Pro12Ala Polymorphism of the *PPARγ2* Gene Interacts With a Mediterranean Diet to Prevent Telomere Shortening in the PREDIMED-NAVARRA Randomized Trial

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Background—The gene variant Pro/Ala (rs1801282) in the *PPARγ2* has been associated with lower cardiovascular risk and greater benefit from lifestyle interventions. This polymorphism also seems to be associated with longer lifespan, but no information on telomere length (TL) is available. Our aim was to study the association between the Ala allele and changes in TL in high cardiovascular risk subjects and the potential interaction with a Mediterranean dietary pattern.

Methods and Results—A total of 521 subjects (55–80 years) participating in the Prevención con Dieta Mediterránea randomized trial were genotyped. Changes in TL, measured by quantitative real-time polymerase chain reaction (PCR), were assessed over 5 years of a nutritional intervention, which promoted adherence to the Mediterranean diet (MeDiet). Interestingly, Ala carriers showed lower telomere shortening after 5 years compared with the Pro/Pro genotype (P=0.031). This association was modulated by MeDiet because those Ala carriers who reported better conformity to the MeDiet exhibited increased TL (P<0.001). Moreover, a reduction in carbohydrate intake (≤9.5 g/d) resulted in increased TL among Ala carriers. Notably, an apparent gene–diet interaction was found through the observed changes in the MUFA+PUFA/carbohydrates ratio: as this ratio increased, TL lengthening was detected to a greater extent in the Ala carriers compared with the Pro/Pro subjects (P for interaction <0.001).

Conclusions—The Pro12Ala polymorphism is associated with TL homeostasis after 5 years follow-up in subjects at high cardiovascular risk. In addition, a higher adherence to the MeDiet pattern strengthens the prevention of telomere shortening among Ala carriers.

Clinical Trial Registration—www.controlled-trials.com; Unique Identifier: ISRCTN35739639.

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Telomeres are tandem TTAGGG repeats of DNA that, together with associated proteins, protect the ends of chromosomes.[1] Importantly, they become shorter during repeated DNA replication, and leukocyte telomere length (TL) has therefore been proposed as a potential biomarker of biological age.[2] There are several endogenous factors that cause telomere shortening, such as inflammation or oxidative stress,[3–5] and many age-related diseases, such as obesity,[6] and cardiovascular disease[7] are linked to shortened telomeres.[8,9] Several studies indicate that patients with early-onset coronary heart disease, hypertension, and diabetes mellitus complications have lower TL, whereas increased left ventricular hypertrophy or ventricular mass is associated with higher TL.[10] Moreover, telomere attrition is reported to be accelerated in endothelial cells in the regions affected by atherosclerosis,[11,12] and in coronary endothelial cells, it may contribute to coronary endothelial dysfunction and the progression of cardiovascular disease in humans.[13] In this context, cardiovascular risk factors can be causes of oxidative stress and endothelial injury, leading to telomere shortening.

Clinical Perspective on p 99

There is evidence that TL is modified by the environment (poor diet, smoking, psychosocial factors)[6] and also by non-environmental influences, such as genetic[14] or epigenetic factors.[15] In healthy individuals, the estimated heritability of TL ranges from 0.36 to 0.84.[16] The effect of telomere pathway gene variants on TL has been widely studied, especially for single-nucleotide polymorphisms encoding telomerase

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subunits.[17,18] However, other genome-wide association studies have identified single-nucleotide polymorphisms associated with TL, which do not contain candidate genes of telomere biology, but rather genes related to several cancers and other diseases.[19,20] Recently, Saxena et al[21] showed that variants in casein kinase II (*CSNK2A2*) are associated with leukocyte TL in a Punjabi Sikh Diabetic Cohort.

The presence of the Ala allele in the peroxisome proliferator–activated receptor γ2 (*PPARγ2*) results in a moderate reduction in transcriptional activity of the *PPARγ2* in adipose tissue, thus improving insulin sensitivity and lipid and glucose homeostasis.[22,23] This gene variant seems to regulate inflammation by controlling downstream inflammatory molecules,[24] and it may decrease resistance to oxidative stress.[25] Moreover, several studies have reported that carriers of the Ala allele are considerably better protected against cardiovascular disease,[26,27] and the effectiveness of primary prevention of cardiovascular disease by a lifestyle intervention even increased among them.[28,29]

Interestingly, it has been suggested that the presence of the Ala allele could be associated with longer lifespan in humans.[30,31] However, no further studies have examined the association of the Pro12Ala polymorphism and TL in high cardiovascular risk subjects after a Mediterranean diet (MeDiet) intervention. We hypothesized that subjects carrying the Ala allele of the *PPARγ2* gene would have lower telomere attrition after 5 years of intervention with a Mediterranean dietary pattern. Thus, our aim was to assess the relationship between the Ala allele of the *PPARγ2* gene and changes in TL in high cardiovascular risk subjects, whereas they received a nutritional intervention with a Mediterranean dietary pattern.

