An amino acid change in the carbohydrate response element binding protein is associated with lower triglycerides and myocardial infarction incidence depending on level of adherence to the Mediterranean diet in the PREDIMED trial

Running title: *MLXIPL* interaction with Mediterranean Diet

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The total word count: 6996; Abstract: 250

Journal Subject Codes: [89]: Genetics of Cardiovascular Disease; [8]: Epidemiology; [90]: Lipid and lipoprotein metabolism

ABSTRACT

Background: A variant (rs3812316, C771G, Gln241His) in the *MLXIPL* (Max-like protein X interacting protein-like) gene encoding the carbohydrate response element binding protein has been associated with lower triglycerides. However, its association with cardiovascular diseases and gene-diet interactions modulating these traits are unknown.

Methods and Results: We studied 7,166 participants in the PREDIMED trial testing a Mediterranean diet (MedDiet) intervention versus a control diet for cardiovascular prevention, with a median follow-up of 4.8 years. Diet, lipids, *MLXIPL* polymorphisms and cardiovascular events were assessed. Data were analyzed at baseline and longitudinally. We used multivariable-adjusted Cox regression to estimate hazard ratios (HR) for cardiovascular outcomes. The *MLXIPL*-rs3812316 was associated with lower baseline triglycerides ($P=5.5x10^{-5}$) and lower hypertriglyceridemia (odds ratio [OR]: 0.73; 95%CI, 0.63-0.85; $P=1.4x10^{-6}$ in G-carriers versus CC). This association was modulated by baseline adherence to MedDiet (AdMedDiet). When AdMedDiet was high, the protection was stronger (OR: 0.63, 95%CI: 0.51-0.77; $P=8.6x10^{-6}$) than when AdMedDiet was low (OR: 0.88, 95%CI: 0.70-1.09;P=0.219). Throughout the follow-up, both the *MLXIPL*-rs3812316 ($P=3.8x10^{-6}$) and the MedDiet intervention (P=0.030) were significantly associated with decreased triglycerides. Likewise in G-carriers MedDiet intervention was associated with greater total cardiovascular risk reduction and specifically for myocardial infarction. In the MedDiet, but not in the control group, we observed lower myocardial infarction incidence in G-carriers versus CC (HR: 0.34; 95%CI:0.12-0.93;P=0.036 and 0.90; 95%CI: 0.35-2.33;P=0.830, respectively).

Conclusion: Our novel results suggest that MedDiet enhances the triglyceride-

lowering effect of the *MLXIPL*-rs3812316 variant and strengthens its protective effect on myocardial infarction incidence.

Clinical Trial Registration: http://www.controlled-trials.com/; number, ISRCTN35739639.

Key Words: Gene-diet interactions, Mediterranean diet, cardiovascular disease, myocardial infarction.

Given that hypertriglyceridemia is re-emerging as an important cardiovascular disease risk factor[1-3], it is essential to gain further understanding about the genes involved and the environmental factors that modulate their expression[4,5]. Outstanding among the genes that have recently been associated with triglyceride metabolism is the *MLXIPL* (Max-like protein X interacting protein-like, also known as Mondo B) that encodes the carbohydrate response element binding protein (CHREBP). In a genome-wide association study (GWAs)[6], we and others reported for the first time that the *MLXIPL* was a new locus associated with plasma triglycerides. We found that the minor allele of the intergenic rs17145738 polymorphism, located downstream of *MLXIPL*, was associated with significantly lower plasma triglyceride concentrations[6]. This observation was in line with previous work in animal models in which the ChREBP, discovered by the group of Uyeda[7], was characterized as an important transcription factor coupling hepatic glucose utilization and lipogenesis[8]. Subsequent studies in animal models confirmed the crucial role of ChREBP in regulating the process that converts excess of dietary carbohydrate into triglycerides and suggested its implication in the development of the metabolic syndrome[9,10]. However, given the intergenic location of the rs17145738 polymorphism between the *MLXIPL/TBL2* genes[6], the direct involvement of this gene in human triglyceride metabolism was not consolidated until Kooner et al[11] identified a functional variant (rs3812316, C771G, amino acid change Gln241His), located in an evolutionarily conserved region encoding a domain implicated in the activation of *MLXIPL*[10]. In view of the association of the minor G allele with low triglyceride concentrations, the authors suggested a reduced *MLXIPL* function for this variant, consistent with the low triglycerides described in Chrebp-deficient mice[9,12].

The two variants rs17145738 and rs3812316 are in high linkage disequilibrium

(LD), so explaining our previous results[6]. However, the existing literature provides a more heterogeneous perspective[6,11,13-21]. Although the GWAs and meta-analyses have consistently found a significant association between the minor allele and lower triglyceride concentrations[6,11,13,14], other experimental approaches have not shown the same consistency[18-21] which may be due to the fact that the expression of this gene and its biological consequences have been shown to be strongly modulated by diet (i.e., total fat, polyunsaturated fatty acids, total carbohydrates, or fructose)[7,8,12,22,23]. Thus, unexplored gene-diet interactions might be affecting the outcomes of human studies. Similarly, the association between *MLXIPL* polymorphisms and cardiovascular diseases is also heterogeneous. Although two studies on Chinese subjects[24, 25] reported significant associations between *MLXIPL* polymorphisms and lower risk of coronary artery disease, such an association has not been supported in meta-analyses[13,26]. Therefore, the aims of the present study were: 1) to assess the impact of the *MLXIPL*-rs3812316 polymorphism on triglyceride concentrations as well as the modulating effects of the Mediterranean diet (MedDiet) at baseline and longitudinally; 2) to study the association between this polymorphism and major cardiovascular events (myocardial infarction and stroke incidence), as well as its modulation through long-term intervention (mean ≈5 years) with MedDiet; and 3) To analyze whether the effects of the MedDiet intervention on cardiovascular events is modulated by the *MLXPIL*-rs3812316 polymorphism.

METHODS

Detailed Methods are included in the Supplement.

Subjects

We studied 7,161 participants (3,049 men and 4,112 women) in the PREDIMED

(PREvención with DIeta MEDiterránea) trial (27) from whom DNA was available and the *MLXIPL*-rs3812316 determined. The PREDIMED is a multi-center, randomized, controlled clinical trial (controlled-trials.com number, ISRCTN35739639) aimed at assessing the effects of the MedDiet on the primary prevention of cardiovascular disease[27,28]. The completion date of this study was December 2010 and the total number of randomized subjects was 7,447. The 7,166 participants included did not differ in the main characteristics from those of the total cohort. From October 2003 physicians in Primary Care Centers selected potential high cardiovascular risk subjects (Detailed in the Supplement). Participants were randomly assigned to these interventions: MedDiet with extra-virgin olive oil (EVOO), MeDiet with mixed nuts and control group (low-fat diet). Participants assigned to both MedDiet groups received intensive training to follow the MedDiet and allotments of either extra virgin olive oil or mixed nuts (30 g/d)[27,28]. Participants assigned to the control diet received recommendations to reduce the intake of all types of fat[27]. Participants were followed for a median of 4.8 years (interquartile range, 2.8 to 5.8 years).

Demographic, clinical, anthropometric and dietary measurements

The baseline examination included assessment of standard cardiovascular risk factors, medication use, socio-demographic factors and lifestyle variables[27,28]. The level of adherence to the MedDiet (AdMedDiet) was measured by a validated 14-item questionnaire[29]. It consisted of 14 questions on food consumption frequency and habits characteristic of the MedDiet (**Supplemental Table 1**). Each question was scored 0 or 1.

