Effects of the Ser326Cys polymorphism in the DNA repair OGG1 gene on cancer, cardiovascular and all-cause mortality in the PREDIMED study: Modulation by Mediterranean diet

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RESEARCH SNAPSHOT

Research Question: Is the lower DNA-repair capacity genotype (homozygous individuals for the Cys326 allele) in the OGG1-rs1052133 (Ser326Cys) polymorphism associated with cancer mortality or other causes and are these associations modulated by Mediterranean diet (MedDiet) or vegetable intake?

Key findings: In the PREDIMED dietary intervention trial including 7,170 participants, the Cys326Cys-OGG1 genotype was associated with higher total mortality, mainly cardiovascular mortality. For cardiovascular and total mortality, no statistically significant interactions were found with the MedDiet intervention. However, when vegetable intake was considered, significant interactions decreasing the risk for cardiovascular mortality in homozygous individuals with higher intake were detected.

ABSTRACT

Background: Oxidatively induced DNA damage, an important factor in cancer etiology, is repaired by oxyguanine glycosylase 1 (OGG1). The lower repair capacity genotype (homozygote Cys326Cys) in the OGG1-rs1052133 (Ser326Cys) polymorphism has been associated with cancer risk. However, no information is available in relation to cancer mortality, other causes of

death and modulation by diet.

Objective: Our aim was to evaluate the association of the OGG1-rs1052133 with total, cancer and cardiovascular (CVD) mortality and to analyze its modulation by the Mediterranean diet (MedDiet), focusing especially on total vegetable intake as one of the main characteristics of this diet.

Design: PREDIMED is a randomized, controlled trial conducted in Spain from 2003 to 2010.

Participants/setting: Study participants (n=7,170) were at high risk for CVD and aged 55-80 years.

Intervention: Participants were randomly allocated to two groups with a MedDiet intervention or to a control diet.

Main Outcome measures: Main outcomes were all-cause, cancer and CVD mortality after a median follow-up of 4.8 years.

Statistical analyses: Multivariable-adjusted Cox regression models were fitted.

Results: 318 deaths were detected (cancer=127, CVD=81 and others=110). Cys326Cys individuals (prevalence 4.2%) presented higher total mortality rates than Ser326-carriers (P=0.009). The multivariable-adjusted Hazard Ratio (HR) for Cys326Cys versus Ser326-carriers was 1.69 (95%CI:1.09-2.62); P=0.018. This association was greater for CVD mortality (P=0.001). No relationship was detected for cancer mortality in the whole population (HR:1.07; 95%CI:0.47-2.45; P=0.867), but a significant age interaction (P=0.048) was observed as Cys326Cys was associated with cancer mortality in participants <66.5 years (P=0.029). Recessive effects limited our ability to investigate Cys326Cys*diet interactions for cancer mortality. No statistically significant interactions for total or CVD mortality were found for the MedDiet intervention. However, significant protective interactions for CVD mortality were found for vegetable intake (HR-interaction per standard deviation: 0.42;95%CI:0.18-0.98, P=0.046).

Conclusions: In this population, the Cys326Cys-OGG1 genotype was associated with all-cause mortality, mainly CVD instead of cancer mortality. Additional studies are needed to provide further evidence on its dietary modulation.

INTRODUCTION

DNA molecules are exposed to the attack of DNA-damaging agents[1], among them reactive oxygen species (ROS)[2]. Oxidatively induced DNA damage can be both mutagenic and cytotoxic[3] and has been implicated in the etiology of cancer[4], neurodegenerative diseases[5] and overall aging[6]. Hydroxyl radicals preferentially react with the C8 atom of purines in DNA to generate 8-oxo-7,8-dihydroguanine (8-oxoG), 8-oxo-7,8-dihydroadenine (8-oxoA) and formamidopyrimidines (Fapy)[7]. The accumulation of unrepaired DNA damage can cause genetic instability and has deleterious effects on cell function[8]. 8-oxoG is a critical mutagenic lesion because of its propensity to mispair with A during DNA replication[7]. Repair of oxidatively damaged bases occurs primarily via the DNA base excision repair (BER) pathway[2]. In the first step of this type of repair, damaged bases are removed from DNA by DNA glycosylases[9]. The oxyguanine glycosylase 1 (OGG1) is the human DNA glycosylase responsible for removal of the highly mutagenic 8-oxoG from DNA[7]. The OGG1 gene is located in chromosome 3p26.2 and this region has frequently been detected as deleted in various tumors suggesting the loss of this gene as a possible contributor to carcinogenesis[7,10-13].

The most studied polymorphism in the human OGG1 gene is the rs1052133 (Ser326Cys), a C to G transversion at nucleotide 1245 in exon 7, leading to a serine to cysteine substitution at residue 326 [14]. This variant is functional and it has been shown that the Cys326 protein has weaker 8-hydroxyguanine-repair capacity than the Ser326 protein[15-17]. The deactivation of the OGG1 gene or the presence of a less active variant such as the Cys326 may lead to a higher risk of cancer and oxidation-related pathologies[7,13,18]. Consequently, this polymorphism has been analyzed as a risk factor in several cancers[19-25] (i.e., breast, prostate, lung, colorectal, aero digestive, gastric, bladder). The results of meta-analyses for each location are heterogeneous[21-25], but where there is more consensus is in the significant association of the Ser326Cys

polymorphism with greater overall risk of cancer when the different locations are pooled[26,27]. Thus, Zou et al[26] in a meta-analysis including 152 case-control studies, concluded that the Cys variant was strongly associated with higher cancer risk. Interestingly, the cancer risk was higher in homozygous individuals for the Cys variant, suggesting a recessive pattern. This observation agrees with several functional studies showing that only homozygous carriers of the Cys allele showed a significantly lower DNA repair activity compared to Ser326Ser[16,18]. A potential source of the observed heterogeneity found among studies may be the exposure to different environmental factors[28-31] (i.e. mainly vegetable intake and other dietary factors).

The Ser326Cys OGG1 polymorphism has also been associated with a greater risk of atherosclerosis[32,33] and incidence of cardiovascular diseases[34,35], although there have been very few studies that have specifically focused on cardiovascular phenotypes.

Whereas many studies have analyzed the influence of the OGG1 Ser326Cys polymorphism on cancer risk, few have analyzed its influence on mortality due to cancer. Moreover, if the OGG1 gene also makes an important contribution to other pathologies, such as cardiovascular diseases, there is compelling interest in knowing whether, in the same cohort, this gene has a greater influence on mortality due to cancer or on mortality due to cardiovascular disease. The aims were, first, to analyze the influence of the OGG1 Ser326Cys polymorphism on cancer mortality, cardiovascular mortality and on total mortality in a high cardiovascular risk Mediterranean population and second to investigate the possible modulation by diet by analyzing the Mediterranean diet (MedDiet) intervention as well as focusing on total vegetable intake as one of the main characteristics of the MedDiet.

METHODS

The present study was conducted within the framework of the PREDIMED trial, the

104 design of which has been described in detail elsewhere[36]. Briefly, the PREDIMED study is a
105 multicenter, randomized and controlled clinical trial aimed at assessing the effects of the
106 MedDiet on the primary cardiovascular prevention[37]. This study was registered at controlled-
107 trials.com (http://www.controlledtrials.com/ISRCTN35739639). Here, 7,170 participants (from
108 a total of 7,447) were included from whom DNA was isolated and the OGG1-rs1052133
109 (Ser326Cys) polymorphism determined. Briefly, from October 2003 to June 2009 physicians in
110 Primary Care Centers located in several Spanish regions selected high-cardiovascular risk
111 participants. Eligible participants were community-dwelling adults at high cardiovascular risk
112 (55-80 years for men; 60-80 years for women) who met at least one of two criteria: diabetes or 3
113 or more cardiovascular risk factors (hypertension, dyslipidemia, overweight or obesity, current
114 smoking, or a family history of premature coronary heart disease)[36]. Exclusion criteria were the
115 presence of any severe chronic illness, previous history of cardiovascular diseases, alcohol or
116 drug abuse, and history of allergy or intolerance to olive oil or nuts. Hence, individuals with
117 incident cancer undergoing treatment were excluded, but individuals that reported having had
118 some form of cancer in previous years but who had no clinical signs of cancer at the time of
119 enrollment were not excluded.
120 Participants were randomly assigned to these interventions: a MedDiet (2 groups, one
121 supplemented with extra-virgin olive oil and the other with nuts) and a control group (advised to
122 follow a low-fat diet). Randomization was performed by means of a computer-generated
123 random-number sequence (randomly assigned in a 1:1:1 ratio to one of three groups).
124 Participants assigned to both MedDiet groups received intensive training to follow the MedDiet
125 and allotments of either extra-virgin olive oil (1L/week) or mixed nuts (30 g/d) throughout the
126 entire study time period, whereas those assigned to the control diet were instructed to reduce the
127 intake of all types of fat[37]. Because both MedDiet intervention groups had a similar effect [37],

these groups were pooled and analyzed together. The primary end point of the PREDIMED trial was cardiovascular disease incidence, including a composite endpoint comprised of myocardial infarction incidence, stroke incidence and cardiovascular death. Total and cause-specific mortality were considered as secondary endpoints. In this study, total and cause-specific mortality will be analyzed, focusing on mortality due to cancer and cardiovascular events.