Methods

Study Design

The Prevención con Dieta Mediterránea (PREDIMED) study was a large, parallel-group, multicenter, randomized, and controlled clinical trial designed to assess the effects of the MeDiet on the primary prevention of cardiovascular disease. All subjects provided informed consent, and the protocol was approved by the Institutional Review Boards at all study locations according to the principles of the Helsinki Declaration. The trial is registered at http://www.controlled-trials.com/ISRCTN 35739639. Participants were randomly assigned 1 of 3 different dietary interventions: MeDiet supplemented with extra virgin olive oil (EVOO), MeDiet supplemented with mixed nuts, or a control group (low-fat diet). The design and methods of this trial have been reported in specific publications,[32,33] and further details are also available at www.predimed.es.

Participants were female (60–80 years old) or male (55–80 years old) without a history of cardiovascular disease, but at high cardiovascular risk. Inclusion criteria were either type-2 diabetes mellitus or ≥3 of the following major cardiovascular risk factors: current smoking, hypertension, elevated low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, overweight/obese, and family history of premature coronary heart disease.

This study was designed to assess TL in a subset of participants from 1 of the 11 recruitment centers (PREDIMED-NAVARRA). The PREDIMED-NAVARRA recruitment center was the first of the 11 centers to complete the enrolment of participants, comprising 1055 of the 7447 subjects taking part in the trial. Individuals from this center were recruited between June 2003 and May 2005, whereas in the rest of the centers, it took place between October 2003 and March 2009. The intervention was stopped in 2011 because of early evidence of benefit according to a joint decision of the Data and Safety Monitoring Board and the Steering Committee of the study.

From the PREDIMED-NAVARRA total sample, participants with either baseline or 5-year missing data or missing DNA samples were excluded from the TL analysis. Therefore, 521 subjects were included in this study. They did not differ from the total cohort (n=1055) in terms of their main characteristics. Among them, 211 participants were allocated to the MeDiet supplemented with EVOO group, 170 to the MeDiet supplemented with mixed nuts group, and 140 to the control group (Figure 1).

Genotyping

DNA was extracted from overnight fasting venous blood samples using a commercial kit (Master PureTM; Epicentre, Madison, WI). All participants were genotyped for the Pro12Ala polymorphism of the

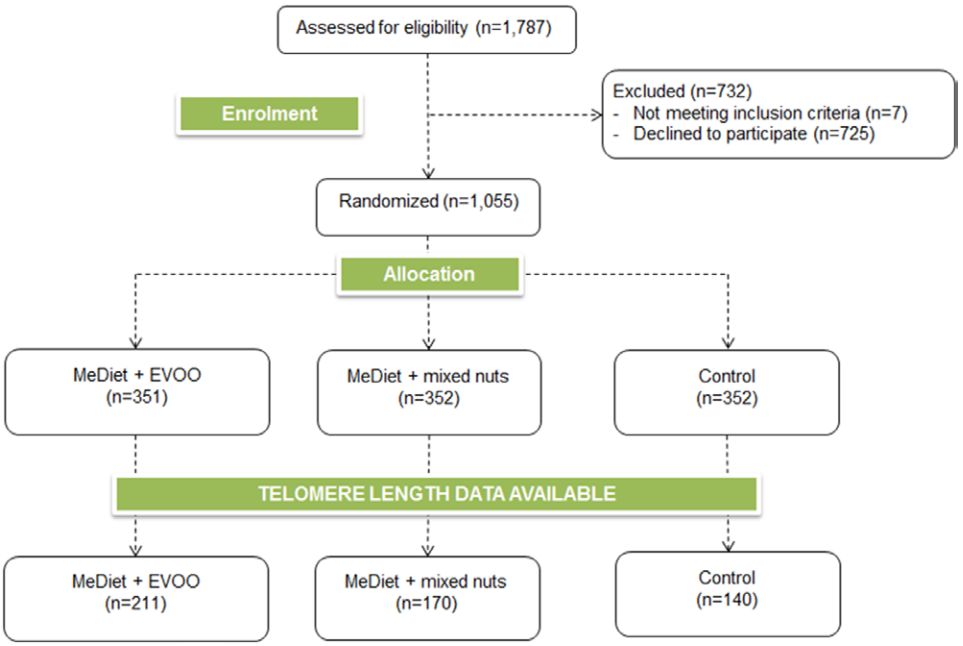

Figure 1. Flowchart of participants in a subsample of the PREDIMED-NAVARRA trial. EVOO indicates extra virgin olive oil; MeDiet, Mediterranean Diet.