Outcome ascertainment

The primary endpoint was the occurrence of the first major cardiovascular event and comprised myocardial infarction, stroke or cardiovascular death as detailed in the Supplement and elsewhere[27]. End-points confirmed by the committee occurring between October 1, 2003, and December 1, 2010 were included in the analyses.

Biochemical determinations, DNA extraction and genotyping

Blood samples were obtained after an overnight fast and were frozen at -80ºC. Fasting glucose, total cholesterol, triglycerides, HDL-C and LDL-C were measured using standard enzymatic methods[28].

Genomic DNA was extracted from buffy-coat. We genotyped the intergenic *MLXIPL*-rs17145738 (in a sub-sample consisting of 1016 participants recruited in the Valencia region) and the nonsynonymous (Gln241His) rs3812316 polymorphism (in the whole sample) on a 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using a fluorescent allelic discrimination TaqManTM assay with a calling rate >95%. 5% of samples were randomly selected and genotyped a second time and no discrepancies were found. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium expectations (P=0.069 for the rs17145738 and P= 0.187 for the rs3812316). We found strong linkage disequilibrium (LD) between both polymorphisms (D': 0.955; r^2: 0.895; P<0.001). Consistent with our previous findings[6], the intergenic rs17145738 polymorphism was significantly associated with fasting triglycerides: 1.52+/-0.93 mmol/L in CC *vs*. 1.34+/-0.77 mmol/L in T carriers; P=0.004, and we decided to continue the main study with the functional *MLXIPL*-rs3812316 polymorphism.

Statistical Analyses

LD was assessed with the Haploview software package. Chi-square tests were used to test differences in percentages. Triglycerides were log-transformed for the statistical analyses. Hypertriglyceridemia was defined as a fasting triglyceride level >1.7 mmol/l. *MLXIPL* polymorphisms were first tested as additive and further, carriers of the variant allele were grouped together (dominant model) due to the low prevalence of the variant allele. To compare crude means we applied t and ANOVA tests. Multivariable adjustments for continuous variables were carried out by covariance analysis. The control for possible confounders has been undertaken sequentially following the PREDIMED study general protocol. Firstly the models were adjusted for basic potential confounders such as age, gender and recruitment center (adding the adjustment for dietary intervention in the longitudinal studies to that basic model). Afterwards an additional control for more potential confounders such as BMI, type 2 diabetes, medications (lipid-lowering and hypoglycemic drugs), alcohol consumption, physical activity, AdMedDiet at baseline, total energy intake, blood pressure, family history of cardiovascular disease, waist circumference or plasma lipids as specifically indicated in the corresponding analyses. The aim of this adjustment was to check whether, following the additional adjustments, the estimations of the polymorphism remained statistically significant or not.

Dichotomous variables for AdMedDiet at baseline and physical activity were created using the sample means as cut-off. Logistic regression methods were also used to estimate the contribution of the polymorphism to predict hypertriglyceridemia at baseline. The interaction between the *MLXIPL*-rs3812316 polymorphism and AdMedDiet at baseline in determining triglycerides was tested in multivariable regression models (lineal or logistic) including the corresponding main effects and

interaction terms. Stratified analyses were also carried out.

The longitudinal influence of the *MLXIPL*-rs3812316 polymorphism and dietary intervention (MedDiet groups were pooled *versus* the control group) on plasma triglyceride concentrations was analyzed by multivariable-ANOVA of repeated measures including those subjects having complete triglyceride data at baseline, 1, 3 and 5 years (n=2,418 subjects). Recruitment of participants lasted from 2003 to 2009; therefore, some participants had not completed three or five years of follow-up by the 1st December, 2010 study closing date. We also analyzed prevalence of hypertriglyceridemia by genotype and dietary intervention (MedDiet versus control) in the follow-up by logistic regression. To examine the association between intervention with MedDiet and cardiovascular events in all subjects (n=7,166), we used Cox regression models with the length of follow-up as the primary time variable. The exposure time was calculated as the time between randomization and the date of a major cardiovascular event, the date of the last interview, December 1st 2010, or the date at death, whichever came first. We evaluated both the association between the *MLXIPL*-rs3812316 polymorphism and cardiovascular events depending on the dietary intervention in which hazard ratios (HR) with 95 % confidence intervals (CI) for the *MLXIPL*-rs3812316 polymorphism, stratified by intervention with MedDiet *versus* control group were calculated, as well as the effect of the dietary intervention with MedDiet during follow-up on major cardiovascular events depending on the *MLXIPL*-rs3812316 polymorphism. In this case, HR and 95% CI for the MedDiet intervention were stratified by the polymorphism. We also analyzed the influence of baseline triglyceride concentrations on cardiovascular events in the Cox regression models. Analyses were based on the intention-to-treat principle. In multivariable model 1 (basic model) we adjusted for sex, age, center and intervention group. Multivariable models 2

and 3 included additional adjustments as detailed. Kaplan-Meier survival curves were plotted to estimate the probability of remaining free of myocardial infarction during follow-up. Statistical analyses were performed with the IBM SPSS Statistics version 21.0, NY. All tests were two-tailed and p values <0.05 were considered statistically significant.

RESULTS

Prevalence of the *MLXIPL*-rs3812316 (C>G, Gln241His) genotypes were: 83.2% CC, 15.4% CG, and 0.9% GG. **Table 1** shows baseline demographic, biochemical, clinical and lifestyle characteristics of the 7,166 participants by the *MLXIPL*-rs3812316 polymorphism. There were no significant differences in sex, age, BMI, dietary intake, smoking, drinking, physical activity, type 2 diabetes, hypertension, or treatment with lipid lowering drugs between genotypes. Likewise, we found no significant differences of genotype distribution among intervention groups (MedDiet groups or control diet). Characteristics of the study participants according to the randomly assigned dietary intervention groups at baseline are shown in **Supplemental Table 2**.

Baseline association between the MLXIPL-rs3812316 polymorphism and plasma lipid concentrations and fasting glucose and type 2 diabetes

We observed (**Table 2**) a strong association between this polymorphism and lower triglyceride concentrations [B: -0.11 mmol/l (-9.86 mg/dl) per variant G-allele, 95% CI: -0.17, -0.06; P=5.5x10^{-5}] in the entire population. This association remained statistically significant after multivariable adjustment for potential confounders (models 3 and 4, Table 2). We also found a protective effect (OR: 0.73; 95%CI: 0.63-0.85)

11

against hypertriglyceridemia (triglycerides>1.7 mmol/l), for carriers of the minor G-allele in comparison with CC homozygotes. Moreover, we detected a significant association with fasting glucose that was higher in GG homozygous subjects, but the effect did not show a linear trend. In a recessive model, we observed a statistically significant association (P=0.014) between the polymorphism and type 2 diabetes. Homozygous subjects for the G-allele had lower type 2 diabetes risk in comparison with CC (OR: 0.52; 95%CI: 0.30-0.89). This association remained statistically significant after further multivariate adjustment (OR: 0.55; 95%CI: 0-32-0.95; P=0.033).

