

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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2 **cardiovascular and all-cause mortality in the PREDIMED study: Modulation by the**
3 **Mediterranean diet**

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6 **RESEARCH SNAPSHOT**

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

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

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20 **ABSTRACT**

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

26 death and modulation by diet.

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

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

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

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

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

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

39 **Results:** 318 deaths were detected (cancer=127, CVD=81 and others=110). Cys326Cys
40 individuals (prevalence 4.2%) presented higher total mortality rates than Ser326-carriers
41 (P=0.009). The multivariable-adjusted Hazard Ratio (HR) for Cys326Cys versus Ser326-carriers
42 was 1.69 (95%CI:1.09-2.62); P=0.018. This association was greater for CVD mortality
43 (P=0.001). No relationship was detected for cancer mortality in the whole population (HR:1.07;
44 95%CI:0.47-2.45; P=0.867), but a significant age interaction (P=0.048) was observed as
45 Cys326Cys was associated with cancer mortality in participants <66.5 years (P=0.029).

46 Recessive effects limited our ability to investigate Cys326Cys*diet interactions for cancer
47 mortality. No statistically significant interactions for total or CVD mortality were found for the
48 MedDiet intervention. However, significant protective interactions for CVD mortality were found
49 for vegetable intake (HR-interaction per standard deviation: 0.42;95%CI:0.18-0.98, P=0.046).

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

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56 INTRODUCTION

57 DNA molecules are exposed to the attack of DNA-damaging agents¹, among them
58 reactive oxygen species (ROS)². Oxidatively induced DNA damage can be both mutagenic and
59 cytotoxic³ and has been implicated in the etiology of cancer⁴, neurodegenerative diseases⁵ and
60 overall aging⁶. Hydroxyl radicals preferentially react with the C8 atom of purines in DNA to
61 generate 8-oxo-7,8-dihydroguanine (8-oxoG), 8-oxo-7,8-dihydroadenine (8-oxoA) and
62 formamidopyrimidines (Fapy)⁷. The accumulation of unrepaired DNA damage can cause genetic
63 instability and has deleterious effects on cell function⁸. 8-oxoG is a critical mutagenic lesion
64 because of its propensity to mispair with A during DNA replication⁷. Repair of oxidatively
65 damaged bases occurs primarily via the DNA base excision repair (BER) pathway². In the first
66 step of this type of repair, damaged bases are removed from DNA by DNA glycosylases⁹. The
67 oxyguanine glycosylase 1 (OGG1) is the human DNA glycosylase responsible for removal of the
68 highly mutagenic 8-oxoG from DNA⁷. The OGG1 gene is located in chromosome 3p26.2 and
69 this region has frequently been detected as deleted in various tumors suggesting the loss of this
70 gene as a possible contributor to carcinogenesis^{7,10-13}.

71 The most studied polymorphism in the human OGG1 gene is the rs1052133 (Ser326Cys),
72 a C to G transversion at nucleotide 1245 in exon 7, leading to a serine to cysteine substitution at
73 residue 326¹⁴. This variant is functional and it has been shown that the Cys326 protein has
74 weaker 8-hydroxyguanine-repair capacity than the Ser326 protein¹⁵⁻¹⁷. The deactivation of the
75 OGG1 gene or the presence of a less active variant such as the Cys326 may lead to a higher risk
76 of cancer and oxidation-related pathologies^{7,13,18}. Consequently, this polymorphism has been
77 analyzed as a risk factor in several cancers¹⁹⁻²⁵ (i.e., breast, prostate, lung, colorectal, aero
78 digestive, gastric, **bladder**). The results of meta-analyses for each location are heterogeneous²¹⁻²⁵,
79 but where there is more consensus is in the significant association of the Ser326Cys

80 polymorphism with greater overall risk of cancer when the different locations are pooled^{26,27}.
81 Thus, Zou et al²⁶ in a meta-analysis including 152 case-control studies, concluded that the Cys
82 variant was strongly associated with higher cancer risk. Interestingly, the cancer risk was higher
83 in homozygous individuals for the Cys variant, suggesting a recessive pattern. This observation
84 agrees with several functional studies showing that only homozygous carriers of the Cys allele
85 showed a significantly lower DNA repair activity compared to Ser326Ser^{16,18}. A potential source
86 of the observed heterogeneity found among studies may be the exposure to different
87 environmental factors²⁸⁻³¹ (i.e. mainly vegetable intake and other dietary factors).