PPARγ2 gene (rs1801282) using the PCR-restriction fragment length polymorphism method as previously described.[34]

Telomere Length Assessment

Telomeres were measured at 2 points: at baseline and after 5 years of the nutritional intervention using a real-time quantitative PCR (RT-PCR), as described by Cawthon.[35] This method displays concentrations of telomere repeat copy number (*T*) and single-copy gene (Ribosomal Protein Large PO) copy number (*S*) as a reference for each sample.

White 384-well plates were used to perform PCRs on an ABI-Applied Biosystems 7900 HT thermal cycler (Applied Biosystems, CA, USA). The total reaction volume was 10 μL containing 10 ng of genomic DNA, and quantiTect SYBR Green PCR kit (Qiagen, Valencia, CA, USA) was used as master mix. The final telomere primer concentrations were as follows: for telomere amplification tel1, 675 nmol/L and for tel2, 1350 nmol/L; and for the amplification of the single copy gene RPLPO: hRPLPO1, 800 nmol/L and hRPLPO2, 800 nmol/L. The primer sequences (Sigma-Aldrich, St.Louis, MO, USA) were tel1 (5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′), tel2 (5′-TCCCGACTATC CCTATCCCTATCCCTATCCCTATCCCTA-3′), hRPLPO1 (5′-CCCATTCTATCATCAACGGGTACAA-3′) and hRPLPO2 (5′-CAGCAAGTGGGAAGGTGTAATCC-3′). This procedure normalizes *T* to *S* by taking the ratio (*T*/*S* ratio) for each sample. The *T*/*S* ratio was calculated as follows: $[2^{CT(\text{telomeres})}/2^{CT(\text{single copy gene})}]=2^{-\Delta CT}$ because the amount of the PCR product approximately doubles in each cycle of the PCR.[35]

A calibration curve of the same DNA reference sample (from 64.00 to 0.25 ng/microliter in 2-fold dilutions) was included for each measurement as a standard to control the day-to-day variations. Standard curve with linearity $R^2 > 0.98$ was accepted. For quality control, all samples were run in duplicate, and concordance between duplicate values was checked. To ensure consistency, samples showing a high variation (>10%) were rerun and reanalyzed. The intra-assay coefficient of variation between duplicates for telomere was 3.0%, and for the single copy gene, it was 2.6%. The interassay coefficient of variation between plates for telomere was 0.8%, and for the single copy gene, it was 1.3%. The small coefficients of variation support the reliability of this procedure.

Dietary Assessment

Dietary habits were evaluated using a semiquantitative 137-item Food frequency Questionnaire previously validated in Spain.[36] Frequency of consumption was registered in 9 categories that ranged from never or almost never to 6 times/day. In addition, a validated 14-item questionnaire was used in this study to appraise adherence of participants to the MeDiet.[37]

The 2 groups which were allocated MeDiets received intensive education to be able to follow the MeDiet and supplemental foods at no cost. EVOO (1 L/week) was provided to the first group and 30 g/day of mixed nuts (15 g walnuts, 7.5 g hazelnuts, and 7.5 g almonds) to the second group. In the control group, participants did not receive education on the MeDiet, but were advised to reduce all types of fat and were given written recommendations according to the American Heart Association guidelines. Each participant received a personal interview with a trained dietician and a group session conducted by the same dietician every 3 months during the 5 years. No energy restrictions were prescribed for any intervention group. Other specific details of the intervention protocol have been published elsewhere.[33]

Confounders Assessment

The baseline interview included information about medical, sociodemographic, and lifestyle variables. Physical activity was assessed through a validated physical activity questionnaire.[38,39] Anthropometric data were obtained by trained dieticians using standardized methods[40] and were measured in each of the yearly follow-up visits. Educational attainment was used as the indicator of socioeconomic status because it does not change much over a lifetime, and – either directly or indirectly – it affects an individual's adoption of health behaviors and their outcomes.

Statistical Analysis

Hardy–Weinberg equilibrium was assessed using the χ^2 test and the allele frequency was determined by direct counting. Participants' baseline characteristics were compared according to the Pro12Ala *PPARγ2* polymorphism (dominant models). All analyses were performed with the dominant genetic model because of the small sample of Ala/Ala homozygotes. At baseline, we calculated means and standard deviations (SD) or percentages for each variable across the 2 genotypes and assessed the statistical significance of the differences among them with the unpaired *t* test and χ^2 tests, respectively.