Baseline modulation of the associations between the MLXIPL-rs3812316 polymorphism and risk of hypertriglyceridemia by AdMedDiet

We found a statistically significant gene-diet interaction between the *MLXIPL*-rs3812316 polymorphism and baseline AdMedDiet on hypertriglyceridemia (P-interaction: 0.025, which remained statistically significant after multivariate adjustment). When AdMedDiet was high (≥9 points), the protective effect of the variant allele against hypertriglyceridemia was stronger (OR: 0.63, 95% CI: 0.51-0.77; P=8.6×10^{-6}). When AdMedDiet was low, the protection was attenuated and did not reach statistical significance (OR: 0.88, 95% CI: 0.70-1.09; P=0.219). These results remained consistent in a sensitivity analysis testing this gene-diet interaction in different sub-groups: men, women, type 2 diabetic and non-diabetic subjects (**Table 3**). We also observed a similar dietary modulation when plasma triglyceride concentrations at baseline were considered as a continuous variable (**Supplemental Figure 1**).

Association between the MLXIPL-rs3812316 polymorphism and plasma triglycerides in the follow-up and modulation by the MedDiet

We analyzed the effect of this polymorphism on on-trial triglyceride concentrations (evolution of triglyceride levels over time) as well as the dietary modulation of this effect using longitudinal data from 5-year follow-up for all subjects having triglycerides measured at baseline, at 1-y, at 3-y and at 5-years (n=2,418 subjects) in a model for repeated measures. In a model adjusted for dietary intervention and other covariables (**Figure 1 A**), we found a strong longitudinal association of the polymorphism and triglycerides (P=0.000004) with carriers of the variant allele having lower triglycerides. This association remained statistically significant at each time-point. We also detected a statistically significant effect of the intervention with MedDiet on decreasing triglyceride concentrations in comparison with the control group (P=0.030 in the multivariable adjusted model) (**Figure 1B**). Although we did not find a statistically significant interaction between the *MLXIPL*-rs3812316 polymorphism and the dietary intervention through the follow-up (P=0.803), we observed a "biological interaction" by which G-allele carriers had lower triglyceride concentrations in the MedDiet intervention group than in the control group (**Supplemental Figure 2**). This effect was similar for hypertriglyceridemia (**Supplemental Figure 3**). Thus, in the multivariate adjusted model we found that after 5-years follow-up, G-carriers that received intervention with MedDiet had a significantly lower hypertriglyceridemia risk (OR: 0.62; 95%CI: 0.44-0.86; P=0.005 compared with CC subjects in the control group). However, consistent with our observation at baseline, G-carriers in the control group had an attenuated protection against hypertriglyceridemia risk (OR: 0.74; 95%CI: 0.46-1.19; P=0.208 compared with CC subjects in the control group).

Effects of dietary interventions on the associations between the *MLXIPL*-rs3812316 polymorphism and incidence of cardiovascular diseases

After a median follow-up of 4.8 years (interquartile range, 2.8 to 5.8 years), 265 major cardiovascular events occurred among the 7,166 participants (30,936 person-years of observation). Of these, 99 were myocardial infarctions, 133 strokes and the other were cardiovascular deaths. Taking the population as a whole we observed no significant associations between the *MLXIPL*-rs3812316 polymorphism and total cardiovascular events (HR: 0.88; 95%CI:0.63-1.24; P=0.472 for G-carriers versus CC in a model adjusted for sex, age, center and dietary intervention group. Similar results were obtained after additional adjustments (not shown). However, the association between the *MLXIPL*-rs3812316 polymorphism and reduced incidence of myocardial infarction tended toward significance (HR: 0.53; 95%CI: 0.27-1.05; P=0.070 for G-carriers versus CC) in the model adjusted for sex, age, center and dietary intervention. This result was similar in the fully adjusted model (HR: 0.51; 95%CI: 0.26-1.02; P=0.058). However, when studying cardiovascular events in the stratified analyses taking dietary intervention with MedDiet versus the control group into account, we obtained statistically significant results. **Table 4** presents the HRs for the incidence of cardiovascular events (total, myocardial infarction and stroke) for the *MLXIPL*-rs3812316 genotypes according to the dietary intervention groups (control group and MedDiet). We found that G-carriers had a significantly lower risk of myocardial infarction than CC homozygotes in the MedDiet intervention group (HR: 0.34; 95%CI: 0.12-0.93; P=0.036, in the model adjusted for sex, age, center, and dietary intervention). No significant association was found in the control group (HR: 0.90; 95%CI: 0.35-2.33; P=0.830). Results were similar after additional multivariable adjustment (Table 4). No statistically significant dietary modulations were found for stroke.

Effects of the *MLXIPL-rs3812316 polymorphism on the association between the*

intervention with MedDiet and incidence of cardiovascular diseases

We examined the effect of the dietary intervention (MedDiet versus control) by genotype on cardiovascular events and found (**Supplemental Table 3**) that the MedDiet intervention appears to be more efficacious among G-allele carriers. Thus, we obtained a statistically higher risk reduction for total cardiovascular diseases in G-carriers (HR: 0.51; 95%CI:0.26-0.99; P=0.045 for the MedDiet intervention group versus control) than in CC homozygotes (HR: 0.69; 95%CI: 0.52-0.91; P=0.008) in the adjusted models. A similar not statistically significant trend was found for stroke. Nevertheless, we observed a statistically significant risk reduction of the MedDiet vs control for myocardial infarction in G-allele carriers (HR: 0.24; 95%CI: 0.06-0.96; P=0.043) in comparison with the effect observed in CC (HR: 0.84; 95%CI:0.54-1.31; P=0.447). After further adjustment of these results for other covariables (hypertension, family history or cardiovascular disease, waist circumference and total cholesterol) or baseline hypertriglyceridemia, the association between the polymorphism and lower risk of myocardial infarction in the MedDiet intervention group remained statistically significant (P<0.05). In these Cox-multivariable models, baseline hypertiglyceridemia was significantly associated with higher risk of cardiovascular diseases, myocardial infarction and stroke in the whole population (all P<0.01). For myocardial infarction, we also expressed the relative risks from Cox regression with a single referent group (controls with CC - and the other 3 groups referent to that) as well as the incidence rates in **Supplemental Table 4**.

DISCUSSION

In this dietary intervention study, conducted in a large cohort of high cardiovascular risk subjects we found that the *MLXIPL*-rs3812316 polymorphism was a

strong genetic determinant of fasting triglycerides and hypertriglyceridemia both at baseline and in the follow-up. Carriers of the variant G-allele presented lower triglyceride concentrations in comparison with homozygous subjects for the common allele, consistent with previous reports at cross-sectional level for either the rs3812316 or for the intergenic rs17145738 polymorphism[6,12-17, 30-32], and added new longitudinal evidence for this association after 5-years follow-up. Moreover, we provide novel evidence supporting the fact that AdMedDiet can modulate the effects of the *MLXIPL*-rs3812316 polymorphism in determining plasma triglyceride concentrations. Thus, when AdMedDiet was low (below the population mean), the genetic effect of the G-allele reducing fasting triglyceride concentrations was attenuated and, when AdMedDiet was high (above the population mean), the decreasing effect of the G-allele on triglyceride concentrations was magnified. This cross-sectional observation at baseline was reinforced by the results of the longitudinal analysis showing a "biological" interaction with the intervention with MedDiet. Although intervention with MedDiet decreased triglyceride concentrations in both CC and G-allele carriers in comparison with the control group, G-allele carriers had lower triglyceride concentrations and a statistically significant lower risk of hypertriglyceridemia in a MedDiet intervention than in a low-fat control diet, so modifying the potential genetic determinism.