The Institutional Review Board of each participating center approved the study protocol, and all participants provided written informed consent. The trial was stopped following the statistical analysis of data obtained up to December 2010, due to early evidence of the benefit of the MedDiet on the prevention of major cardiovascular events[37]. This study is based on the data obtained from this follow-up period (median follow-up of 4.8 years) with dietary intervention throughout the entire study time period.

Demographic, clinical, anthropometric and dietary measurements

The baseline examination included assessment of standard cardiovascular risk factors, medication use, socio-demographic factors and lifestyle variables by validated questionnaires[36,38,39]. Adherence to the MedDiet was measured by a validated 14-item questionnaire[38]. Food and beverage consumption was reported using a validated 137-item semiquantitative food-frequency questionnaire (FFQ)[39]. Dietary data from the FFQ were obtained for 7,122 participants. Weight and height were measured with calibrated manual or digital scales and a wall-mounted stadiometer, respectively[36]. Body mass index (BMI) was calculated as kg/m^2.

Biochemical determinations, DNA extraction and genotyping

Fasting glucose and lipids were measured as previously described[40]. Biochemical

measures were available for nearly 7000 participants at baseline. Genomic DNA was extracted from buffy-coat and the OGG1-rs1052133 (Ser326Cys) polymorphism was genotyped in the whole cohort with DNA available on a 7900HT Sequence Detection System (Applied Biosystems, FosterCity, CA, USA) using a fluorescent allelic discrimination TaqManTM assay. Valid genotype results for 7,170 participants were obtained. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations (P=0.882).

Outcomes and Follow-up

The end points of interest in the present analysis were cancer mortality, cardiovascular mortality and all-cause mortality after the follow-up period. We used the following 4 sources of information to identify deaths: contacts with families of participants, contacts with general practitioners who were responsible for the routine clinical care of participants, yearly consultation of the National Death Index, and a comprehensive yearly review of medical records of all participants by medical doctors who were blinded with respect to the group allocation and all nutritional information. All medical records related to endpoints were examined by the Event Adjudication Committee, whose members were unaware of the dietary information[37]. Only endpoints that were confirmed by the Event Adjudication Committee were included in the analyses. In this follow-up, all deaths detected in the 7,170 patients analyzed (those that had genotype OGG1 data), and that occurred between 1 October 2003 and 1 December 2010 were included: Total deaths (n=318), per total cancer (n=127) and per cardiovascular diseases (n=81).

Statistical analyses

The OGG1-rs1052133 polymorphism was first tested as codominant with the three genotypes considered and taking into account the Ser326Ser genotype as reference. Given that, in

the total and cause-specific association models, the effects of the Ser326Ser and Ser326Cys genotypes were similar and no statistically significant differences were found between them, carriers of the Ser326 allele were grouped together and compared to those of Cys326Cys participants (recessive model). Triglycerides were log-transformed for statistical analyses. Vegetable intake was standardized for further Cox regression analyses. ANOVA tests were used to compare means of continuous variables by the OGG1 polymorphism and cause of death. The association between the OGG1-rs1052133 polymorphism and the different causes of death were analyzed by means of the Chi Square test, using both codominant and recessive models.

To examine the longitudinal association between the OGG1-rs1052133 polymorphism and mortality (separated models for all-cause, cancer and cardiovascular mortality) in the 4.8 years median follow-up, Cox regression models were used with length of follow-up as the primary time variable. The exposure time was calculated as the time between randomization and the date at death, the date when the last interview was completed on 1 December 2010, whichever came first. Firstly, the mortality rate for the 3 genotypes and fitted codominant models were estimated. After having checked that there were no significant differences between the estimates of genotypes Ser326Ser and Ser326Cys, both genotypes were grouped together as Ser-carriers. This group was used as the category of reference and homozygous Cys326Cys were compared with it using a recessive model. Hazard Ratios (HRs) with 95% CIs for the OGG1-rs1052133 genotypes were estimated. Models were sequentially adjusted for covariates as indicted. Model 1 was adjusted for age, sex, field center and dietary intervention group (three groups). Model 2 was additionally adjusted for type-2 diabetes, BMI, and self-reported personal history of a previously diagnosed cancer at baseline. Model 3 was additionally adjusted for alcohol consumption, smoking, physical activity, hypertension, dyslipidemia, medications (lipid-lowering, hypoglycemic, and antihypertensive drugs) adherence to MedDiet and total energy

intake in the models analyzing diet.

Also evaluated was the heterogeneity of the OGG1-rs1052133 associations with mortality by age groups. Two age groups were considered, taking into account the median age of the population (66.5 years). Formal tests for the interaction between the OGG1 polymorphism and age group in determining mortality (total, cancer and cardiovascular deaths) were carried out by analyzing the product term of these variables in the corresponding hierarchical Cox regression model. Testing this interaction in a Cox regression model estimates the departure from multiplicativity instead of the departure from additivity[41,42]. Stratified analyses of both age groups were carried out. Finally, the modulation by Mediterranean diet of the associations between the OGG1-rs1052133 polymorphism and CVD mortality and total mortality were evaluated. First of all, the randomized and controlled clinical trial design (MedDiet intervention compared with the control diet) was used. Analyses were based on the intent-to-treat principle. Models were sequentially adjusted for covariates as previously indicated (model 1, model 2 and model 3). Multiplicative tests for the interaction between the OGG1 polymorphism and MedDiet intervention in determining mortality (total and cardiovascular mortality) were carried out in the multivariable adjusted Cox regression models. Stratified analyses by dietary intervention groups were undertaken.

In addition to the modulation by MedDiet intervention, as secondary analysis, the influence of total vegetable intake at baseline (observational cohort design) was investigated, as vegetables are a main food of the MedDiet previously reported to statistically interact with the OGG1-rs1052133 polymorphism[28]. Vegetable intake was used as categorical (dichotomously, using the consumption median of the population as the cut-off point) and as a continuous variable (in grams/day). For the continuous variable, the HRs of mortality per standard deviation (SD) of vegetable intake were calculated. Multivariable Cox regression models were fitted and interaction

terms analyzed. Taking into account the relevance of age in mortality, dietary interactions by age groups were also explored.

Kaplan-Meier survival curves were plotted to estimate the probability of remaining free of mortality (total or causes) during follow-up. Statistical analyses were performed with the IBM SPSS Statistics version 24.0[43]. All tests were 2-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Descriptive characteristics of participants and causes of death by OGG1-rs1052133 (Ser326Cys) genotypes

Table 1 presents demographic, clinical and lifestyle characteristics at baseline of the 7,170 PREDIMED participants according to their genotype in the OGG1-rs1052133 (Ser326Cys) polymorphism. Overall, there were no differences among genotypes in the main characteristics analyzed. The only statistically significant differences were observed in BMI and triglycerides. The OGG1 genotypes were equally distributed into the three dietary intervention groups. Following 4.8 years of median follow-up, 318 deaths were confirmed, of which the majority were from cancer (n=127), followed by cardiovascular diseases (n=81) and other causes (n=110 deaths). **Table 2** presents the baseline characteristics of the participants depending on whether participants were still alive or had died after 4.8 years of median follow-up. Within the mortality group, the cause of death was also reported. The mean age at baseline of the individuals still living was lower than that of the deceased. Among the deceased, the mean age was lower in those who died from cancer than from cardiovascular diseases. Although, in this study, individuals with a recently diagnosed cancer were not included, there were 184 participants with a prior diagnosis of cancer (in any location), presumably cancer-free at enrollment according to self-reports.