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

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

101

102 **METHODS**

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

104 design of which has been described in detail elsewhere³⁶. 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 prevention³⁷. 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 fat³⁷. Because both MedDiet intervention groups had a similar effect³⁷,

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

133 The Institutional Review Board of each participating center approved the study protocol,
134 and all participants provided written informed consent. The trial was stopped following the
135 statistical analysis of data obtained up to December 2010, due to early evidence of the benefit of
136 the MedDiet on the prevention of major cardiovascular events³⁷. This study is based on the data
137 obtained from this follow-up period (median follow-up of 4.8 years) with dietary intervention
138 throughout the entire study time period.

139

140 **Demographic, clinical, anthropometric and dietary measurements**

141 The baseline examination included assessment of standard cardiovascular risk factors,
142 medication use, socio-demographic factors and lifestyle variables by validated
143 questionnaires^{36,38,39}. Adherence to the MedDiet was measured by a validated 14-item
144 questionnaire³⁸. Food and beverage consumption was reported using a validated 137-item
145 semiquantitative food-frequency questionnaire (FFQ)³⁹. Dietary data from the FFQ were
146 obtained for 7,122 participants. Weight and height were measured with calibrated manual or
147 digital scales and a wall-mounted stadiometer, respectively³⁶. Body mass index (BMI) was
148 calculated as kg/m².

149

150 **Biochemical determinations, DNA extraction and genotyping**

151 Fasting glucose and lipids were measured as previously described⁴⁰. Biochemical

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

158

159 **Outcomes and Follow-up**

160 The end points of interest in the present analysis were cancer mortality, cardiovascular
161 mortality and all-cause mortality after the follow-up period. We used the following 4 sources of
162 information to identify deaths: contacts with families of participants, contacts with general
163 practitioners who were responsible for the routine clinical care of participants, yearly
164 consultation of the National Death Index, and a comprehensive yearly review of medical records
165 of all participants by medical doctors who were blinded with respect to the group allocation and
166 all nutritional information. All medical records related to endpoints were examined by the Event
167 Adjudication Committee, whose members were unaware of the dietary information³⁷. Only
168 endpoints that were confirmed by the Event Adjudication Committee were included in the
169 analyses. In this follow-up, all deaths detected in the 7,170 patients analyzed (those that had
170 genotype OGG1 data), and that occurred between 1 October 2003 and 1 December 2010 were
171 included: Total deaths ($n=318$), per total cancer ($n=127$) and per cardiovascular diseases ($n=81$).

172

173 **Statistical analyses**

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

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

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

200 intake in the models analyzing diet.

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

217 In addition to the modulation by MedDiet intervention, as secondary analysis, the
218 influence of total vegetable intake at baseline (observational cohort design) was investigated, as
219 vegetables are a main food of the MedDiet previously reported to statistically interact with the
220 OGG1-rs1052133 polymorphism²⁸. Vegetable intake was used as categorical (dichotomously,
221 using the consumption median of the population as the cut-off point) and as a continuous variable
222 (in grams/day). For the continuous variable, the HRs of mortality per standard deviation (SD) of
223 vegetable intake were calculated. Multivariable Cox regression models were fitted and interaction

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

226 Kaplan-Meier survival curves were plotted to estimate the probability of remaining free of
227 mortality (total or causes) during follow-up. Statistical analyses were performed with the IBM
228 SPSS Statistics version 24.0⁴³. All tests were 2-tailed, and $P < 0.05$ was considered statistically
229 significant.

230

231 **RESULTS**

232 **Descriptive characteristics of participants and causes of death by OGG1-rs1052133** 233 **(Ser326Cys) genotypes**

234 **Table 1** presents demographic, clinical and lifestyle characteristics at baseline of the
235 7,170 PREDIMED participants according to their genotype in the OGG1-rs1052133 (Ser326Cys)
236 polymorphism. Overall, there were no differences among genotypes in the main characteristics
237 analyzed. The only statistically significant differences were observed in BMI and triglycerides.
238 The OGG1 genotypes were equally distributed into the three dietary intervention groups.
239 Following 4.8 years of median follow-up, 318 deaths were confirmed, of which the majority were
240 from cancer (n=127), followed by cardiovascular diseases (n=81) and other causes (n=110
241 deaths). **Table 2** presents the baseline characteristics of the participants depending on whether
242 participants were still alive or had died after 4.8 years of median follow-up. Within the mortality
243 group, the cause of death was also reported. The mean age at baseline of the individuals still
244 living was lower than that of the deceased. Among the deceased, the mean age was lower in those
245 who died from cancer than from cardiovascular diseases. Although, in this study, individuals with
246 **a recently diagnosed cancer** were not included, there were 184 participants with a prior diagnosis
247 of cancer (in any location), presumably cancer-free at enrollment according to self-reports.