In addition to the differences in linkage disequilibrium between the several variants in different populations, the observed gene-diet interaction may help explain some ethnic- and population-specific associations described for the *MLXIPL* polymorphisms with triglycerides in different settings and dietary intakes[6,11-21,30-32]. Thus, Musunuru et al[32] observed that the *MLXIPL* polymorphisms were strongly associated with triglycerides in European American populations (P<0.001), but they failed to find significant associations in African Americans. Also, inter-population

16

specific differences were noted in the multiethnic United States National Health and Nutrition Examination Survey III[18]. In the present study we focused on the overall dietary pattern by analyzing the level of adherence to the MedDiet at baseline (or MedDiet intervention in the follow-up), a healthy dietary pattern characterized by a high intake of vegetables, fruits, cereals, olive oil, fish, legumes, and nuts that is low in saturated fats and simple carbohydrates and rich in MUFA and fiber[27]. As far as we know, ours is the first study to report a gene-diet interaction between *MLXIPL*-rs3812316 polymorphism and AdMedDiet on plasma triglycerides in humans. However, although the detailed regulation of ChREBP remains unknown, several studies in animal models have reported that diet modulates ChREBP gene expression as well as the effects on triglyceride and carbohydrate metabolism[7,8,22,23,33,34], so supporting our results. In the first murine ChREBP-knockout model[8] it was observed that ChREBP deficiency caused intolerance of simple carbohydrates (60% glucose or sucrose) and although ChREBP -/- mice were viable and appear to have a normal life span on a standard diet, when ChREBP-knockout mice were fed with a high-fructose (70%) diet they died within a few days. In addition to this extreme gene-diet interaction the authors also observed differences in liver triglycerides, glucose and other metabolites when ChREBP-knockout mice fed with a standard diet (standard rodent chow, Harlan Teklad Mouse/Rat Diet 7002) were compared with ChREBP-knockout mice fed with a high-starch diet (60% starch, 20% casein, 15% cellulose, 2.5% vitamins, and 2.5% minerals) and the statistical differences with wild-type mice were analyzed[8]. However, no significant effect on plasma triglycerides was detected[8]. The effect the ChREBP deficiency on reduced plasma triglycerides was more clearly observed in another work using a ChREBP-null ob/ob murine model[12], in which a normalization of plasma triglyceride levels was detected in comparison to the high triglycerides found in ob/ob

17

mice. Erion et al[33], using a murine model in which expression of ChREBP was decreased with a specific antisense oligonucleotide, also observed a reduction of plasma triglycerides. As suggested by others[11], the amino acid change caused by the *MLXIPL*-rs3812316 polymorphism could be related with a lower CHERBP activity in humans mimicking the phenotypes observed in deficient murine models. Moreover Erion et al[33] compared the effect of high-safflower oil versus a high-fructose diet on plasma triglycerides in the ChREBP deficient mice and observed lower levels in the high-fat than in the high-fructose group. Our results in humans showing lower triglyceride concentrations in G-carriers when consuming a high-MUFA MedDiet are in agreement with this observation. Likewise, Dentin et al[22] demonstrated in mice that PUFA suppressed ChREBP activity. However, additional work is needed both to better characterize the mechanisms involved and to specifically analyze the components of the MedDiet (high MUFA, fiber intake, PUFA, low saturated fat, etc.) responsible for the observed gene-diet interaction on triglyceride concentrations.

Furthermore, silencing of ChREBP in ob/ob mice[9] has been associated with improvements in fasting glucose and insulin tolerance. In line with this observation, we have described here a novel association between the *MLXIPL*-rs3812316 polymorphism and lower type 2 diabetes risk in humans (recessive model). Although the ChREBP is now considered a key player in insulin resistance[10,33,34], there are still paradoxical results regarding underexpression or overexpression of this gene depending on the liver or the adipose tissue associated with this phenotype.

Another novel and important finding from our study is the modulation by the MedDiet of the association between the *MLXIPL*-rs3812316 polymorphism and incident myocardial infarction. Few studies have analyzed the association between the *MLXIPL*-rs3812316 polymorphism and cardiovascular diseases, and their results have been

inconsistent[13,24-26,31,35] probably because dietary modulation was not taken into consideration. Thus, while some[24,25] reported a significant lower risk of coronary artery disease in carriers of the minor allele; the results of large meta-analyses (including several populations and more heterogeneity) did not confirm these findings[13,26,31,35]. The protective effect of the *MLXIPL*-rs3812316 polymorphism on myocardial infarction can be partially explained by the interaction of MedDiet on triglycerides, but other mechanisms are probably involved. Thus, the *MLXIPL* locus has also been associated with VLDL particle size and concentrations[30], coagulation Factor VII levels[36] and plasma concentrations of liver enzymes[37].

In conclusion, we reported three novel findings on the relationships of the nonsynonymous *MLXIPL*-rs3812316 polymorphism with cardiometabolic outcomes. First, in addition to triglyceride concentrations, we observed a lower risk of type 2 diabetes in homozygous subjects for the variant G-allele. Second, its association with fasting triglycerides was modulated by AdMedDiet at baseline, the protective effect of the variant G-allele against hypertriglyceridemia being stronger when AdMedDiet was high. The results obtained in the intervention study reinforced these findings. Third, intervention with MedDiet increased the beneficial effect of the variant allele against cardiovascular diseases, specifically for myocardial infarction. Given that the results stem from a large randomized intervention trial, they provide a high level of evidence, which may be important for future nutrigenetic studies.

Funding Sources

Disclosures

JSS is a non paid member of the Scientific Advisory Board of the International Nut Council, Reus, Spain. ER is a non paid member of the Scientific Advisory Committee of the California Walnut Commission, Sacramento, CA. The other authors have no conflict of interest.

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Table 1: Demographic, clinical, lifestyle characteristics and randomization to dietary intervention of study participants by the *MLXIPL*-rs3812316 polymorphism at baseline

	CC (n=6003)	CG+GG (n=1102+61)	P
Age (years)	66.9±6.2	66.8±6.4	0.623
BMI (Kg/m^2)	30±3.9	29.8±3.9	0.257
Gender (% women)	3422(57.1)	689(59.2)	0.158
Current smokers	834(13.9)	169(14.5)	0.688
Type 2 diabetes	2891(48.2)	576(49.5)	0.393
Hypertension	4969(82.8)	957(82.3)	0.687
Systolic blood pressure (mmHg)	149.4±20.8	148.7±20.3	0.305
Diastolic blood pressure (mmHg)	83.4±10.9	83.1±11.3	0.406
Lipid lowering drugs, statins	2408(40.1)	463(39.8)	0.847
Lipid lowering drugs, fibrates and others	319(5.3)	60(5.2)	0.829
Adherence to the Mediterranean diet	8.6±2	8.7±2	0.066
Energy intake (kcal/d)	2280.5±610.5	2258.7±585.1	0.262
Total fat (% energy)	39.1±6.8	39.4±6.7	0.113
Saturated fat (% energy)	10±2.3	10±2.2	0.758
MUFA (% energy)	19.4±4.6	19.7±4.5	0.082
PUFA (% energy)	6.2±2.1	6.3±2	0.356
Carbohydrates (% energy)	41.9±7.1	41.6±7	0.104
Physical activity* (kcal/d)	230.7±238.9	235.4±242.3	0.539
Alcohol consumption (g/d)	8.4±14.3	8.5±14	0.810
Non-drinkers	2183(36.7)	426(36.8)	0.919
Dietary intervention groups			
Mediterranean diet + EVOO	2070(34.5)	398(34.2)	0.915
Mediterranean diet + nuts	1970(32.8)	389(33.4)	
Control group	1963(32.7)	376(32.6)	

Values are mean±SD for continuous variables and number (%) for categorical variables. BMI indicates body mass index;