To determine modulation by dietary components, changes in macronutrients after the 5-year nutritional intervention were dichotomized based on individual's median levels, with the exception of the 14-item questionnaire that was divided into tertiles. The ratio unsaturated fats to carbohydrates ((MUFA+PUFA)/CHO) was also evaluated as a continuous variable. Indicated interactions between the gene variant (dichotomous) and all the dietary components were tested for TL changes using the likelihood ratio tests. When the dietary component was a dichotomous variable, the interaction test had 1 degree of freedom, whereas the interaction test had 2 degrees of freedom in the case of a dietary component that was categorized into 3 groups, without assuming any linear effect. Changes in TL and dietary habits were defined as the difference between the 2 points in time (baseline and 5-year follow-up).

The comparison between genotype differences in TL within each dietary category was assessed using ANCOVA techniques after adjusting for the following potential confounders measured at baseline: age, sex, body mass index (kg/m^2), TL, smoking habit (current smoker or never smoker), physical activity (METS-min/d), total energy intake (kcal/d), and the score obtained from the 14-item MeDiet tool. In additional analyses, the model was also adjusted for each initial dietary component as indicated in the Results section.

We achieved a statistical power >80% (α-level=0.05 and β-level=0.20), with a sample size of 521 and a prevalence of 13% in Ala allele carriers, when detecting significant mean differences of 40 arbitrary units in TL between both genetic variants, after adjusting for potential confounders. All *P* values presented are 2-tailed, and statistical significance was defined at $P<0.05$. Data analyses were performed using STATA version 12.0 (StataCorp, College Station, TX, USA).

Results

The mean age of participants was: 67.0 years (SD, ±6.0), and 54.7% of them were female. The overall frequencies of the Pro/Pro, Pro/Ala, and Ala/Ala genotypes were 86.7%, 13.1%, and 0.2%, respectively. The minor allele frequency of Ala allele was 0.067, which is similar to 0.076 in HapMap-CEU (European) subjects as recorded in the NCBI single-nucleotide polymorphism database.[41] The genotype frequency did not deviate from the Hardy–Weinberg equilibrium expectations ($P=0.344$). All analyses were undertaken with a dominant genetic model because of the small number of Ala/Ala homozygotes.

Baseline characteristics of the 521 participants according to the Pro12Ala polymorphism are presented in Table 1. Most of them were overweight or obese (92%), nonsmokers, and >3 quarters had hypertension. No significant differences were observed for the Pro12Ala polymorphism in this population. Specifically, no differences were observed for adherence to MeDiet with the 14-item questionnaire, within the

Table 1. Baseline Characteristics According to the *PPARγ2* Genotype Among All the Participants

	Pro/Pro	Ala Carriers	*P* Value
N	451	70	
Male/female, %	46/54	36/64	0.106
Age, y	66.9 (5.9)	68.1 (5.7)	0.101
Socioeconomic status			0.395
Low level, %	75.8	71.1	
High level, %	24.2	29.0	
BMI, kg/m^2	29.2 (3.2)	28.9 (3.2)	0.482
Waist circumference, cm	95.3 (10.1)	94.0 (7.9)	0.313
Physical activity, METs-min per day	276.2 (197.3)	283.9 (189.2)	0.763
Adherence to the MeDiet, points	9.1 (1.9)	9.3 (1.7)	0.616
Dietary intake			
Energy intake, kcal/d	2258.2 (526.8)	2210.5 (530.5)	0.485
Carbohydrates, % energy	40.0	41.2	0.150
Fat, % energy	40.6	40.0	0.460
Protein, % energy	15.9	16.1	0.560
Glycemic load	125.0 (43.3)	124.8 (49.2)	0.977
Intervention groups			0.732
MeDiet+EVOO %	39.9	44.9	
MeDiet+nuts, %	32.8	30.4	
Control, %	27.3	24.6	
Current smokers, %	15.3	11.6	0.407
Diabetes mellitus, %	36.1	39.1	0.633
Hypertension, %	83.6	81.2	0.619
Dyslipidemia, %	65.0	73.9	0.136
Telomere length, T/S ratio	258.3 (453.8)	368.3 (781.9)	0.095

Values are means and standard deviations (SD) for continuous variables or percentages for categorical variables.

BMI indicates body mass index; EVOO, extra virgin olive oil; and MeDiet, Mediterranean Diet.

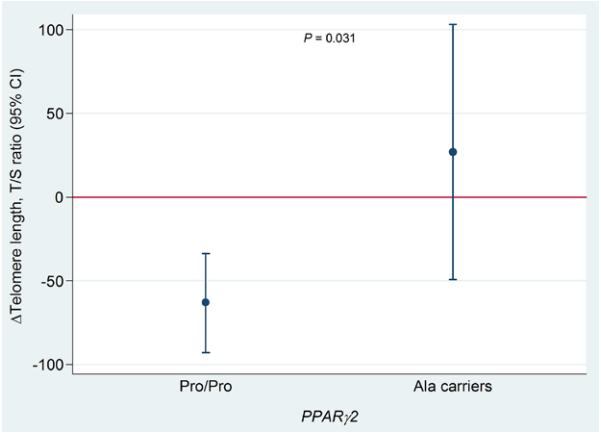

Figure 2. Effect of the rs1801282 polymorphism of the *PPARγ2* gene on changes in telomere length after 5 years of a nutritional intervention in the frame of the PREDIMED-NAVARRA trial (n=521). Multivariate adjusted means for telomere length changes depending on the polymorphism, adjusted for age, sex, baseline telomere length, body mass index (kg/m^2), physical activity (METS-min per day), total energy intake (kcal per day), total 14-point score at baseline, and smoking status (dichotomous).