Greater mortality due to cancer was detected in individuals who had previously been diagnosed with cancer compared to those who had not (P<0.001). The effect was high (HR: 5.91; 95%CI: 3.52-9.92; P<0.001, for cancer mortality and HR: 3.13; 95%CI: 2.04-4.80; P<0.001 for total mortality, in model 1), so this variable was included as an adjustment variable in the later multivariable Cox regression models. Table 2 also presents the frequencies of the OGG1-rs1052133 polymorphism according to vital status and cause of death. In the model in which the three genotypes were analyzed separately, genotypes Ser326Ser and Ser326Cys were distributed equally among the different causes of death (P>0.05). However, the Cys326Cys genotype differed in some causes of death (P<0.05) and when comparing total mortality. In the recessive model, the Cys326Cys genotype was associated with all-cause mortality (P=0.006), being more frequent in mortality cases than in non-cases, while the highest frequency of the Cys326Cys genotype occurred in cardiovascular diseases. The detection of this recessive effect will limit the statistical power of subsequent comparisons.

Multivariable-adjusted associations of the OGG1-rs1052133 polymorphism with total, cancer and cardiovascular mortality

Table 3 presents mortality rates, HRs and 95% CI for the OGG1 genotypes for total, cancer and cardiovascular mortality after 4.8 years of median follow-up (maximum follow-up of 7.4 years) obtained in the multivariable-adjusted Cox-regression models (model 1, model 2 and model 3). For all-cause mortality, higher total mortality rates in homozygous Cys326Cys were detected in comparison with the other genotypes (Ser-carriers): HR for total mortality in Cys326Cys participants: 1.77; 95% CI: 1.16-2.71; P=0.009, in the minimally adjusted model 1 (adjusted for sex, age, field center and dietary intervention group). After additional multivariable adjustment in model 3 (including BMI, diabetes, self-reported history of cancer, smoking,

drinking, physical activity, adherence to the MedDiet and medications), this association remained statistically significant (HR: 1.69; 95% CI:1.09-2.62; P=0.018). On analyzing the specific causes of death separately, a strong association was found between the OGG1 polymorphism and cardiovascular mortality (HR: 3.31; 95% CI: 1.68-6.53; P=0.001 for Cys363Cys participants in comparison with Ser-carriers in the multivariable adjusted model 3). However, on studying the overall association of the OGG- rs1052133 polymorphism with mortality from cancer, even though in this population there were more deaths from cancer than from cardiovascular diseases (n=127 compared to n=81, respectively), no statistically significant association was detected in the case of cancer. Also using a recessive model, the HR for cancer mortality in Cys326Cys individuals in comparison with Ser-carriers was 1.07; 95%CI: 0.47-2.45; P=0.867. Comparing the Cys326Cys with the Ser326Ser, the results of no association were similar. **Figure 1** shows Kaplan Meier curves of cumulative mortality–free survival for total mortality (**A**) cardiovascular (**B**) and cancer mortality (**C**) by the three OGG1-rs1052133 genotypes in the whole population. Bearing in mind that mortality from cancer occurs in younger individuals, whereas mortality from cardiovascular diseases occurs in older individuals, the influence of the age group (two groups according to the median of age at baseline) on the associations of the OGG1 polymorphism was analyzed (**Table 4**). It was observed that there was heterogeneity by age in the association of the OGG1- rs1052133 polymorphism with cancer mortality, in such a way that in younger individuals (less than 66.5 years at baseline), the Cys326Cys genotype was significantly associated (Table 4 and Figure **1D**) with higher cancer mortality (HR: 3.27; 95%CI: 1.13-9.47; P=0.029 for Cy362Cys participants compared to Ser-carriers in model 3). Nevertheless, in those 66.5 years or older, no significant association was detected (P=0.285). One important limitation in this estimation is the small number of cases of fatal cancer in Cys326Cys homozygous individuals. However, despite this limitation of sample size, a statistically

significant interaction term between age group and the OGG1 polymorphism on cancer mortality (P-interaction=0.048 in model 3) was obtained. Cancer deaths (n=41) in participants <66.5 years at baseline were as follows: lung (26.8%), pancreatic-biliary (12.2%), colorectal (9.8%), gastric (7.3%), prostate (7.3%), liver (2.4%), ovary-endometrial (2.4%) and other locations (31.7%).

For cardiovascular mortality, an opposite effect was observed. Most of the mortality and the greatest association with the OGG1 polymorphism occurred in the older age group (>=66.5 years). However, on testing the interaction per age, no statistically significant value (P=0.234 in model 3) was detected, as although the risk is lower in the younger group, the association goes in the same direction. Neither was a statistically significant heterogeneity of the association of the polymorphism by age group with total mortality found (P-interaction=0.570 in model 3).

Effect of the MedDiet intervention on the association between the OGG1-rs1052133 polymorphism and mortality

The influence that diet had on modulating the Cys326Cys genotype association with greater mortality (total and cardiovascular) was analyzed. Modulation by diet in mortality due to cancer was not analyzed owing to the small number of Cys326Cys participants dying from cancer (n=6) and, besides, an additional interaction per age group had been detected that presents heterogeneity and limits statistical power still further (n=4 cancer deaths in Cys326Cys participants aged <66.5 years at baseline). **Table 5** presents the results of the modulation of the Cys326Cys genotype associations with total mortality and per cardiovascular diseases depending on the intervention with MedDiet (both groups considered jointly) or the control diet. For total mortality, no statistically significant interaction between the genotype and intervention with MedDiet (P-interaction=0.752, in model 3) was found. Likewise, for cardiovascular mortality, the interaction term between intervention with the MedDiet and the OGG1 polymorphism did not

reach statistical significance (P-interaction=0.181 in model 1 and P-interaction=0.200 in model 3).

In subgroup analysis by age we found that for total mortality the interaction term between the OGG1 polymorphism and MedDiet intervention reached statistical significance in participants aged ≥ 66.5 years in model 1 (P-interaction=0.049). However, in model 3 after additional multivariable adjustment (HR for Cys326Cys in the MedDiet group: 1.30; 95%CI: 0.65-2.60; P=0.451 versus HR for Cys326Cys in the control group: 2.99; 95%CI:1.34-6.67; P=0.008, in the stratified analysis), the statistical significance of the interaction term for this comparison was lost (P-interaction=0.112). Likewise, for cardiovascular mortality, the interaction terms in this group did not reach statistical significance (P-interaction=0.082 in model 1 and P=0.086 in model 3).

Effect of vegetable intake on the association between the OGG1-rs1052133 polymorphism and mortality

Finally, vegetable intake at baseline (**Table 6**) was focused on. No statistically significant interactions between vegetable intake and the OGG1-rs1055133 polymorphism in determining total mortality were found (P-interaction=0.491 for categorical and P=0.367 for continuous variables in model 3). However, when cardiovascular mortality was analyzed, a statistically significant interaction term between vegetable intake (as continuous variable) and the OGG1 polymorphism in determining cardiovascular mortality in the whole population (P-interaction=0.035 in model 1, which remained statistically significant in model 3, P-interaction=0.046) was detected. According to this interaction, a high vegetable intake decreased the risk of cardiovascular mortality more in Cys326Cys individuals than in Ser-carriers: HR-interaction: 0.42; 95%CI: 0.18-0.98, per 1 SD (150 g/d) of vegetable intake. When vegetable

intake was analyzed as dichotomous (2 groups according to the median intake of the population), it was observed that the Cys326Cys genotype was associated with higher cardiovascular mortality in comparison with Ser-carriers (P<0.001 in model 3), in participants having a low vegetable intake (<314 g/d). However, Cys326Cys participants having a high vegetable intake (>=314 grams/d) did not present a statistically significant higher risk of cardiovascular mortality in comparison with Ser-carriers (P=0.671). Although the P-value for the corresponding interaction term did not reach the statistical significance (P=0.101, in model 3) for the dichotomous variable of vegetable intake, due to very small number of Cys326Cys participants, this observation was supported by the statistical significance of the interaction term between the OGG1 genotype and vegetable intake as continuous variable.

In the subgroup analysis in participants aged ≥ 66.5 years, a statistically significant interaction between vegetable intake (as continuous variable) and the OGG1 polymorphism in determining total mortality (HR-interaction: 0.49; 95%CI: 0.25-0.96; P=0.037 per SD, in model 3) was obtained. Also in participants aged ≥ 66.5 years, the interaction term between vegetable intake (as continuous) and the OGG1 polymorphism was statistically significant for cardiovascular mortality (HR-interaction: 0.30; 95%CI: 0.11-0.83; P=0.021, per SD, in model 3).

DISCUSSION

In this study the influence of the OGG1-rs1052133 (Ser326Cys) polymorphism on total and cause-specific mortality, including cancer and cardiovascular mortality, has been longitudinally investigated in a cohort of older participants in the PREDIMED study. This polymorphism, in which the Cys326Cys genotype has been associated with a lower damage repair capacity in DNA[15-17], has also been associated with a higher risk of cancer and other diseases related to DNA repair in many studies[7,18-27]. However, no previous study has jointly

analyzed the impact of this polymorphism on total mortality and in a comparative manner on cancer and cardiovascular mortality in the same population. In this sense, the current study results on the contribution of the OGG1-rs1052133 genotypes to the mortality rate per 1,000 (person-years of follow-up) as well as to the mortality risk, are novel.