248 Greater mortality due to cancer was detected in individuals who had previously been diagnosed
249 with cancer compared to those who had not ($P < 0.001$). The effect was high (HR: 5.91; 95%CI:
250 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
251 mortality, in model 1), so this variable was included as an adjustment variable in the later
252 multivariable Cox regression models. Table 2 also presents the frequencies of the OGG1-
253 rs1052133 polymorphism according to vital status and cause of death. In the model in which the
254 three genotypes were analyzed separately, genotypes Ser326Ser and Ser326Cys were distributed
255 equally among the different causes of death ($P > 0.05$). However, the Cys326Cys genotype
256 differed in some causes of death ($P < 0.05$) and when comparing total mortality. In the recessive
257 model, the Cys326Cys genotype was associated with all-cause mortality ($P = 0.006$), being more
258 frequent in mortality cases than in non-cases, while the highest frequency of the Cys326Cys
259 genotype occurred in cardiovascular diseases. The detection of this recessive effect will limit the
260 statistical power of subsequent comparisons.

261

262 **Multivariable-adjusted associations of the OGG1-rs1052133 polymorphism with total,** 263 **cancer and cardiovascular mortality**

264 **Table 3** presents mortality rates, HRs and 95% CI for the OGG1 genotypes for total,
265 cancer and cardiovascular mortality after 4.8 years of median follow-up (maximum follow-up of
266 7.4 years) obtained in the multivariable-adjusted Cox-regression models (model 1, model 2 and
267 model 3). For all-cause mortality, higher total mortality rates in homozygous Cys326Cys were
268 detected in comparison with the other genotypes (Ser-carriers): HR for total mortality in
269 Cys326Cys participants: 1.77; 95% CI: 1.16-2.71; $P = 0.009$, in the minimally adjusted model 1
270 (adjusted for sex, age, field center and dietary intervention group). After additional multivariable
271 adjustment in model 3 (including BMI, diabetes, self-reported history of cancer, smoking,

272 drinking, physical activity, adherence to the MedDiet and medications), this association remained
273 statistically significant (HR: 1.69; 95% CI:1.09-2.62; P=0.018). On analyzing the specific causes
274 of death separately, a strong association was found between the OGG1 polymorphism and
275 cardiovascular mortality (HR: 3.31; 95% CI: 1.68-6.53; P=0.001 for Cys363Cys participants in
276 comparison with Ser-carriers in the multivariable adjusted model 3). However, on studying the
277 overall association of the OGG- rs1052133 polymorphism with mortality from cancer, even
278 though in this population there were more deaths from cancer than from cardiovascular diseases
279 (n=127 compared to n=81, respectively), no statistically significant association was detected in
280 the case of cancer. Also using a recessive model, the HR for cancer mortality in Cys326Cys
281 individuals in comparison with Ser-carriers was 1.07; 95% CI: 0.47-2.45; P=0.867. Comparing
282 the Cys326Cys with the Ser326Ser, the results of no association were similar. **Figure 1** shows
283 Kaplan Meier curves of cumulative mortality-free survival for total mortality (**A**) cardiovascular
284 (**B**) and cancer mortality (**C**) by the three OGG1-rs1052133 genotypes in the whole population.
285 Bearing in mind that mortality from cancer occurs in younger individuals, whereas mortality
286 from cardiovascular diseases occurs in older individuals, the influence of the age group (two
287 groups according to the median of age at baseline) on the associations of the OGG1
288 polymorphism was analyzed (**Table 4**). It was observed that there was heterogeneity by age in
289 the association of the OGG1- rs1052133 polymorphism with cancer mortality, in such a way that
290 in younger individuals (less than 66.5 years at baseline), the Cys326Cys genotype was
291 significantly associated (Table 4 and Figure **1D**) with higher cancer mortality (HR: 3.27; 95% CI:
292 1.13-9.47; P=0.029 for Cys326Cys participants compared to Ser-carriers in model 3).
293 Nevertheless, in those 66.5 years or older, no significant association was detected (P=0.285). One
294 important limitation in this estimation is the small number of cases of fatal cancer in Cys326Cys
295 homozygous individuals. However, despite this limitation of sample size, a statistically

296 significant interaction term between age group and the OGG1 polymorphism on cancer mortality
297 (P-interaction=0.048 in model 3) was obtained. Cancer deaths (n=41) in participants <66.5 years
298 at baseline were as follows: lung (26.8%), pancreatic-biliary (12.2%), colorectal (9.8%), gastric
299 (7.3%), prostate (7.3%), liver (2.4%), ovary-endometrial (2.4%) and other **locations (31.7%)**.