Pro12Ala gene variant (*P*=0.616), meaning that they have a similar adherence to MeDiet at enrolment. However, it should be highlighted that carriers of the Ala allele tended to have higher TL (*P*=0.095).

As expected, TL was negatively associated with age in this population both at baseline (rho=-0.104, *P*=0.018) and after the 5-years nutritional intervention (rho=–0.124; *P*=0.004; data not shown). Remarkably, the 5-year MeDiet intervention did prevent telomere shortening and even increased TL among Ala carriers, whereas this effect was not observed in Pro/Pro homozygotes after adjusting for potential confounders (P_{ANCOVA}=0.031; Figure 2).

We assessed the effects of changes in the dietary pattern, as categorical factors, after the intervention on telomere lengthening linked to the *PPARγ2* Pro/Ala gene variant, using multivariable-adjusted analysis (Table 2). Interestingly, an increased TL was detected when subjects reported better conformity to the MeDiet (14-item score) after the intervention, but only in subjects carrying the Ala allele of the *PPARγ2* gene (P_{ANCOVA}<0.001). The interaction for modifications in TL between changes in adherence to MeDiet and the *PPARγ2* gene was also statistically significant (*P* for interaction =0.016). Moreover, the presence of the Ala allele increased TL in subjects with a greater reduction in CHO intake (≤9.5 g/day) after 5 years in comparison with Pro/Pro participants (P_{ANCOVA}= 0.006). Notably, there was evidence of statistical interaction (*P* for interaction =0.007). Similar results were observed when individuals with the Pro/Ala genotype reduced glycemic load intake (≤5; P_{ANCOVA}=0.004; *P* for interaction =0.004).

As the nutritional intervention promoted increased intake of unsaturated fats (MUFA+PUFA) at the expense of reduced CHO intake, we assessed whether changes in the ratio unsaturated fat:CHO modulated the relationship between Pro12Ala polymorphism and changes in TL.We found a modulation of the genetic predisposition to TL changes by the unsaturated fat:CHO ratio (Figure 3). The prediction plot showed a gene–nutrient interaction: as the unsaturated fat:CHO ratio increased, TL was predicted to increase only in the Ala carriers and not in the Pro/Pro homozygotes (*P* for interaction <0.001).

When considering the items included in the 14-item MeDiet score separately, we found that higher consumptions of olive oil, vegetables, fruits and the use of *sofrito* sauce during follow-up were significantly associated with increased TL, but again, this association was only present in the Ala carriers, whereas a lower consumption of red meat, butter/margarine/cream, soda drinks, commercial sweets, and confectionery led to decreased telomere attrition, also only among these subjects (Table I in the Data Supplement). Contrary to expectations, subjects carrying the Ala allele consuming less wine, legumes, fish/seafood, and tree nuts exhibited an increase in TL after adjusting for potential confounders.

Analysis performed on an intention-to-treat basis showed no differences in TL changes after 5 years in any of the intervention groups (EVOO, Nuts, and Control) after splitting

Table 2. Interactions Between *PPARγ2* and Changes in Adherence to MeDiet, CHO Intake, Glycemic Load and Ratio (MUFA+PUFA)/CHO in Association With Changes in Telomere Length After 5 Years of the Nutritional Intervention

	Δ Telomere Length			
	Pro/Pro	Ala Carriers	*P* Value*	*P* for Interaction
Δ Adherence MeDiet, score points				0.016
Low <1 (n=240)	−57.2 (−88.0 to −26.3)	−98.1 (−176.3 to −20.0)	0.342	
Medium 2–3 (n=180)	−40.8 (−109.6 to 27.8)	141.0 (−40.9 to 323.0)	0.070	
High >4 (n=101)	−121.9 (−176.5 to −67.3)	160.4 (9.5 to 311.3)	<0.001	
Δ CHO intake,† g/d				0.007
Below median: ≤9.5 (n=260)	−76.6 (−128.7 to −24.6)	131.2 (−6.8 to 269.1)	0.006	
Above median: >9.5 (n=261)	−47.0 (−74.7 to −19.3)	−86.8 (−157.3 to −16.3)	0.307	
Δ Glycemic load,‡ units				0.004
Below median: ≤5.0 (n=260)	−80.4 (−132.0 to −28.9)	144.4 (−0.7 to 289.4)	0.004	
Above median: >5.0 (n=261)	−44.8 (−73.3 to −16.3)	−68.1 (−136.9 to 0.6)	0.541	
Δ (MUFA+PUFA)/Δ CHO§				0.092
Below median: ≤0.03 (n=260)	−39.1 (−69.4 to −8.8)	−33.7 (−106.7 to 39.3)	0.894	
Above median: >0.03 (n=261)	−84.8 (−134.8 to −34.8)	92.0 (−48.9 to 232.9)	0.022	