Overall, a statistically significant association of the OGG1-rs1052133 (Ser326Cys) polymorphism with all-cause mortality has been found; the mortality risk of Cys326Cys participants being 1.69 times higher than that of the other genotypes (recessive effects). This association was stronger for cardiovascular mortality, whereas for cancer mortality no association was detected for the OGG1-rs1052133 polymorphism in the whole population. The association with cancer was only statistically significant in participants aged less than 66.5 years at baseline. The observation of recessive effects limited the statistical power of our subsequent gene-diet interaction analyses[44]. Moreover, the small number of Cys326Cys participants may have led to an overestimation of effect size in some associations, in the so-called winner's curse[45]. This term refers to the phenomenon by which studies that first find evidence of an effect often provide inflated estimates of the size of that effect[45]. Effect inflation is worse for small, low-powered studies. However, despite some inflation of the effects, a true association effect can be present in large, well-designed prospective studies[46,47]. Therefore, it can be assumed that some associations found in the present study, mainly those obtained in subgroup analyses, may be overestimated due to the low number of Cys326Cys carriers. Supporting a true association, the current study's results are consistent with dozens of previous studies in animal models that show harmful health effects associated with a reduced DNA repair capacity of the variants in the OGG1 gene[7,48-52] They are also consistent with work in humans that associate the Cys326 variant with a higher risk of cancer[11-27] as well as other diseases[2,33,53-55]. However, as far as we know, no previous study has estimated the influence of this polymorphism on total mortality. One of the factors that can help

explain the strong associations found between the OGG- rs1052133 polymorphism and mortality is that a high cardiovascular risk population is being analyzed. In a subsample of this population[56], higher levels of the DNA- damaged product 8-oxo-7′8′-dihydro-2′-deoxyguanosine (8-oxo-dG) were previously detected in nucleated blood cells in comparison with participants from the general population (not at high cardiovascular risk) paired by age and sex (5.61±1.17 in PREDIMED participants versus 3.71±0.65 in non-high cardiovascular risk participants, expressed as 8-oxo-dG/10^6dG; P<0.001). This is relevant considering the reports on the impaired DNA repair capacity of the Cys326Cys variant being enhanced under conditions of oxidative stress[17], largely increasing the risk of oxidative patothologies[18].

Although several studies have analyzed the influence of the OGG1-rs1052133 on cancer incidence or prevalence[19-27], no previous study at the population level has analyzed the association of such polymorphism with cancer mortality. Some studies have analyzed the influence of the OGG1-rs1052133 polymorphism on the survival or prognosis of selected groups of patients receiving cancer treatment[57, 58], but there are no estimates of mortality rates in a general population cohort. Although in our cohort at high cardiovascular risk deaths from cancer outnumbered those from cardiovascular disease, no association between the Cys326Cys genotype and cancer mortality was observed in the whole population. However, a strong association was detected between the Cys326Cys risk genotype and cardiovascular mortality. Although in comparison to studies that have examined the possible association between the OGG1-rs1052133 polymorphism and cancer[18-31], very few have examined its association with cardiovascular disease[33-35,54], studies in animal models on OGG1 function strongly support this association[32,59,60]. Thus, in a study by Tumurkhuu et al[32] in Ogg1(-/-) mice, the authors observed a more atherogenic profile of the different markers analyzed in comparison with mice with a normal Ogg1 gene expression. In the Ogg1 (-/-) mice, higher serum IL-1β and IL-18 levels,

higher oxidized mitochondrial DNA and higher inflammasome activation were detected. Taking into account that OGG1 is the major DNA glycosylase responsible for removing the most abundant products of oxidative DNA damage, it is not surprising to find a pro-atherosclerotic phenotype in mice deficient in the ogg1 gene. Interestingly, these authors also reported higher levels of triglycerides in deficient mice[32]. Interestingly, in PREDIMED participants, higher plasma triglycerides in Cys326Cys participants were also detected. Overall, OGG1 may play a protective role in atherogenesis by preventing excessive inflammasome activation[32]. In humans, most of the few studies carried out on cardiovascular disease[33-35,54] also have found a higher risk associated with the Cys326 allele. Thus, Izzoti et al[33] examined the survival of patients with severe atherosclerosis and concluded that those bearing the OGG1 homozygous slow polymorphism had increased levels of two bulky DNA adducts, being more susceptible than other individuals to the genotoxic consequences of oxidative stress in the arterial wall. Orhan et al[35] also concluded that the OGG1-rs1052133 played a role in stroke risk, and Shyu et al[34] reported an effect of smoking increasing stroke risk in Chinese carriers of the Cys allele. The present study results showing a strong association between the OGG1-rs1052133 polymorphism and cardiovascular mortality in Cys326Cys homozygotes concur with these findings. Because a high cardiovascular risk population was analyzed, it is no surprise that the association of the OGG1-rs1052133 polymorphism was stronger for cardiovascular disease mortality than cancer mortality.

Of note, cardiovascular mortality is also gaining in importance in cancer patients[61,62], as their increased survival allows them to reach older ages in which their risk of death may be determined by cardiovascular risk factors. For instance, in a population-based cohort study conducted among 98,999 women diagnosed with early-stage breast cancer, those 66 years or older who survived 5 years or more after diagnosis had cardiovascular disease as the leading

cause of death, exceeding breast cancer mortality rates at 10 years after diagnosis[62].

Age is an important determinant of mortality. The mean age of the deceased due to cancer in the PREDIMED cohort was significantly lower than the mean age of the deceased due to cardiovascular diseases. Interestingly, it was found that, in the younger age group (<66.5 years), the OGG1-rs1052133 polymorphism was indeed more associated with cancer mortality than cardiovascular mortality. Conversely, the association of the OGG1 polymorphism with higher cardiovascular mortality was mainly detected in the older age group. This may be explained by the age-dependent reduction of the DNA repair efficiency, enhanced in Cys326Cys participants[2]. In younger participants, the increased cancer mortality associated with this polymorphism may be associated with an additional genetic component related to specific locations (i.e. BRCA1, BRCA2, etc.) in which the OGG1-risk genotype may contribute to enhance the genome instability that increases the risk, being also considered as a cancer risk modifier[63].

When analyzing gene-diet interactions, sample size limitations due to the recessive effect and the relatively low prevalence of the Cys326Cys genotype in this population (4.2%) prevented examination of the dietary modulation of the effects of the OGG1-rs1052133 polymorphism on cancer mortality (only 6 deaths with the Cys326Cys genotype were detected). Related to this, it is known that the prevalence of the OGG1-rs1052133 polymorphism is lower in white (1.8-8.6 per cent Cys326Cys participants) than in Asian populations (13.4-38.2 per cent Cys326Cys)[64]. However, bearing this limitation in mind, it was possible to explore dietary modulation in determining all-cause mortality and cardiovascular mortality (involving more homozygotes). When testing whether intervention with the MedDiet modulated the effect of the Cys326Cys genotype increasing total mortality a statistically significant interaction was not found. Likewise, for cardiovascular mortality in the whole population, the interaction term between the OGG1 genotype and MedDiet did not reach statistical significance. Further studies are needed to provide

464 further evidence on the modulation of the MedDiet intervention on the effects of the OGG1-
465 rs1052133 polymorphism on mortality risk.

466 The MedDiet is characterized by a high intake of vegetables[37,65]. Vegetables are very rich
467 in antioxidants and other phytochemicals[66,67] that may contribute to a better DNA protection from
468 oxidation in Cys326Cys individuals who have less capacity for repairing it[68-70]. Recent meta-
469 analyses[71,72] have shown that high vegetable consumption is associated with a lower risk of all-
470 cause mortality[71,72], particularly cardiovascular mortality[72]. Although no previous study has
471 analyzed the interaction between vegetable consumption and the OGG1-rs1052133
472 polymorphism in determining total or cause-specific mortality, this gene-diet interaction on
473 cancer risk has been analyzed in some reports[28,73,74]. Noteworthy is the work of Sorensen et al[28],
474 showing a statistically significant interaction between vegetable intake and the OGG1-rs1052133
475 polymorphism on lung cancer incidence, with a 54% decrease in cancer risk per 50% increase in
476 vegetable consumption among Cys326Cys participants and no decrease in risk among Ser326Ser
477 or Ser326Cys individuals. In the PREDIMED study, a similar interaction between theOGG1-
478 rs1052133 polymorphism and vegetable intake in determining cardiovascular mortality in the
479 whole population has been detected, in such a way that a high vegetable intake was associated
480 with a greater reduction of cardiovascular mortality in Cys326Cys homozygotes in comparison
481 with Ser-carriers. This effect had a similar trend for total mortality but only reached significance
482 in the older age group.