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

306

307 **Effect of the MedDiet intervention on the association between the OGG1-rs1052133** 308 **polymorphism and mortality**

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

320 reach statistical significance (P-interaction=0.181 in model 1 and P-interaction=0.200 in model
321 3).

322 In subgroup analysis by age we found that for total mortality the interaction term between
323 the OGG1 polymorphism and MedDiet intervention reached statistical significance in
324 participants aged ≥ 66.5 years in model 1 (P-interaction=0.049). However, in model 3 after
325 additional multivariable adjustment (HR for Cys326Cys in the MedDiet group: 1.30; 95%CI:
326 0.65-2.60; P=0.451 versus HR for Cys326Cys in the control group: 2.99; 95%CI:1.34-6.67;
327 P=0.008, in the stratified analysis), the statistical significance of the interaction term for this
328 comparison was lost (P-interaction=0.112). Likewise, for cardiovascular mortality, the interaction
329 terms in this group did not reach statistical significance (P-interaction=0.082 in model 1 and
330 P=0.086 in model 3).

331

332 **Effect of vegetable intake on the association between the OGG1-rs1052133 polymorphism** 333 **and mortality**

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

344 intake was analyzed as dichotomous (2 groups according to the median intake of the population),
345 it was observed that the Cys326Cys genotype was associated with higher cardiovascular
346 mortality in comparison with Ser-carriers ($P < 0.001$ in model 3), in participants having a low
347 vegetable intake (< 314 g/d). However, Cys326Cys participants having a high vegetable intake
348 (≥ 314 grams/d) did not present a statistically significant higher risk of cardiovascular mortality
349 in comparison with Ser-carriers ($P = 0.671$). Although the P-value for the corresponding
350 interaction term did not reach the statistical significance ($P = 0.101$, in model 3) for the
351 dichotomous variable of vegetable intake, due to very small number of Cys326Cys participants,
352 this observation was supported by the statistical significance of the interaction term between the
353 OGG1 genotype and vegetable intake as continuous variable.

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

360

361 **DISCUSSION**

362 In this study the influence of the OGG1-rs1052133 (Ser326Cys) polymorphism on total
363 and cause-specific mortality, including cancer and cardiovascular mortality, has been
364 longitudinally investigated in a cohort of older participants in the PREDIMED study. This
365 polymorphism, in which the Cys326Cys genotype has been associated with a lower damage
366 repair capacity in DNA¹⁵⁻¹⁷, has also been associated with a higher risk of cancer and other
367 diseases related to DNA repair in many studies^{7,18-27}. However, no previous study has jointly

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

372 Overall, a statistically significant association of the OGG1-rs1052133 (Ser326Cys)
373 polymorphism with all-cause mortality has been found; the mortality risk of Cys326Cys
374 participants being 1.69 times higher than that of the other genotypes (recessive effects). This
375 association was stronger for cardiovascular mortality, whereas for cancer mortality no association
376 was detected for the OGG1-rs1052133 polymorphism in the whole population. The association
377 with cancer was only statistically significant in participants aged less than 66.5 years at baseline.
378 The observation of recessive effects limited the statistical power of our subsequent gene-diet
379 interaction analyses⁴⁴. Moreover, the small number of Cys326Cys participants may have led to an
380 overestimation of effect size in some associations, in the so-called winner's curse⁴⁵. This term
381 refers to the phenomenon by which studies that first find evidence of an effect often provide
382 inflated estimates of the size of that effect⁴⁵. Effect inflation is worse for small, low-powered
383 studies. However, despite some inflation of the effects, a true association effect can be present in
384 large, well-designed prospective studies^{46,47}. Therefore, it can be assumed that some associations
385 found in the present study, mainly those obtained in subgroup analyses, may be overestimated
386 due to the low number of Cys326Cys carriers. Supporting a true association, the current study's
387 results are consistent with dozens of previous studies in animal models that show harmful health
388 effects associated with a reduced DNA repair capacity of the variants in the OGG1 gene^{7,48-52}
389 They are also consistent with work in humans that associate the Cys326 variant with a higher risk
390 of cancer¹¹⁻²⁷ as well as other diseases^{2,33,53-55}. However, as far as we know, no previous study has
391 estimated the influence of this polymorphism on total mortality. One of the factors that can help