Adjusted for age, sex, basal BMI, basal telomere length, physical activity (METS-min per day), total energy intake (kcal per day), total 14-point score at baseline, and smoking status (dichotomous).

BMI indicates body mass index; CHO, carbohydrates; MeDiet, Mediterranean Diet; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids.

**P* values are for the comparison between genotype differences in telomere length within each dietary category after our corresponding adjustment for potential confounders.

†Additionally adjusted for basal CHO intake.

‡Additionally adjusted for basal Glycemic load.

§Additionally adjusted for basal ratio (MUFA+PUFA)/CHO.

the sample according to the Pro/Pro and Pro/Ala genotypes (Table 3). However, when distributing subjects according to the intervention group and changes in adherence to MeDiet, only Ala carriers with higher scores in the MeDiet screener and allocated to one intervention group, either EVOO or Nuts, prevented telomere shortening (Figure 4).

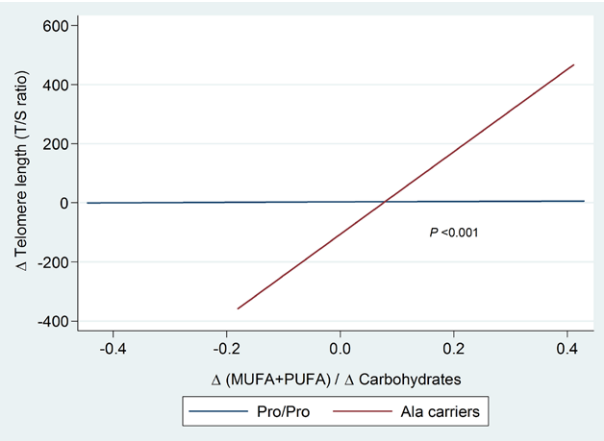

Figure 3. Interaction between the rs1801282 polymorphism of the *PPARγ2* gene with changes in the ratio (MUFA+PUFA)/carbohydrates in determining changes in telomere length after 5 years of the nutritional intervention in the frame of the PREDIMED-NAVARRA trial (n=521). *P* for interaction was adjusted for age, sex, baseline telomere length, baseline ratio MUFA+PUFA/carbohydrates, body mass index (kg/m^2), physical activity (METS-min per day), total energy intake (kcal per day), total 14-point score at baseline, and smoking status (dichotomous). The 2 following equations correspond to both regression lines, Pro/Pro genotype (blue line): y=2.83+6.52x; Ala carriers (red line): y=−106.76+1398x.

Discussion

To our knowledge, this is the first study to report an association between the common genetic variant at the *PPARγ2* locus, rs1801282, and TL, in which the Ala allele prevented telomere shortening associated with age after a 5-year nutritional intervention in high cardiovascular risk subjects. In addition, it has been shown that a Mediterranean dietary pattern interacts with the Ala allele, resulting in higher TL.

This Ala variant has been associated with lower cardiovascular disease risk in some studies.[27,28] Regieli et al[27] showed that Ala allele carriers had less widespread coronary artery disease and were protected against 10-year cardiovascular morbidity and mortality, with a hazard ratio of 0.24 (0.08–0.74; *P*=0.013) for vascular death, per each added copy of Ala allele. It is also reported that lifestyle interventions for cardiovascular prevention are more effective in carriers of the Ala allele of the *PPARγ2* gene than in those with the Pro/Pro genotype.[28,29] Because shorter telomeres have been found in subjects with cardiovascular disease,[7] it could be suggested that the Pro/Ala genotype may potentially protect against telomere attrition, as observed in our population at high cardiovascular risk.

Of particular interest is the reported relationship between the presence of the Ala allele and longevity, as TL is considered a biomarker for age.[2] Work conducted by Luo et al[25]

Table 3. Changes in Telomere Length After 5 Years of the Nutritional Intervention Associated With Pro12Ala Polymorphism According to Groups of Intervention

	Δ Telomere Length			
	Pro/Pro	Ala Carriers	*P* Value*	*P* for Interaction
Group of intervention				0.737
Control	7.5 (–17.7 to 32.8)	39.1 (–31.0 to 109.1)	0.412	
EVOO	–137.5 (–199.7 to –75.2)	–52.6 (–204.7 to 99.4)	0.315	
Nuts	–26.2 (–68.9 to 16.6)	93.2 (–22.5 to 208.9)	0.061	

Adjusted for age, sex, basal BMI, basal telomere length, physical activity (METS-min per day), total energy intake (kcal per day), total 14-point score at baseline, and smoking status (dichotomous).