483 **CONCLUSIONS**

484 In conclusion, in a Mediterranean population at high cardiovascular disease risk, an association of
485 the OGG1-rs1052133 polymorphism with higher total and cardiovascular mortality in
486 Cys326Cys homozygotes has been found, while higher cancer mortality was only detected in the
487 lower age group. Recessive effects limited the study of gene-diet interactions. Non-significant

interaction terms were detected for the MedDiet intervention. Nevertheless, a significant gene-diet interaction with vegetable consumption in determining cardiovascular mortality has been observed, in such a way that higher consumption decreased the risk more in Cys326Cys participants, supporting the beneficial role of the antioxidant compounds present in vegetables in providing protection from DNA damage and mortality risk in genetically susceptible individuals. However, replication of these results in other studies is needed to confirm these associations and dietary modulations.

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678

LEGEND TO FIGURE

Figure 1: Cumulative mortality-free survival by the OGG1-rs1052133 (Ser326Cys) polymorphism for total mortality in the whole population (**A**), cardiovascular mortality in the whole population (**B**), cancer mortality in the whole population (**C**) and cancer mortality in participants aged less than 66.5 years (**D**). Kaplan-Meier curves were depicted for the three genotypes, the one letter code was used for the amino acids (S indicated serine and C indicates cysteine) (n = 4519 SS, n = 2349 SC and n = 302 SS in the whole population). In the group of participants aged less than 66.5 years, n=3515 individuals. Multivariable Cox regression models were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI). Models were adjusted for age, sex, field center, dietary intervention group, type-2 diabetes, BMI, self-reported personal history of a previously diagnosed cancer at baseline, alcohol consumption, smoking, physical activity, hypertension, dyslipidemia, medications (lipid-lowering, hypoglycemic, and antihypertensive drugs) and adherence to the Mediterranean Diet. P^1 indicates the P-value for the comparison between CC and CS genotypes in the multivariable Cox regression model. HR and CI were estimated in the corresponding multivariable Cox regression models for CC participants in comparison with SS (P^2) or in comparison with SS and SC grouped together (recessive model) (P^3) for each cause of death.

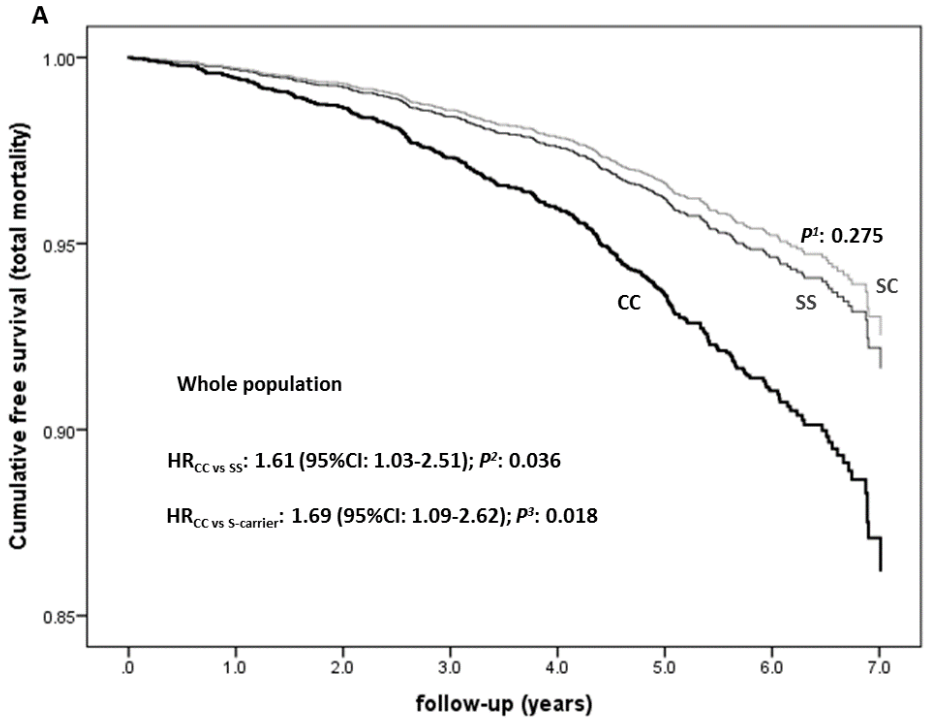
CONSORT 2010 checklist of information to include when reporting a randomised trial*

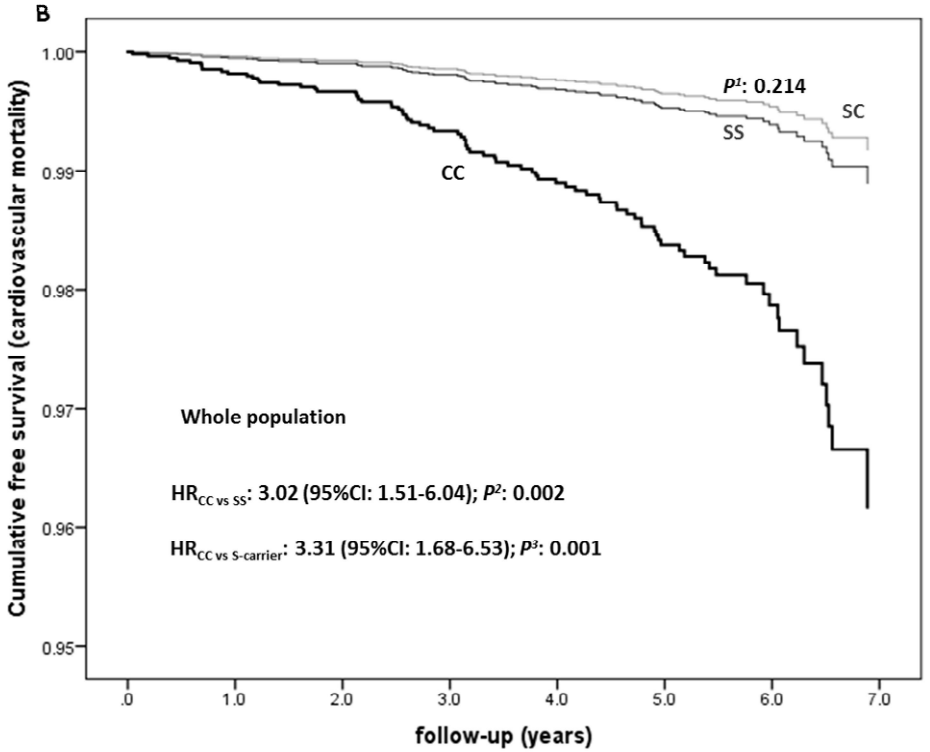
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1-3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	8-11
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

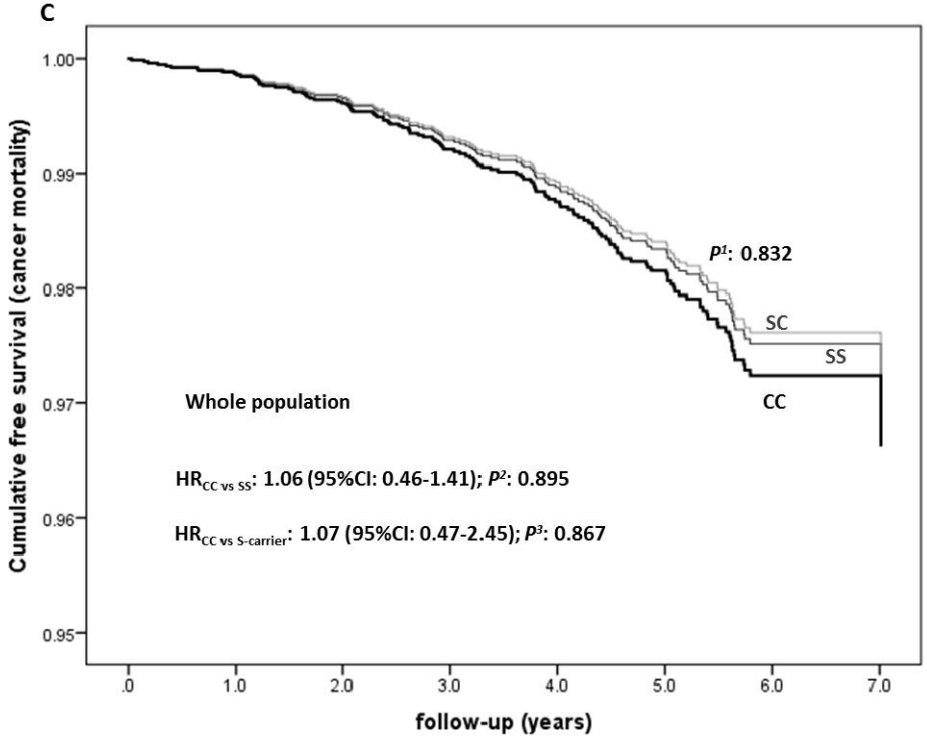
		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	7
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10-11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Table 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	N/A
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8,11
	14b	Why the trial ended or was stopped	7
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 5
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Tables 3, 5
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	11-16
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 6
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	22
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	22
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	22
Other information			
Registration	23	Registration number and name of trial registry	3,6
Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	N/A