MUFA, Monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EVOO, extra virgin olive oil

P: P-value obtained in the tests for differences between genotypes

*Leisure time physical activity

Table 2: Association between the *MLXIPL*-rs3812316 polymorphism and plasma lipids and glucose. Crude means, odds ratios (OR) for hypertriglyceridemia*, unadjusted and adjusted linear regression coefficients (B)

| | *MLXIPL* Genotypes[†] | | | | Crude | | Adjusted | | |
| | CC (n=5607) | CG (n=1037) | GG (n=56) | | | | | | |
	Mean SD	Mean SD	Mean SD	P^1	B (95%CI)	P^2	B^3 (95%CI)	P^3	P^4
Total cholesterol (mmol/l)	5.47±1.02	5.46±1.05	5.57±0.87	0.699	0.002(0.059, 0.063)	0.954	-0.04(-0.062, -0.053)	0.878	0.876
LDL-C (mmol/l)	3.37±0.91	3.41±0.92	3.5±0.75	0.269	0.044(-0.011, 0.099)	0.116	0.038(-0.013,0.090)	0.114	0.182
HDL-C (mmol/l)	1.39±0.37	1.4±0.35	1.55±0.41	0.004	0.020(-0,002, 0.042)	0.073	0.018(-0,002, 0.039)	0.081	0.061
Triglycerides (mmol/l)	1.57±0.89	1.47±0.93	1.25±0.77	<0.001	-0.112(-0.166, -0.057)	<0.001	-0.109(-0.162, -0.056)	<0.001	<0.001
Fasting glucose (mmol/l)	6.75±2.26	6.92±2.5	5.96±1.81	0.003	0.056(-0.085, 0.197)	0.439	0.047(-0.064, 0.159)	0.406	0.562
Hypertriglyceridemia (%)	32.1	25.8	21.8	<0.001					
Hypertriglyceridemia, OR (95%CI)	1(ref.)	0.74(0.63-0.86)	0.59(0.31-1.12)	0.001	0.74(0.65-0.85)	<0.001	0.75(0.65-0.86)	<0.001	<0.001

*Fasting triglycerides >1.7 mmol/l. P-values for triglycerides were obtained after log transformation

Values are means and standard deviations (SD), odds ratio (OR) and 95% confidence intervals (CI) or regression coefficients (B) and 95%CI

[1]: Unadjusted P values for the polymorphisms for the comparison of means or OR as categorical (three categories)

B: Regression coefficient per-variant allele effects (genotypes coded as 0, 1 and 2 according to the number of minor alleles; additive effects)

[2]: P-values obtained for the unadjusted regression coefficients considering additive effects

3: P-values and regression coefficients obtained considering additive effects in models adjusted for sex, age, center, type 2 diabetes, BMI, lipid lowering drugs, hypoglycemic drugs, smoking, drinking, physical activity and total energy intake

4: P-values for regression coefficients in model 3 additionally adjusted for blood pressure, waist circumference and family history of premature cardiovascular disease (model 4).
[†]Biochemical data were available in 6700 subjects for total cholesterol, 6628 subjects for HDL-C, 6619 subjects for triglycerides, 6594 subjects for LDL-C and 6327 subjects for glucose

Table 3. Association between the _MLXIPL_-rs3812316 polymorphism* and hypertriglyceridemia[†] depending on the adherence to the Mediterranean diet (MedDiet) in the whole population and in relevant sub-groups[‡]

	Adherence to MedDiet	
	Low (<9 points)	**High (>=9 points)**
Strata	OR 95%CI	OR 95%CI
**Whole population** (n=6619)		
MLXIPL genotypes (Model 1)		
CC	1.00 (reference)	1.00 (reference)
G-carriers	0.88 (0.70-1.09)	0.63 (0.51-0.77)
	P^1=0.219	P^1<0.001
MLXIPL genotypes (Model 2)		
CC	1.00(reference)	1.00 (reference)
G-carriers	0.88 (0.70-1.10)	0.62 (0.50-0.77)
	P^2=0.248	P^2<0.001
**Males** (n=2829)		
MLXIPL genotypes (Model 2)		
CC	1.00 (reference)	1.00 (reference)
G-carriers	0.89 (0.63-1.27)	0.65 (0.48-0.89)
	P^2=0.519	P^2=0.006
Females (n=3790)		
MLXIPL genotypes (Model 2)		
CC	1.00 (reference)	1.00 (reference)
G-carriers	0.85 (0.64-1.14)	0.62 (0.46-0.82)
	P^2=0.273	P^2=0.001
**Type-2 diabetic subjects** (n=3189)		
MLXIPL genotypes (Model 2)		
CC	1.00 (reference)	1.00 (reference)
G-carriers	0.90 (0.66-1.23)	0.59 (0.44-0.79)
	P^2=0.508	P^2=0.001
Non-diabetic subjects (n=3430)		
MLXIPL genotypes (Model 2)		
CC	1.00 (reference)	1.00 (reference)
G-carriers	0.83 (0.63-1.14)	0.67 (0.48-0.89)
	P^2=0.247	P^2=0.008

*Dominant model

[†]Fasting triglycerides >1.7 mmol/l; Available in 6619 subjects

[‡]Odds ratios (OR) and 95% Confidence Intervals (CI)

Model 1: Adjusted for sex, age and center

Model 2: Additionally adjusted for type 2 diabetes, lipid lowering and hypoglycemic drugs, alcohol, smoking, physical activity and total energy intake

P^1: P value obtained for the genotype effect (dominant model) of the polymorphism in the multivariable logistic regression Model 1 stratified by MedDiet

P^2: P value obtained in Model 2

Additional adjustment of Model 2 for blood pressure, family history of cardiovascular disease and waist circumference did not change the level of the statistical significance of results (P^3: 0.242 for the whole population in the low adherence stratum and P^3<0.001 in the high stratum)

Table 4. Association between the *MLXIPL*-rs3812316 polymorphism and incidence of cardiovascular diseases depending on the dietary intervention* groups after the follow-up[†]. Hazard ratios (HR) and 95% confidence intervals (CI)

	Intervention group			
	Control (n=2339)		**Mediterranean diet*** (n=4827)	
	HR	95% CI	HR	95% CI
Total cardiovascular outcomes[‡]				
MLXIPL genotypes (Model 1)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	1.04	(0.62-1.76)	0.78	(0.50-1.23)
		P^1=0.876		P^1=0.292
MLXIPL genotypes (Model 2)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	1.03	(0.61-1.73)	0.78	(0.49-1.23)
		P^2=0.926		P^1=0.290
Myocardial Infarction				
MLXIPL genotypes (Model 1)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	0.90	(0.35, 2.33)	0.34	(0.12, 0.93)
		P^1=0.830		P^1=0.036
MLXIPL genotypes (Model 2)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	0.85	(0.33-2.21)	0.33	(0.12-0.91)
		P^2=0.739		P^2=0.033
Stroke				
MLXIPL genotypes (Model 1)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	1.36	(0.73-2.59)	1.03	(0.57-1.88)
		P^1=0.346		P^1=0.922
MLXIPL genotypes (Model 2)				
CC	1.00	(reference)	1.00	(reference)
G-carriers	1.43	(0.75-2.72)	1.04	(0.57-1.89)
		P^2=0.279		P^2=0.898

*: Pooled group: MedDiet supplement with extra virgin olive oil (n=2468) and MedDiet plus nuts (n=2359)

[†]: After a median follow-up of 4.8 years (interquartile range, 2.8-5.8 years)