BMI indicates body mass index; and EVOO, extra virgin olive oil.

**P* values are for the comparison between genotype differences in telomere length within each group of intervention after our corresponding adjustment for potential confounders.

showed that *PPARγ2* heterozygous mice exhibited improvement in insulin sensitivity and increased resistance to oxidative stress, underlying an extended lifespan. Another study indicated that Pro/Ala mice seem to be leaner, with improved insulin sensitivity and plasma lipid profiles, and have longer lifespans.[42] Notably, this evidence has been supported in a human study performed on a group of centenarian male who had increased frequency of the Pro/Ala genotype.[30] In contrast, however, a recent study proposed the opposite, having found a high frequency of the Ala allele in male <90 years old.[31] Our findings give valuable information in longitudinal terms, by suggesting *PPARγ2* as a possible longevity candidate gene because the Ala allele was associated with telomere lengthening in multiple-adjusted models after 5 years of a nutritional intervention.

The response of subjects to dietary components might be different depending on the genotype. In the population studied, we found a consistent gene–diet interaction between the Pro/Ala gene variant and adherence to MeDiet for changes in TL after the intervention. There are few examples in the literature on the subject of the influence of a MeDiet on maintaining TL. There are 2 potential mechanisms that account for changes in TL: one may occur through telomerase-mediated elongation and the other by mechanisms resulting in pseudotelomeric lengthening, as might result from changes in cell type distribution.[43] Boccardi et al[44] showed that people with the highest adherence to a MeDiet have longer telomeres and higher telomerase activity levels in peripheral white blood cells, independently of multiple confounding variables. This positive effect of the MedDiet could be direct, through some