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Figure 1


A
Cumulative free survival (total mortality)
1.00
0.95
0.90
0.85
.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
follow-up (years)
P1: 0.275
SC
SS
CC
Whole population
HRCC vs SS: 1.61 (95%CI: 1.03-2.51); P2: 0.036
HRCC vs S-carrier: 1.69 (95%CI: 1.09-2.62); P3: 0.018


B
Cumulative free survival (cardiovascular mortality)
1.00
0.99
0.98
0.97
0.96
0.95
.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
follow-up (years)
P1: 0.214
SC
SS
CC
Whole population
HRCC vs SS: 3.02 (95%CI: 1.51-6.04); P2: 0.002
HRCC vs S-carrier: 3.31 (95%CI: 1.68-6.53); P3: 0.001


C
Cumulative free survival (cancer mortality)
1.00
0.99
0.98
0.97
0.96
0.95
.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
follow-up (years)
P1: 0.832
SC
SS
CC
Whole population
HRCC vs SS: 1.06 (95%CI: 0.46-1.41); P2: 0.895
HRCC vs S-carrier: 1.07 (95%CI: 0.47-2.45); P3: 0.867

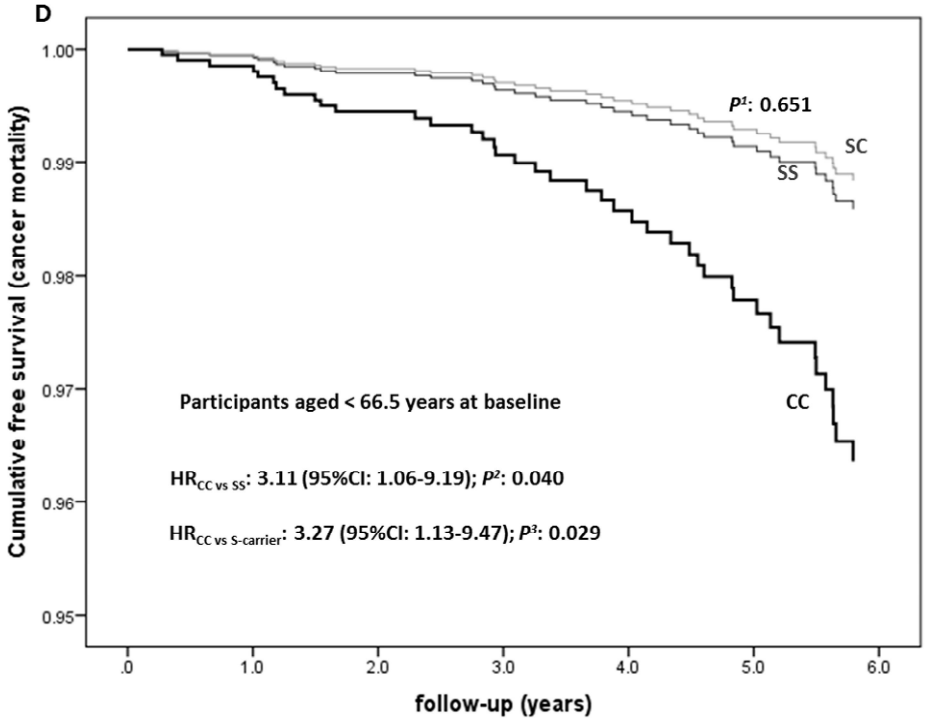

D
Cumulative free survival (cancer mortality)
1.00
0.99
0.98
0.97
0.96
0.95
.0
1.0
2.0
3.0
4.0
5.0
6.0
follow-up (years)
P1: 0.651
SC
SS
CC
Participants aged < 66.5 years at baseline
HRCC vs SS: 3.11 (95%CI: 1.06-9.19); P2: 0.040
HRCC vs S-carrier: 3.27 (95%CI: 1.13-9.47); P3: 0.029

Table 1

Table 1: Demographic, clinical, lifestyle and genetic characteristics of the PREDIMED study participants at baseline according to the OGG1-rs1052133 genotype (n = 7,170)[a]

	Ser326Ser (n=4,519)	Ser326Cys (n=2,349)	Cys326Cys (n=302)	P[b]
Age (years)	66.9 (6.2)	67.0 (6.2)	67.3 (6.2)	0.526
BMI (kg/m^2)[c]	29.9 (3.9)	30.1 (3.8)	29.5 (3.7)	0.016
Female sex : n, %	2601 (57.6)	1346 (57.3)	171 (56.6)	0.939
Current smokers: n, %	661 (14.6)	300 (12.8)	41 (13.6)	0.204
Type 2 diabetes: n, %	2195 (48.6)	1127 (48.0)	142 (47.0)	0.807
Hypertension: n, %	3729 (82.5)	1959 (83.4)	248 (82.1)	0.626
Dyslipidemia: n, %	3259 (72.1)	1707 (72.7)	225 (74.5)	0.627
OGG1-rs1052133: n, %				0.069
MedDiet with EVOO[d]	1550 (62.7)	817 (33.0)	106 (4.3)	
MedDiet with Nuts	1525 (64.5)	729 (32.6)	110 (4.7)	
Control group	1444 (61.9)	803 (34.4)	86 (3.7)	
SBP (mm Hg)[e]	149.3 (20.5)	149.5 (21.3)	148.1 (20.1)	0.543
DBP (mm Hg)[f]	83.3 (11.0)	83.4 (11.0)	83.9 (11.2)	0.676
Total cholesterol (mg/dL)[g]	210.4 (38.4)	210.7 (37.8)	208.6 (38.3)	0.697
LDL-C (mg/dL)[g,h]	129.4 (33.8)	130.4 (33.4)	125.9 (34.1)	0.083
HDL-C (mg/dL)[g,i]	53.9 (14.1)	53.7 (13.4)	53.4 (14.5)	0.762
Triglycerides (mg/dL)[j]	136.7 (74.9)	135.1 (70.9)	149.6 (89.7)	0.018
Fasting glucose (mg/dL)[k]	121.9 (40.5)	122.5 (41.7)	122.6 (46.1)	0.838
Energy intake (kcal/d)	2273 (598)	2275 (614)	2321 (647)	0.411
Total fat (g/d)	98.6 (30.1)	98.7 (30.7)	100.6 (30.9)	0.554
Saturated fat (g/d)	25.2 (9.1)	25.4 (9.2)	25.8 (10.1)	0.368
MUFA (g/d)[l]	48.9 (16.0)	48.7 (16.1)	49.8 (15.2)	0.530
PUFA (g/d)[m]	15.8 (7.0)	15.9 (7.1)	16.2 (6.9)	0.663
Protein (g/d)	92.2 (22.9)	93.0 (23.4)	94.9 (25.0)	0.087
Carbohydrate (g/d)	239.4 (79.9)	238.8 (82.5)	245.6 (87.0)	0.395
Fat (% energy)	39.2 (6.8)	39.2 (6.8)	39.2 (6.8)	0.554
Carbohydrate (% energy)	41.9 (7.2)	41.7 (7.1)	42.0 (7.0)	0.395
Protein (% energy)	92.2 (22.9)	93.0 (23.4)	94.9 (25.0)	0.087
Fiber (g/d)	25.6 (9.0)	25.7 (9.4)	26.0 (8.9)	0.604