392 explain the strong associations found between the OGG- rs1052133 polymorphism and mortality
393 is that a high cardiovascular risk population is being analyzed. In a subsample of this
394 population⁵⁶, higher levels of the DNA- damaged product 8-oxo-7'8'-dihydro-2'-deoxyguanosine
395 (8-oxo-dG) were previously detected in nucleated blood cells in comparison with participants
396 from the general population (not at high cardiovascular risk) paired by age and sex (5.61±1.17 in
397 PREDIMED participants versus 3.71±0.65 in non-high cardiovascular risk participants,
398 expressed as 8-oxo-dG/10⁶dG; P<0.001). This is relevant considering the reports on the impaired
399 DNA repair capacity of the Cys326Cys variant being enhanced under conditions of oxidative
400 stress¹⁷, largely increasing the risk of oxidative pathologies¹⁸.

401 Although several studies have analyzed the influence of the OGG1-rs1052133 on cancer
402 incidence or prevalence¹⁹⁻²⁷, no previous study at the population level has analyzed the
403 association of such polymorphism with cancer mortality. Some studies have analyzed the
404 influence of the OGG1-rs1052133 polymorphism on the survival or prognosis of selected groups
405 of patients receiving cancer treatment^{57, 58}, but there are no estimates of mortality rates in a
406 general population cohort. Although in our cohort at high cardiovascular risk deaths from cancer
407 outnumbered those from cardiovascular disease, no association between the Cys326Cys genotype
408 and cancer mortality was observed in the whole population. However, a strong association was
409 detected between the Cys326Cys risk genotype and cardiovascular mortality. Although in
410 comparison to studies that have examined the possible association between the OGG1-rs1052133
411 polymorphism and cancer¹⁸⁻³¹, very few have examined its association with cardiovascular
412 disease^{33-35,54}, studies in animal models on OGG1 function strongly support this
413 association^{32,59,60}. Thus, in a study by Tumurkhuu et al³² in Ogg1(-/-) mice, the authors observed
414 a more atherogenic profile of the different markers analyzed in comparison with mice with a
415 normal Ogg1 gene expression. In the Ogg1 (-/-) mice, higher serum IL-1β and IL-18 levels,

416 higher oxidized mitochondrial DNA and higher inflammasome activation were detected. Taking
417 into account that OGG1 is the major DNA glycosylase responsible for removing the most
418 abundant products of oxidative DNA damage, it is not surprising to find a pro-atherosclerotic
419 phenotype in mice deficient in the *ogg1* gene. Interestingly, these authors also reported higher
420 levels of triglycerides in deficient mice³². Interestingly, in PREDIMED participants, higher
421 plasma triglycerides in Cys326Cys participants were also detected. Overall, OGG1 may play a
422 protective role in atherogenesis by preventing excessive inflammasome activation³². In humans,
423 most of the few studies carried out on cardiovascular disease^{33-35,54} also have found a higher risk
424 associated with the Cys326 allele. Thus, Izzoti et al³³ examined the survival of patients with
425 severe atherosclerosis and concluded that those bearing the OGG1 homozygous slow
426 polymorphism had increased levels of two bulky DNA adducts, being more susceptible than
427 other individuals to the genotoxic consequences of oxidative stress in the arterial wall. Orhan et
428 al³⁵ also concluded that the OGG1-rs1052133 played a role in stroke risk, and Shyu et al³⁴
429 reported an effect of smoking increasing stroke risk in Chinese carriers of the Cys allele. The
430 present study results showing a strong association between the OGG1-rs1052133 polymorphism
431 and cardiovascular mortality in Cys326Cys homozygotes concur with these findings. Because a
432 high cardiovascular risk population was analyzed, it is no surprise that the association of the
433 OGG1-rs1052133 polymorphism was stronger for cardiovascular disease mortality than cancer
434 mortality.

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

440 cause of death, exceeding breast cancer mortality rates at 10 years after diagnosis⁶².