[‡]: Major cardiovascular events (myocardial infarction, stroke and cardiovascular deaths)

Model 1: Adjusted for sex, age, center and intervention groups

Model 2: Additionally adjusted for type 2 diabetes, BMI, lipid lowering and hypoglycemic drugs, alcohol, smoking, AdMedDiet, physical activity, total energy intake and intervention group

P^1: P-value for the genotype effect (dominant) in Model 1. Cox-regression

P^2: P-value for the genotype effect in Model 2

Further adjustments for blood pressure, waist circumference and family history of cardiovascular disease (Model 3) did not change the statistical significance of estimates (OR:0.35 and P^3=0.040) for G-carriers versus CC for myocardial infarction in the MedDiet stratum

Figure Legends

Figure 1: Longitudinal effect of the *MLXIPL*-rs3812316 polymorphism on plasma triglycerides. Adjusted means of triglycerides depending on the polymorphism (**A**) or the intervention group (**B**) at baseline, 1-y, 3-y and 5-years of follow-up in all subjects having data for all the four measurements (n=2,418). Adjusted means were estimated from a repeated-measures ANOVA model with interaction terms adjusted for dietary intervention (MedDiet *versus* control), sex, age, center, BMI, type 2 diabetes, AdMedDiet, medications, smoking, drinking and physical activity. Adjusted P values for the overall effect of the polymorphism and diet are shown. P-interaction polymorphism x Diet x Time: 0.803 in the multivariable model. *Statistically significant adjusted P-values for the polymorphism or diet at every specific time point. P-values were obtained with log-transformed triglycerides.

Figure 2: **Cumulative myocardial infarction free-survival by *MLXIPL*-rs3812316 genotypes (CC versus G-carriers) in the MedDiet intervention group** (n=4,827). Cox regression model with outcome of myocardial infarction onset and the polymorphism adjusted by sex, age, center, type 2 diabetes, adherence to MedDiet, BMI, lipid lowering drugs, hypolipemic drugs, alcohol, smoking, physical activity, total energy intake and intervention group. HR estimated in the multivariable model.

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Subject characteristics

Eligible subjects were community-dwelling people (55-80 years for men; 60-80 years for women) who fulfilled at least one of two criteria: type 2 diabetes[1-3] or more cardiovascular risk factors (hypertension, dyslipidemia, body mass index [BMI] ≥25 kg/m^2, current smoking, or a family history of premature cardiovascular disease[2]. Exclusion criteria included a personal history of cardiovascular disease, any severe chronic illness, and drug or alcohol addiction[2].
The Institutional Review Board of each participating center approved the study protocol, and all participants provided written informed consent.

Demographic, clinical, anthropometric and dietary measurements

The baseline examination included assessment of standard cardiovascular risk factors, medication use, socio-demographic factors and lifestyle variables[2].
Food consumption was determined by a validated semi-quantitative food frequency questionnaire[3]. Physical activity was estimated by the validated Minnesota Leisure-Time Physical Activity Questionnaire as previously reported[2]. Weight and height were measured with calibrated scales and a wall-mounted stadiometer, respectively[2].

Outcome ascertainment

The primary endpoint was the occurrence of the first major cardiovascular event and comprised myocardial infarction, stroke or cardiovascular death[2]. We used four sources of information to identify end-points: 1) direct participant contact; 2) family physicians; 3) yearly review of medical records; and 4) consultation of the National Death Index. Medical records related to end-points were examined by the end-point adjudication committee, whose members were blind to treatment allocation. End-points confirmed by the committee occurring between October 1, 2003, and December 1, 2010 were included in the analyses.

Supplemental Methods References

1.   American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;31:S55–S60.

2.   Martínez-González MA, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fiol M, et al. Cohort Profile: Design and methods of the PREDIMED study. Int J Epidemiol. 2012;41:377-385.

3.   Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010;103:1808-1816.

Table S1. Quantitative Score of Adherence to the Mediterranean Diet (14-items)

	Foods and frequency of consumption	Criteria for 1 point*
1	Do you use olive oil as main culinary fat?	Yes
2	How much olive oil do you consume in a given day (including oil used for frying, salads, out of house meals, etc.)?	4 or more tablespoons
3	How many vegetable servings do you consume per day? (1 serving = 200g - consider side dishes as 1/2 serving)	2 or more (at least 1 portion raw or as salad)
4	How many fruit units (including natural fruit juices) do you consume per day?	3 or more
5	How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving = 100-150 g)	Less than 1
6	How many servings of butter, margarine, or cream do you consume per day? (1 serving = 12 g)	Less than 1
7	How many sweet/carbonated beverages do you drink per day?	Less than 1
8	How much wine do you drink per week?	7 or more glasses
9	How many servings of legumes do you consume per week? (1 serving = 150 g)	3 or more
10	How many servings of fish or shellfish do you consume per week? (1 serving: 100-150 g fish, or 4-5 units or 200 g shellfish)	3 or more
11	How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard?	Less than 3
12	How many servings of nuts (including peanuts) do you consume per week? (1 serving = 30 g)	3 or more
13	Do you preferentially consume chicken, turkey or rabbit meat instead of veal, pork, hamburger or sausage?	Yes
14	How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic, simmered with olive oil)?	2 or more

* 0 points if these criteria are not met.

Table S2: Demographic, clinical, lifestyle and genetics characteristics of the study participants at baseline according to the dietary intervention groups

	MedDiet with EVOO (n=2468)	MedDiet with Nuts (n=2359)	Control group (n=2339)
Age (years)	66.9 ± 6.2	66.6 ± 6.1	67.3 ± 6.3
BMI (Kg/m^2)	29.9 ± 3.7	29.7 ± 3.8	30.2 ± 4.0
Waist circumference (cm)	100.2 ± 10.4	100.2 ± 10.5	100.8 ± 10.9
Female sex* : n, %	1447 (58.6)	1269 (53.8)	1395 (59.6)
Current smokers: n, %	345 (14.7)	337 (14.3)	321 (13.7)
Type 2 diabetes: n, %	1236 (50.1)	1099 (46.6)	1132 (48.4)
Hypertension: n, %	2022 (81.9)	1947 (82.6)	1957 (83.7)
Lipid lowering drugs, statins: n, %	1011 (41.0)	922 (39.1)	938 (40.1)
Lipid lowering drugs, fibrates and others: n, %	118 (4.8)	142 (6.0)	119 (5.1)
MLXIPL-rs3812316 genotypes: n, %			
CC	2070 (83.9)	1970 (83.5)	1963 (83.9)
G-carriers	398 (16.2)	389 (16.5)	376 (16.1)
Energy intake (kcal/d)	2288 ± 608	2317 ± 607	2224 ± 600
Total fat (% energy)	39.2 ± 6.9	39.4 ± 6.5	39.0 ± 6.9
Saturated fat (% energy)	10.0 ± 2.2	10.0 ± 2.2	10.0 ± 2.3
MUFA (% energy)	19.6 ± 4.6	19.5 ± 4.3	19.3 ± 4.8
Proteins (% energy)	16.6 ± 2.9	16.5 ± 2.7	16.6 ± 2.9
Carbohydrates (% energy)	41.8 ± 7.3	41,6 ± 7.0	42.3 ± 7.2
Adherence to the MedDiet (points)	8.7 ± 2.0	8.7 ± 2.0	8.4 ± 2.0
Alcohol consumption (g/d)	8.6 ± 14.4	9.2 ± 15.0	7.5 ± 13.2
Physical activity (kcal/d)	232 ± 233	248 ± 246	215 ± 240

Values are mean±SD for continuous variables and number (%) for categorical variables. BMI indicates body mass index; MUFA, Monounsaturated fatty acids; EVOO, extra virgin olive oil; MedDiet, Mediterranean diet.