Vegetable (g/d)	334.9	(146.4)	340.9	(156.8)	339.8	(151.0)	0.281
Fruit (g/d)	371.2	(206.2)	373.2	(210.3)	364.7	(192.1)	0.780
Meat (g/d)	131.8	(60.0)	133.1	(58.4)	139.7	(62.7)	0.075
Olive oil (g/d)	39.5	(17.9)	38.8	(18.2)	40.2	(16.7)	0.266
Adherence to the MedDiet (points)[n]	8.6	(2.0)	8.6	(1.9)	8.7	(1.9)	0.969
Alcohol consumption (g/d)	8.4	(14.2)	8.5	(14.4)	7.7	(13.0)	0.685
Physical activity (MET-min/day)[o]	233	(239)	230	(243)	228	(225)	0.904

[a]: Values are mean(SD) for continuous variables and number (%) for categorical variables. Food intake, total energy and macronutrients were available in 7,122 participants. Biochemical determinations were available for almost 7,000 participants (from 6,767 for LDL-C to 6,903 for Total cholesterol).
[b]: *P* unadjusted.
[c]: BMI: body mass index;
[d]: EVOO: extra virgin olive oil;
[e]: SBP: Systolic blood pressure,
[f]: DBP: Diastolic blood pressure;
[g]: Cholesterol conversion units: 1 mg/dL = (1/38.610039) mmol/L;
[h]: LDL-C: Low-Density Lipoprotein Cholesterol;
[i]: HDL-C: High-Density Lipoprotein Cholesterol;
[j]: Triglycerides conversion units: 1 mg/dL = (1/88.495575) mmol/L;
[k]: Glucose conversion units: 1 mg/dL = (1/18.018018) mmol/L;
[l]: MUFA: Monounsaturated fatty acids;
[m]: PUFA: Polyunsaturated fatty acids;
[n]: MedDiet: Mediterranean diet; Adherence to the MedDiet (ADM) score based on a 14-point screener of adherence: a higher score represents greater ADM[38];
[o]: MET: metabolic equivalent of physical activity in leisure time;

Table 2: Baseline characteristics at the time of entry and future cause of death after 4.8 years of median follow-up of the PREDIMED study participants by vital status[a]

	Alive (n=6,852)	Cancer (n=127)	CVD[b] (n=81)	Other (n=110)	P[c]	Total deaths (n=318)	P[d]
Age (years)	66.8 (6.1)	68.2 (6.0)	71.8 (6.4)	71.4 (6.6)	< 0.001	70.6 (6.4)	< 0.001
BMI (kg/m^2)[e]	30.0 (3.8)	29.8 (3.9)	29.9 (4.2)	29.2 (4.3)	0.202	30.0 (3.8)	0.101
SBP (mm Hg)[f]	149.2 (20.7)	152.5 (20.5)	156.9 (22.1)	149.6 (22.6)	0.003	152.8 (21.9)	0.003
DBP (mm Hg)[g]	83.4 (11.0)	83.2 (11.0)	82.9 (11.5)	82.8 (11.5)	0.915	83.1 (11.4)	0.629
Energy intake (kcal/d)	2273 (602)	2309 (636)	2423 (699)	2269 (700)	0.154	2323 (675)	0.161
Total fat (% energy)	39.2 (6.8)	38.1 (6.7)	39.2 (6.5)	39.9 (7.7)	0.270	39.0 (7.1)	0.754
Saturated fat (% energy)	10.0 (2.2)	9.8 (2.4)	10.6 (2.4)	10.5 (2.3)	0.003	10.3 (2.4)	0.023
MUFA (% energy)[h]	19.5 (4.5)	19.0 (4.2)	18.9 (4.5)	20.1 (5.7)	0.199	19.3 (4.8)	0.613
PUFA (% energy)[i]	6.2 (2.1)	6.1 (2.2)	6.0 (2.4)	6.2 (2.0)	0.567	6.1 (2.2)	0.186
Protein (% energy)	16.6 (2.8)	16.3 (2.9)	16.3 (3.2)	16.7 (3.4)	0.486	16.5 (3.2)	0.437
Carbohydrate (% energy)	41.9 (7.1)	42.1 (7.0)	41.3 (7.2)	41.3 (7.7)	0.766	41.6 (7.3)	0.465
ADM (points)[j]	8.7 (2.0)	8.5 (1.9)	8.1 (2.0)	8.5 (2.0)	0.068	8.4 (2.0)	0.029
Sex : n, %					< 0.001		< 0.001
Male : n, %	2857 (93.6)	77 (2.5)	52 (1.7)	66 (2.2)		195 (6.4)	
Female : n, %	3996 (97.0)	50 (1.2)	29 (1.0)	43 (1.0)		123 (3.0)	
History of cancer: n, %					< 0.001		< 0.001
Yes : n, %	184 (88.9)	17 (8.2)	2 (1.0)	4 (1.9)		23 (11.1)	
No : n, %	6668 (95.8)	110 (1.6)	79 (1.1)	105 (1.5)		295 (4.2)	
Type 2 diabetes: n, %					< 0.001		< 0.001
Yes : n, %	3269 (94.4)	68 (2.0)	52 (1.5)	75 (2.2)		196 (5.7)	
No : n, %	3584 (96.7)	59 (1.6)	29 (0.8)	34 (0.9)		122 (3.3)	
OGG1-rs1052133: n, %					0.003		0.016
Ser326Ser	4318 (95.6)	81 (1.8)	50 (1.1)	70 (1.5)		201 (4.4)	
Ser326Cys	2256 (96.0)	40 (1.7)	20 (0.9)	34 (1.4)		94 (4.0)	
Cys326Cys	279 (92.4)	6 (2.0)	11 (3.6)	6 (2.0)		23 (7.6)	
OGG1-rs1052133: n, %					< 0.001		0.006
Ser-carrier	6574 (95.7)	121 (1.8)	70 (1.0)	104 (1.5)		295 (4.3)	
Cys326Cys	279 (92.4)	6 (2.0)	11 (3.6)	6 (2.0)		23 (7.6)	

[a]: Values are mean(SD) for continuous variables and number (%) for categorical variables;
[b]: CVD: Cardiovascular diseases;
[c]: Unadjusted *P*-value for the comparison among the 4 groups;
[d]: Unadjusted *P*-value for the comparison between total deaths and alive;
[e]: BMI: body mass index;
[f]: SBP: Systolic blood pressure,
[g]: DBP: Diastolic blood pressure;
[h]: MUFA: Monounsaturated fatty acids;
[i]: PUFA: Polyunsaturated fatty acids;
[j]: MedDiet: Mediterranean diet; Adherence to the MedDiet (ADM) score based on a 14-point screener of adherence: a higher score represents greater ADM[38].

Table 3. Mortality rate and hazard ratios (HR) for total mortality and cause-specific mortality (cancer and cardiovascular) in the PREDIMED participants depending on the OGG1-rs1052133 polymorphism, after 4.8 years of median follow-up

	Whole population (n = 7,170)									
			Model 1[a]			Model 2[b]			Model 3[c]	
OGG1-rs1052133 genotypes	Deaths / person-y	Mortality rate[d]	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Total mortality (deaths: 318)											
Codominant model											
Ser326Ser	201/19502	10.3	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Ser326Cys	94/10085	9.3	0.89	(0.70-1.14)	0.356	0.88	(0.68-1.12)	0.285	0.87	(0.68-1.03)	0.275
Cys326Cys	23/1302	17.7	1.70	(1.10-2.63)	0.016	1.70	(1.10-2.61)	0.017	1.61	(1.03-2.51)	0.036
Recessive model											
Ser-carriers	295/29587	10.0	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	23/1302	17.7	1.77	(1.16-2.71)	0.009	1.77	(1.16-2.71)	0.009	1.69	(1.09-2.62)	0.018
Cancer mortality (deaths: 127)											
Recessive model											
Ser-carriers	121/29587	4.1	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	6/1302	4.6	1.13	(0.50-2.57)	0.771	1.12	(0.49-2.53)	0.796	1.07	(0.47-2.45)	0.867
Cardiovascular mortality (deaths: 81)											
Recessive model											
Ser-carriers	70/29587	2.4	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	11/1302	8.4	3.87	(2.03-7.36)	< 0.001	3.86	(2.02-7.35)	< 0.001	3.31	(1.68-6.53)	0.001

[a]: Model 1: Adjusted for sex, age, center and dietary intervention group.

[b]: Model 2: Adjusted for variables in model 1 plus body mass index, type-2 diabetes and self-reported cancer history at baseline.

c: Model 3: Adjusted for variables in model 2 plus drinking, smoking, physical activity, adherence to Mediterranean diet and medications (hypertension, dyslipemia and type-2 diabetes) at baseline.

[d]: Mortality rates were expressed per 1000 person-years of follow-up.

