests to examine whether the difference in likelihood ratios between the full model including the interaction block and the model without the interaction block are statistically significant, following the standard Aiken and West procedure (Aiken and West, 1991).

Table 3. Statistical tests to assess rGE correlation between the FOXO1A-209 genotypes and tea drinking for all synergistic significant FOXO-by-tea-drinking interaction terms discovered in Tables 2

	Drink tea, binary variable			**Drink tea, ordered variable**			
	Never E=0	Drink E=1	P Value [see (2)]	Never E=0	Sometimes E=1	Often E=2	P Value [see (3)]
% Carrying FOXO1A-209 (recessive mode)	6.3	5.6	0.487	6.3	3.6	6.9	0.127
Average copies of minor allele FOXO1A-209 (additive mode)	0.466	0.492	0.131	0.466	0.465	0.509	0.100

Note: (1) “Drink” in the binary tea drinking variable includes often and sometimes drinking tea; (2) the P values concerning the binary tea drinking variable are based on the two-sample t-tests; (3) The P values concerning the ordered tea drinking variable are based on the Pearson’s chi-squared tests.

Figure 1. Comparisons of estimated hazards ratios of mortality risk (HR_{GE}) by tea drinking status (E, binary) and the status of carrying the minor allele of FOXO1A-209 (G) based on the *recessive* model and on data from the two cohorts combined

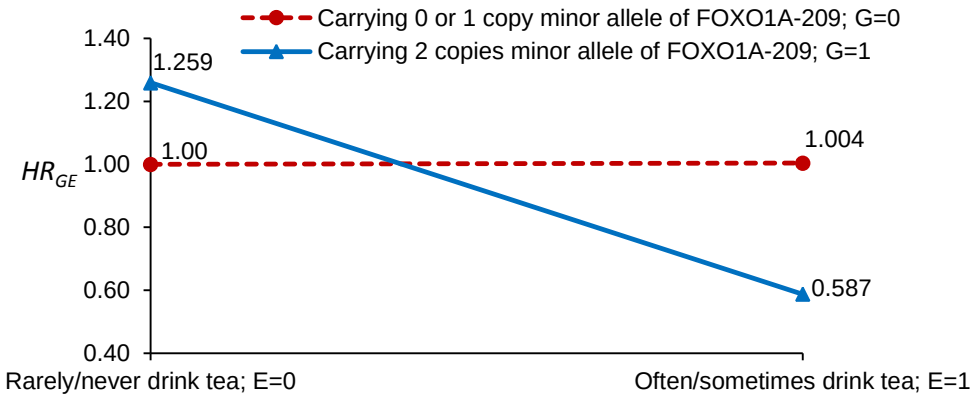

Figure 2. Comparisons of estimated hazards ratios of mortality risk (HR_{GE}) by tea drinking status (E, ordered) and the status of carrying the minor allele of FOXO1A-209 (G) based on the *recessive* model and on data from the two cohorts combined

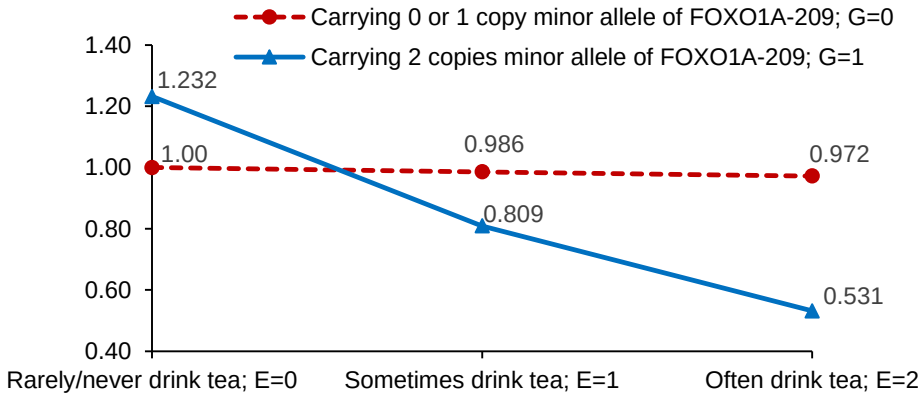

Figure 3. Comparisons of estimated hazards ratios of mortality risk (HR_{GE}) by tea drinking status (E, ordered) and the status of carrying the minor allele of FOXO1A-209 (G) based on the *additive* model (G) and data from the two cohorts combined