Figure S1. Adjusted means of fasting triglyceride concentrations at baseline depending on the *MLXIPL*-rs3812316 polymorphism and the level of adherence to the Mediterranean Diet (AdMedDiet). Means were adjusted for age, sex, center, BMI, type 2 diabetes, medications, total energy intake, physical activity, smoking and drinking. P values were obtained for the comparison of means between *MLXIPL*-rs3812316 genotypes depending on the strata of AdMedDiet (Low and High). Triglycerides were log-transformed for statistical analyses. Error bars: SE of means (n=6619).
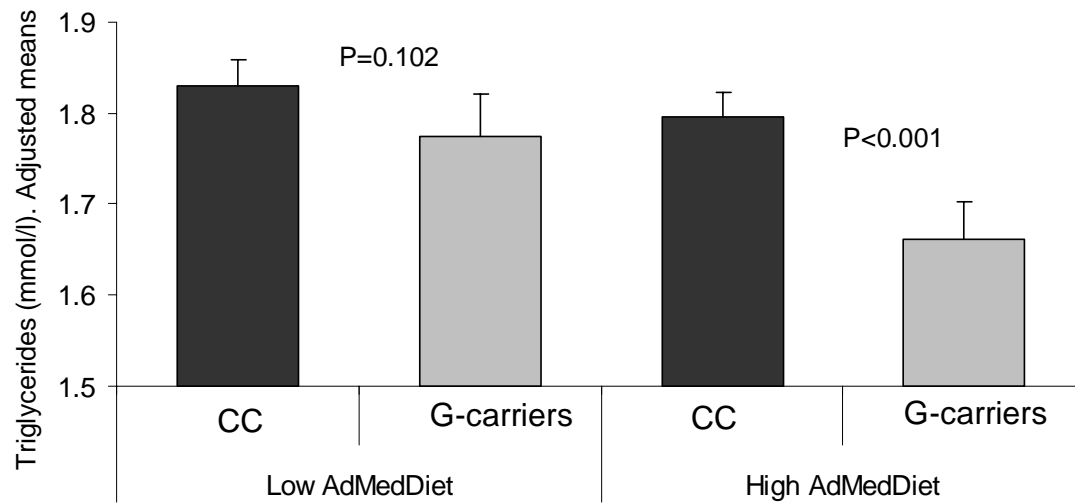
Figure S2: Longitudinal effect of the MLXIPL-rs3812316 polymorphism on plasma triglycerides. Adjusted means of triglycerides depending on the polymorphism (dominant model) and dietary intervention group at baseline, 1-y, 3-y and 5-years of follow-up in all subjects having data for all the four measurements (n=2,418). Adjusted means were estimated from a repeated-measures ANOVA model with interaction terms adjusted for dietary intervention (MedDiet versus control), sex, age, center, BMI, type 2 diabetes, AdMedDiet, medications, smoking, drinking and physical activity. Adjusted P values for the overall effect of the polymorphism diet and for the interaction among the polymorphism x Diet x Time, were obtained in the multivariable model. *P value for lineal trend between the four combined categories genotype and diet at 5-year follow-up. P-values were obtained with log-transformed triglycerides.
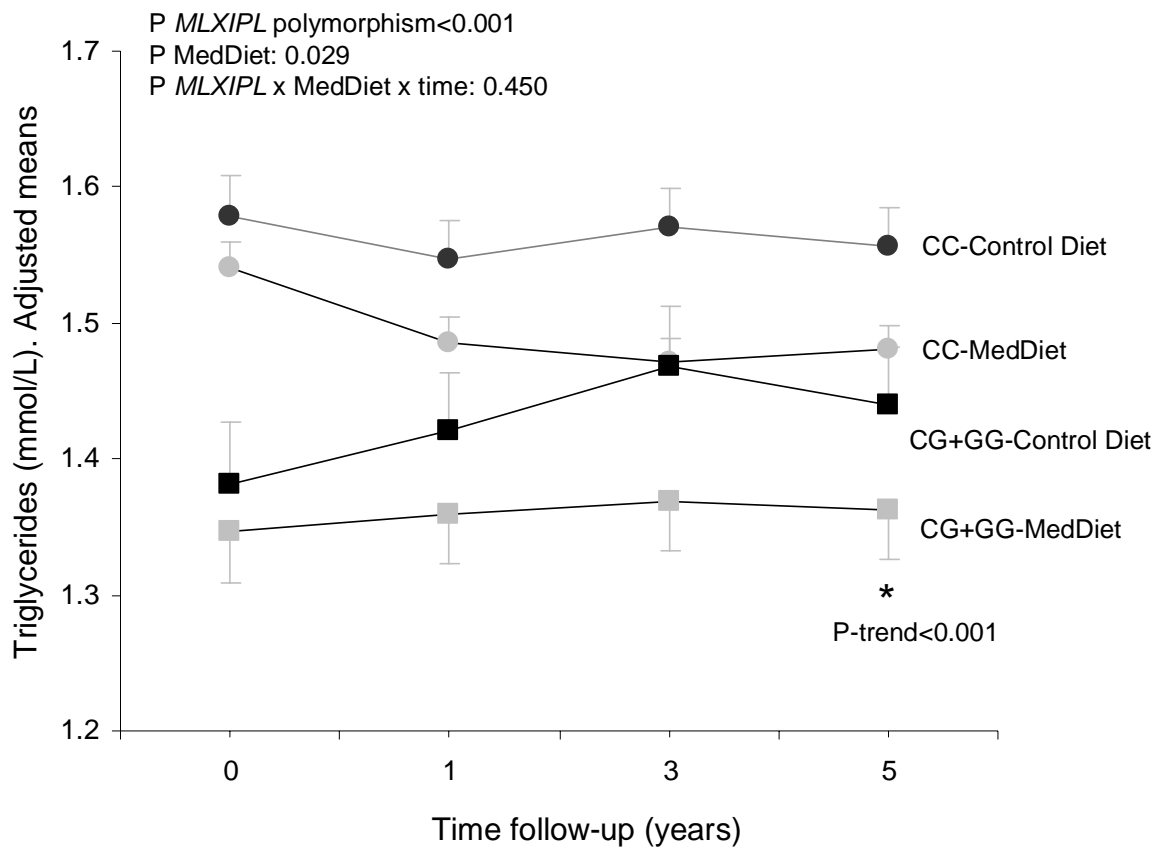
Figure S3: Longitudinal effect of the *MLXIPL*-rs3812316 polymorphism on hypertriglyceridemia (triglycerides >1.7mmol/L). Prevalence of hypertriglyceridemia depending on the polymorphism (dominant model) and dietary intervention group at baseline, 1-y, 3-y and 5-years of follow-up in all subjects having data for all the four measurements (n=2,418). OR for triglyceridemia after 5-year follow-up were estimated. CC subjects in the control group were considered the reference category. Crude and multivariable adjusted models were fitter. Models were adjusted for sex, age, center, BMI, type 2 diabetes, AdMedDiet, medications, smoking, drinking and physical activity.
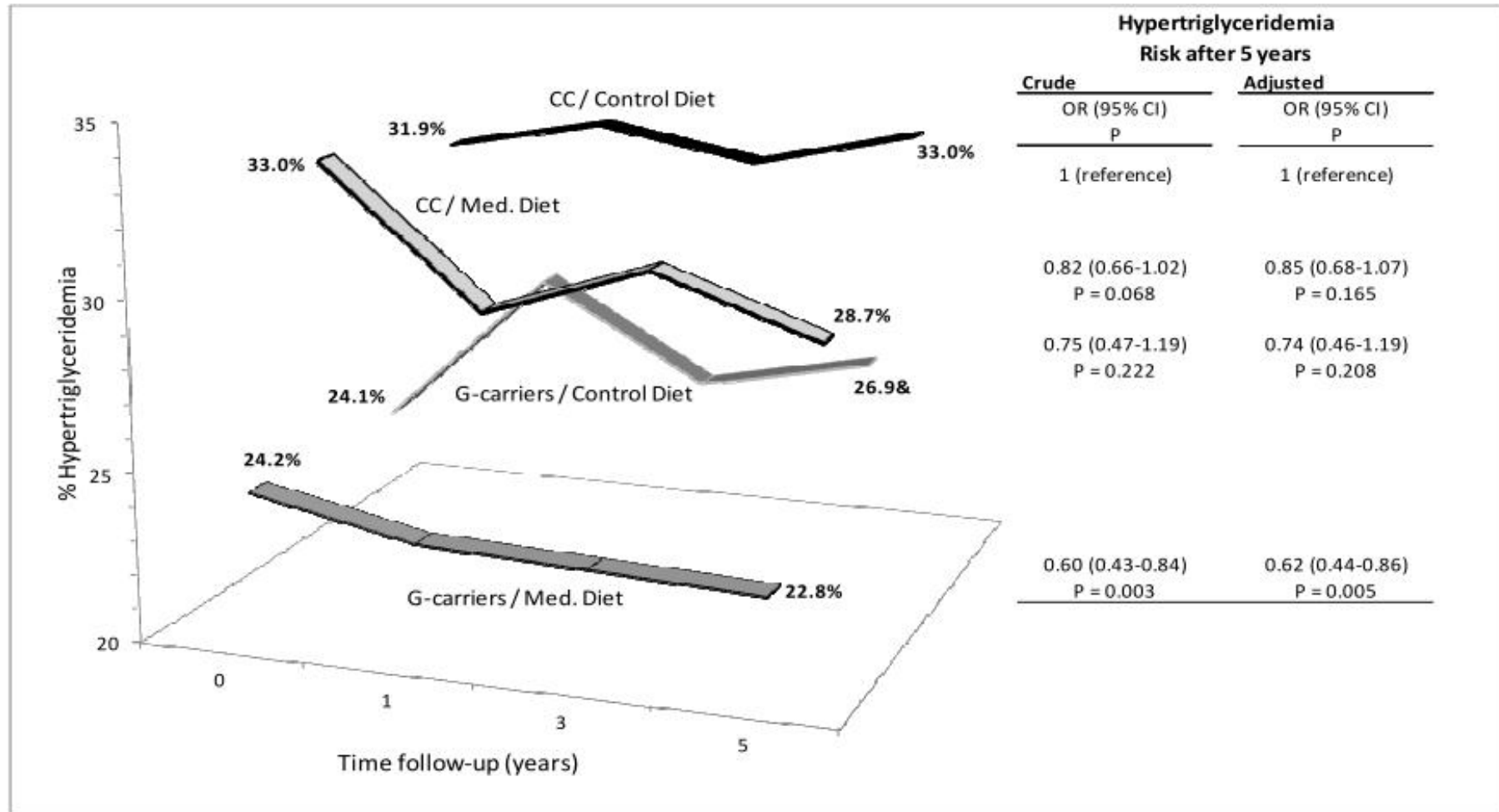
Table S3. Effect of the intervention with Mediterranean diet* on the incidence of cardiovascular diseases** stratified by the *MLXIPL*-rs3812316 polymorphism. Hazard ratios (HR) and 95% confidence intervals (CI)