441 Age is an important determinant of mortality. The mean age of the deceased due to cancer
442 in the PREDIMED cohort was significantly lower than the mean age of the deceased due to
443 cardiovascular diseases. Interestingly, it was found that, in the younger age group (<66.5 years),
444 the OGG1-rs1052133 polymorphism was indeed more associated with cancer mortality than
445 cardiovascular mortality. Conversely, the association of the OGG1 polymorphism with higher
446 cardiovascular mortality was mainly detected in the older age group. This may be explained by
447 the age-dependent reduction of the DNA repair efficiency, enhanced in Cys326Cys participants².
448 In younger participants, the increased cancer mortality associated with this polymorphism may be
449 associated with an additional genetic component related to specific locations (i.e. BRCA1,
450 BRCA2, etc.) in which the OGG1-risk genotype may contribute to enhance the genome
451 instability that increases the risk, being also considered as a cancer risk modifier⁶³.

452 When analyzing gene-diet interactions, sample size limitations due to the recessive effect
453 and the relatively low prevalence of the Cys326Cys genotype in this population (4.2%) prevented
454 examination of the dietary modulation of the effects of the OGG1-rs1052133 polymorphism on
455 cancer mortality (only 6 deaths with the Cys326Cys genotype were detected). Related to this, it is
456 known that the prevalence of the OGG1-rs1052133 polymorphism is lower in white (1.8-8.6 per
457 cent Cys326Cys participants) than in Asian populations (13.4-38.2 per cent Cys326Cys)⁶⁴.
458 However, bearing this limitation in mind, it was possible to explore dietary modulation in
459 determining all-cause mortality and cardiovascular mortality (involving more homozygotes).
460 When testing whether intervention with the MedDiet modulated the effect of the Cys326Cys
461 genotype increasing total mortality a statistically significant interaction was not found. Likewise,
462 for cardiovascular mortality in the whole population, the interaction term between the OGG1
463 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 it⁶⁸⁻⁷⁰. 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 mortality⁷². 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 al²⁸,
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 the OGG1-
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

488 interaction terms were detected for the MedDiet intervention. Nevertheless, a significant gene-
489 diet interaction with vegetable consumption in determining cardiovascular mortality has been
490 observed, in such a way that higher consumption decreased the risk more in Cys326Cys
491 participants, supporting the beneficial role of the antioxidant compounds present in vegetables in
492 providing protection from DNA damage and mortality risk in genetically susceptible individuals.
493 However, replication of these results in other studies is needed to confirm these associations and
494 dietary modulations.
495

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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 CC 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. P¹ 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 (P²) or in comparison with SS and SC grouped together (recessive model) (P³) 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	
Statistical methods	11b	If relevant, description of the similarity of interventions	7
	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