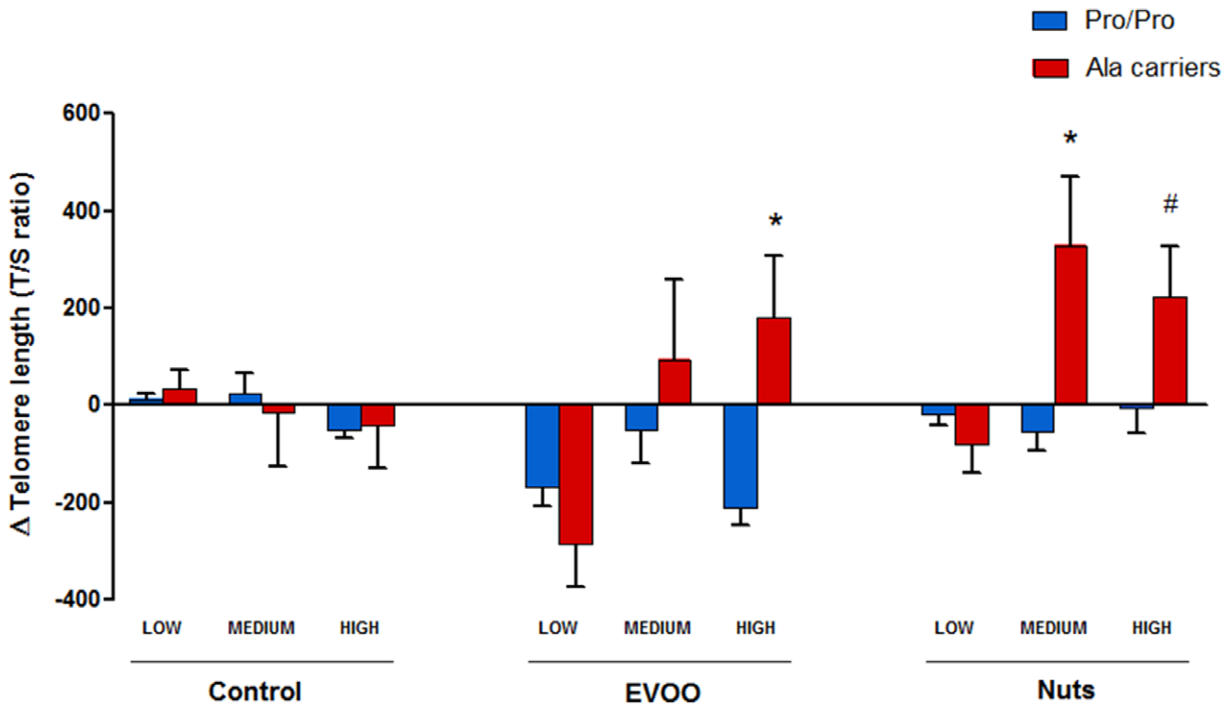

Figure 4. Changes in telomere length after 5 years of the nutritional intervention associated with Pro12Ala polymorphism, according to the group of intervention and tertiles of adherence to the Mediterranean Diet (14-item questionnaire) at follow-up. Means and SD for telomere length are adjusted for age, sex, basal telomere length, body mass index (kg/m^2), physical activity (METS-min per day), total energy intake (kcal per day), total 14-point score at baseline and smoking status (dichotomous). *P<0.025, #P=0.070 are P values comparing Ala carriers vs Pro/Pro subjects within the 3 groups of intervention. EVOO indicates extra virgin olive oil.

specific nutrients that may stimulate telomerase activity, or indirect, through the global effect of the diet on the modulation of inflammation and oxidative status.[44]

With regard to this, oxidative stress and inflammation might be proposed as the underlying causes of accelerated telomere shortening.[5] Interestingly, the Ala allele has been associated with lower levels of inflammation and oxidative stress[24,25] On the other hand, activation of *PPARγ2* can inhibit telomerase activity,[45] elucidating a potential relationship between this gene and TL. Because the Pro12Ala variant is associated with lower *PPARγ2* activity,[23] it may be hypothesized that this effect of the Ala allele is mediated by telomerase activity. Nevertheless, we could not rule out inflammation and oxidative stress as other mechanisms implicated in the change. Our findings need to be further explored in other populations to better understand this biological mechanism.

Surprisingly, no significant interaction was observed between the intervention groups established in the PREDIMED study (on an intention-to-treat basis) and the polymorphism for changes in TL. However, when analyzing the adherence to the MeDiet within each group, we observed a significantly more increased TL in Ala carriers than in Pro/Pro homozygotes when the compliance to MeDiet was high, both in the EVOO and in the nuts group, but not in the control group. A possible explanation could be that these 2 groups presented a higher adherence to MeDiet because they received intensive education to follow MeDiet and supplemental foods at no cost. Thus, it should be highlighted that the Mediterranean dietary pattern itself had the positive, principal effect on the association between the Pro/Ala genotype and TL changes.

Several limitations should be acknowledged. First, that TL has several weaknesses given the extent of interindividual variation at a given chronological age.[46,47] Moreover, there are some methodological problems because of TL measurement techniques. For this reason, we looked after the experimental conditions carefully to avoid potential errors in measurement.[48] Genomic DNA was processed after a standardized protocol and kept to preserve its stability, and the 2 DNA samples per participant (at baseline and after 5 years of recruitment) were run on the same plate. Second, the lack of several biological measurements, such as telomerase activity and others, related to inflammation and oxidative stress. Nor do we have information on the epigenetics profile status of participants.

Another weakness of this study was the self-reported dietary consumption using questionnaires that might have led to misclassifications of exposure. Our participants lived in a MeDiet country and had high cardiovascular risk, which may also limit the generalizability of our findings. On the other hand, the prospective nature of our study, with a long follow-up period, enabled us to measure TL at 2 points in the context of a MeDiet intervention and thus makes this research particularly robust. Other strengths include the randomized design, the high compliance of the participants to the intervention, and the reproduction of real-time conditions with home-prepared foods, as in usual clinical practice.

In summary, our results are in line with previous findings on the great beneficial effect of a lifestyle intervention on Ala carriers who are high cardiovascular risk subjects. Specifically, we demonstrated an increased TL in Ala carriers after 5 years of a MeDiet intervention, suggesting that the Mediterranean dietary pattern seems to have a beneficial effect in modulating the association between the polymorphism and changes in TL. Understanding the underlying mechanism of this gene–diet interaction may help to improve personalized dietary recommendations to prevent cardiovascular risk factors. Nevertheless, to date, this is apparently the only study to report these results, and therefore, further investigation is warranted to confirm our findings.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The presence of the Ala allele in the *PPARγ2* gene has been associated with lower cardiovascular risk and longer lifespan in humans. However, the underlying genetic and molecular mechanisms implicated in these associations remain unclear. In this study, we assessed the relationship between the Pro12Ala polymorphism and leukocyte telomere length, which is considered a potential biomarker of biological age. We observed that the Ala allele prevented telomere attrition associated with aging after a 5-year nutritional intervention in high cardiovascular risk subjects. We further identified that a closer adherence to a Mediterranean dietary pattern had a potentially beneficial effect because together with the presence of the Ala allele, those subjects had longer telomeres. Thus, the discovery of this gene-diet interaction may help to improve personalized dietary recommendations according to the genetic background to prevent aging and to reduce cardiovascular risk.