	MLXIPL-rs3812316 genotypes	
	CC (n=6003)	**G-carriers** (n=1163)
	HR 95% CI	HR 95% CI
Total cardiovascular outcomes*		
Intervention group (Model 1)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.68 (0.52-0.90)	0.52 (0.27-0.99)
	P^1=0.007	P^1=0.046
Intervention group (Model 2)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.69 (0.52-0.91)	0.51 (0.26-0.99)
	P^2=0.008	P^2=0.045
Myocardial Infarction		
Intervention group (Model 1)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.84 (0.54-1.31)	0.29 (0.08-1.10)
	P^1=0.440	P^1=0.070
Intervention group (Model 2)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.84 (0.54-1.32)	0.24 (0.06-0.96)
	P^2=0.447	P^2=0.043
Stroke		
Intervention group (Model 1)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.58 (0.39-0.85)	0.48 (0.21-1.07)
	P^1=0.005	P^1=0.069
Intervention group (Model 2)		
Control group	1.00 (reference)	1.00 (reference)
Mediterranean diet	0.58 (0.39-0.86)	0.51 (0.23-1.15)
	P^2=0.007	P^2=0.105

*: Pooled group: Mediterranean diet supplemented with extra virgin olive oil (n=2468) and Mediterranean (
supplemented with nuts (n=2359)

**: After a median follow-up of 4.8 years (interquartile range, 2.8 to 5.8 years)

***: Major cardiovascular events (myocardial infarction, stroke and cardiovascular deaths)

Model 1: Adjusted for sex, age and center

Model 2: Adjusted for sex, age, center, type 2 diabetes, BMI, lipid lowering drugs, hypoglycemic drugs, drinking, smoking, adherence to MedDiet, physical activity and total energy intake

P^1: P value obtained for diet effect in multivariable Cox regression Model 1

P^2: P value obtained for diet effect in multivariable Cox regression Model 2

Further adjustment of Model 2 for blood pressure, family history of cardiovascular disease, waist circumference or lipids did not change the statistical significance of the estimates

Table S4. Association between the *MLXIPL*-rs3812316 polymorphism and incidence of myocardial infarction depending on the dietary intervention* groups after the follow-up. Incidence rates, hazard ratios (HR) and 95% confidence intervals (CI)**

	Incidence rate***	HR and 95% CI
Myocardial infarction		
Groups (Model 1)		
CC /control group	3.82	1.00 (reference)
G-carriers/ control group	3.33	0.90 (0.35-2.33)
CC /MedDiet group	3.30	0.82 (0.53-1.27)
G-carriers/ MedDiet group	1.10	0.28 (0.10-0.79)
MLXIPL genotypes (Model 2)		
CC	3.82	1.00 (reference)
G-carriers	3.33	0.85 (0.33-2.21)
CC /MedDiet group	3.30	0.83 (0.55-1.28)
G-carriers/ MedDiet group	1.10	0.29 (0.11-0.80)

*: Pooled group: Mediterranean diet supplement with extra virgin olive oil (n=2468) and Mediterranean diet supplemented with nuts (n=2359) vs control group (n=2339)

**: After a median follow-up of 4.8 years (interquartile range, 2.8 to 5.8 years)

***: Crude incedence rates expressed per 1000 person-y follow-up

Model 1: Adjusted for sex, age, center and intervention groups

Model 2: Adjusted for sex, age, center, type 2 diabetes,BMI, lipid lowering drugs, hypoglycemic drugs, alcohol, smoking, AdMedDiet, physical activity, total energy intake and intervention group

P[1]: P-value for the genotype effect (dominant) in Model 1

P[2]: P-value for the genotype effect in Model 2