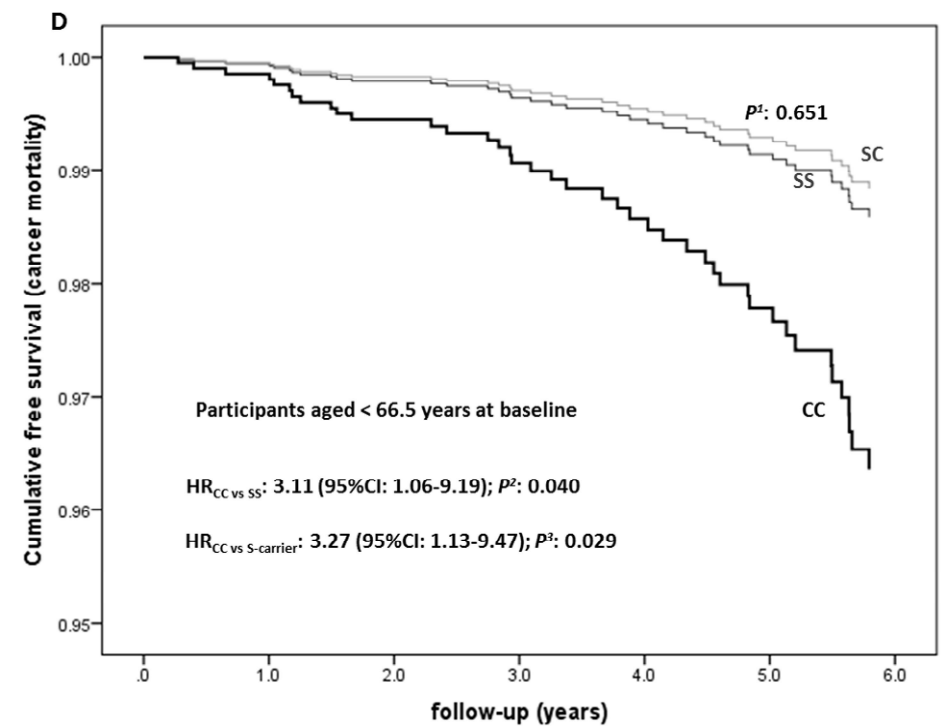
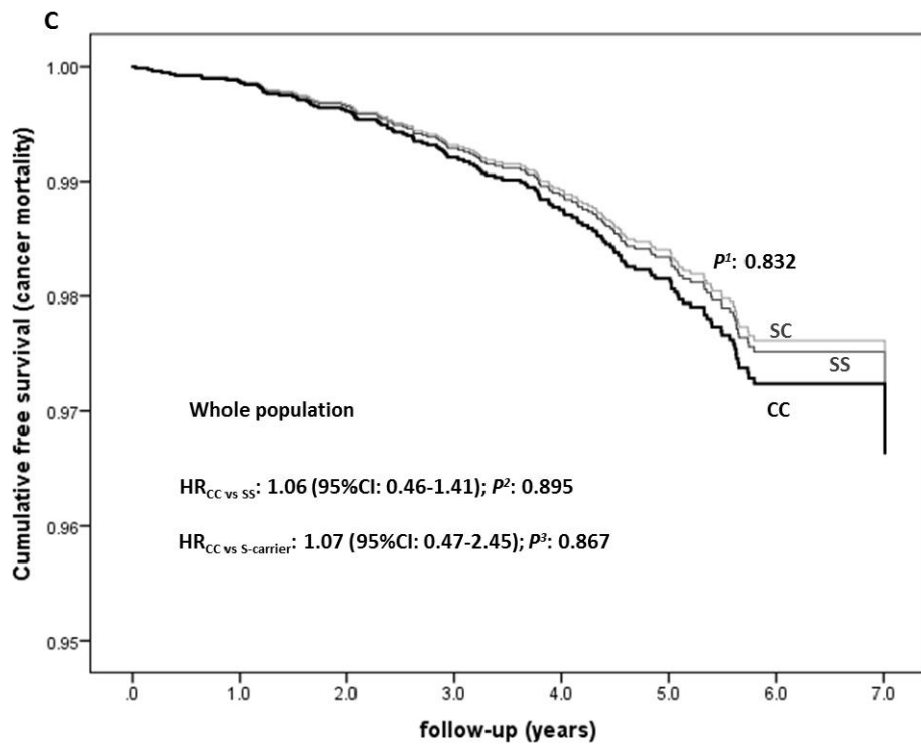
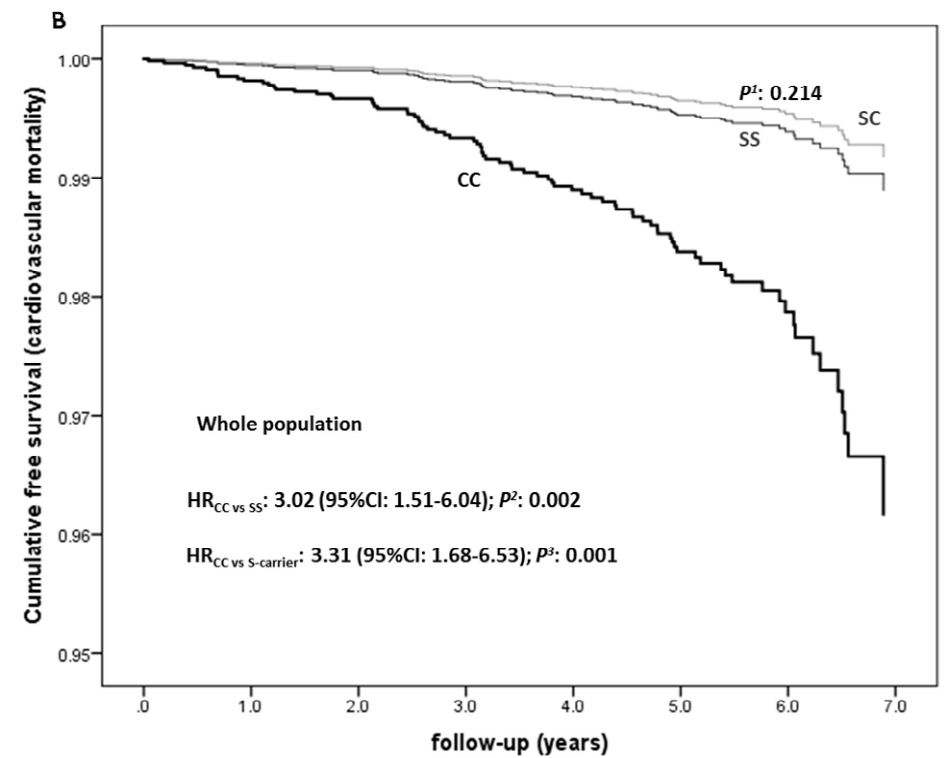
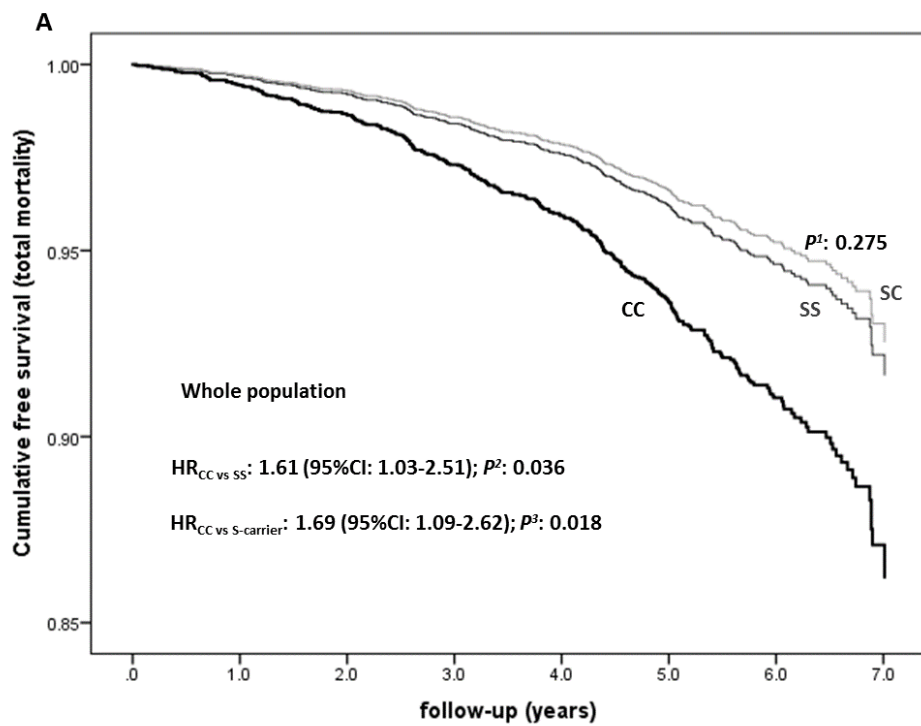