Table 4

Table 4. Mortality rate and hazard ratios (HR) for total mortality and cause-specific mortality (cancer and cardiovascular) in the PREDIMED participants depending on the OGG1-rs1052133 polymorphism, after 4.8 years of median follow-up. Stratified analysis by age group[a]

			Model 1[b]			Model 2[c]			Model 3[d]		
OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate[e]	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Total mortality (deaths: 318)											
Age group < 66.5 years (n = 3515)											
Ser-carriers	80/14402	5.6	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	7/584	12.0	2.27	(1.04-4.95)	0.039	2.33	(1.07-5.08)	0.034	2.63	(1.19-5.83)	0.017
Age group ≥ 66.5 years (n = 3655)											
Ser-carriers	215/15216	14.1	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	16/719	22.3	1.67	(1.00-2.78)	0.051	1.67	(1.00-2.78)	0.051	1.62	(0.95-2.75)	0.077
P (interaction OGG1 x Age group)[f]					0.627			0.570			0.570
Cancer mortality (deaths: 127)											
Age group < 66.5 years (n = 3515)											
Ser-carriers	37/14402	2.6	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	4/584	6.8	2.77	(0.98-7.84)	0.055	3.00	(1.05-8.54)	0.040	3.27	(1.13-9.47)	0.029
Age group ≥ 66.5 years (n = 3655)											
Ser-carriers	84/15216	5.5	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	2/719	2.8	0.52	(0.13-2.14)	0.360	0.50	(0.12-2.04)	0.333	0.46	(0.11-1.90)	0.285
P (interaction OGG1 x Age group)[f]					0.063			0.047			0.048
Cardiovascular mortality (deaths: 81)											
Age group < 66.5 years (n = 3515)											
Ser-carriers	19/14402	1.3	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	1/584	1.7	1.37	(0.19-10.36)	0.761	1.40	(0.19-10.60)	0.744	1.88	(0.23-15.20)	0.555
Age group ≥ 66.5 years (n = 3655)											
Ser-carriers	51/15216	3.4	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	10/719	13.9	4.89	(2.43-9.78)	<0.001	5.00	(2.48-10.01)	<0.001	4.60	(2.18-9.71)	<0.001
P (interaction OGG1 x Age group)[f]					0.219			0.212			0.234

[a]: Age groups were considered taking into account the median of age at baseline.
[b]: Model 1: Adjusted for sex, age, center and dietary intervention group.
[c]: Model 2: Adjusted for variables in model 1 plus body mass index, type-2 diabetes and self-reported cancer history at baseline.
d: Model 3: Adjusted for variables in model 2 plus drinking, smoking, physical activity, adherence to Mediterranean diet and medications (hypertension, dyslipemia and type-2 diabetes) at baseline.

[e]: Mortality rates were expressed per 1000 person-years of follow-up.
[f]: *P*-values obtained for multiplicative interaction terms in the corresponding multivariable-adjusted Cox regression model.

Table 5. Mortality rate and hazard ratios (HR) for total mortality and cardiovascular mortality in the PREDIMED participants according to the OGG1-rs1052133 polymorphism, after 4.8 years of median follow-up, depending on the Mediterranean diet intervention[a]

	Whole population (n = 7,170)									
			Model 1[b]			Model 2[c]			Model 3[d]	
OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate[e]	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Total mortality (deaths: 318)											
Mediterranean diet (n = 4837)											
Ser-carriers	202/20655	9.8	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	16/952	16.8	1.61	(0.97-2.69)	0.068	1.66	(0.99-2.76)	0.071	1.61	(0.96-2.69)	0.070
Control group (n = 2333)											
Ser-carriers	93/8963	10.4	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	7/349	20.0	2.09	(0.97-4.54)	0.061	2.04	(0.94-4.43)	0.071	2.14	(0.98-4.65)	0.056
P (interaction OGG1 x Intervention group)[f]					0.469			0.558			0.752
Cardiovascular mortality (deaths: 81)											
Mediterranean diet (n = 4837)											
Ser-carriers	45/20655	2.2	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	6/952	6.3	2.73	(1.16-6.48)	0.020	2.78	(1.17-6.60)	0.020	2.60	(1.07-6.22)	0.034
Control group (n = 2333)											
Ser-carriers	25/8963	2.8	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	5/349	14.3	7.48	(2.77-20.16)	<0.001	8.16	(3.00-22.20)	<0.001	7.89	(2.48-25.11)	<0.001
P (interaction OGG1 x Intervention group)[f]					0.181			0.167			0.200

[a]: Both, Mediterranean diet intervention groups, were analyzed together.
[b]: Model 1: Adjusted for sex, age, center and dietary intervention group.
[c]: Model 2: Adjusted for variables in model 1 plus body mass index, type-2 diabetes and self-reported cancer history at baseline.
[d]: Model 3: Adjusted for variables in model 2 plus drinking, smoking, physical activity, adherence to Mediterranean diet, medications (hypertension, dyslipemia and type-2 diabetes) and total energy intake at baseline. Energy intake data in Model 3 were only available in 7,122 participants.
[e]: Mortality rates were expressed per 1000 person-years of follow-up.
[f]: *P*-values obtained for multiplicative interaction terms in the corresponding multivariable-adjusted Cox regression model.

Table 6. Mortality rate and hazard ratios (HR) for total mortality and cardiovascular mortality in the PREDIMED participants according to the OGG1-rs1052133 polymorphism, after 4.8 years of median follow-up, depending on vegetable intake[a]

	Whole population (n = 7,122)										
			Model 1[b]			Model 2[c]			Model 3[d]		
OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate[e]	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Total mortality (deaths: 313)											
Vegetable intake (2 groups)											
Low intake (< 314 g/d) (n = 3532)											
Ser-carriers	165/14910	11.1	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	14/650	21.5	2.01	(1.16-3.49)	0.013	1.97	(1.14-3.41)	0.016	1.92	(1.16-3.36)	0.022
High intake (>=314 g/d) (n = 3580)											
Ser-carriers	126/14517	8.7	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	8/639	12.5	1.36	(0.66-2.79)	0.407	1.43	(0.69-2.93)	0.333	1.37	(0.66-2.81)	0.395
P (interaction OGG1 x Vegetable intake)[f]					0.446			0.444			0.491
Vegetable intake (as continuous)											
Interaction term OGG1 x Vegetables[g]			0.75	(0.45-1.25)	0.268	0.79	(0.48-1.30)	0.360	0.80	(0.49-1.30)	0.367
Cardiovascular mortality (deaths: 80)											
Vegetable intake (2 groups)											
Low intake (< 314 g/d) (n = 3532)											
Ser-carriers	39/14910	2.6	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	8/650	12.3	5.23	(2.40-11.38)	<0.001	5.15	(2.36-11.24)	<0.001	5.21	(2.36-11.52)	<0.001
High intake (>=314 g/d) (n = 3580)											
Ser-carriers	31/14517	2.1	1.00	(ref.)		1.00	(ref.)		1.00	(ref.)	
Cys326Cys	2/639	3.1	1.18	(0.28-5.05)	0.823	1.26	(0.29-5.40)	0.757	1.38	(0.31-6.19)	0.671
P (interaction OGG1 x Vegetable intake)[f]					0.096			0.120			0.101
Vegetable intake (as continuous)											
Interaction term OGG1 x Vegetables[g]			0.37	(0.15-0.93)	0.035	0.38	(0.15-0.96)	0.041	0.42	(0.18-0.98)	0.046

[a]: Vegetable intake were analyzed as categorical (2 groups based on the median population intake) and as continuous variable (g/d). This variable was standardized and HRs were expressed per 1 standard deviation (approx. 150 g/d). Vegetable intake data were only available in 7,122 participants. In PREDIMED, one average serving of vegetables was estimated in 125 g/d. Then, 314 g/d of vegetables are equivalent to 2.5 servings/d.

[b]: Model 1: Adjusted for sex, age, center and dietary intervention group.

[c]: Model 2: Adjusted for variables in model 1 plus body mass index, type-2 diabetes and self-reported cancer history at baseline.

[d]: Model 3: Adjusted for variables in model 2 plus drinking, smoking, physical activity, adherence to Mediterranean diet, medications (hypertension, dyslipemia and type-2 diabetes) and total energy intake at baseline.

[e]: Mortality rates were expressed per 1000 person-years of follow-up.

[f]: *P*-values obtained for multiplicative interaction terms between the OGG1 genotype and vegetable intake, as categorical, in the corresponding multivariable-adjusted Cox regression model.

[g]: HR 95% confidence interval and *P*-value for multiplicative interaction terms, between the OGG1 genotype and vegetable intake (as continuous), in the corresponding multivariable-adjusted Cox regression model. HRs are expressed per 1 standard deviation increase in vegetable intake.