Table 1: Demographic, clinical, lifestyle and genetic characteristics of the **PREDIMED study participants at baseline according to the OGG1-rs1052133 genotype (n = 7,170)^a**

	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
<i>OGG1-rs1052133</i> : 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 ADM³⁸;

^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)	
<i>OGG1-rs1052133</i> : 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)	
<i>OGG1-rs1052133</i> : 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 ADM³⁸.

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

OGG1-rs1052133 genotypes	Deaths / person-y	Mortality rate ^d	Whole population (n = 7,170)									
			Model 1 ^a			Model 2 ^b			Model 3 ^c			
			HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	
Total mortality (deaths: 318)												
<i>Codominant model</i>												
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	
<i>Recessive model</i>												
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)												
<i>Recessive model</i>												
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)												
<i>Recessive model</i>												
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, dyslipidemia and type-2 diabetes) at baseline.

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

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

OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate ^e	Model 1 ^b			Model 2 ^c			Model 3 ^d		
			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
<i>P (interaction OGG1 x Age group)^f</i>					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
<i>P (interaction OGG1 x Age group)^f</i>					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
<i>P (interaction OGG1 x Age group)^f</i>					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

OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate ^e	Whole population (n = 7,170)									
			Model 1 ^b			Model 2 ^c			Model 3 ^d			
			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	
<i>P (interaction OGG1 x Intervention group)^f</i>					0.469				0.558			
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	
<i>P (interaction OGG1 x Intervention group)^f</i>					0.181				0.167			

^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

OGG1-rs1052133 genotypes	Deaths / person-years	Mortality rate ^e	Whole population (n = 7,122)								
			Model 1 ^b			Model 2 ^c			Model 3 ^d		
			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
<i>P (interaction OGG1 x Vegetable intake)^f</i>					0.446				0.444		
Vegetable intake (as continuous)											
<i>Interaction term OGG1 x Vegetables^g</i>			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
<i>P (interaction OGG1 x Vegetable intake)^f</i>					0.096				0.120		
Vegetable intake (as continuous)											
<i>Interaction term OGG1 x Vegetables^g</i>			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.