Interplay between the Mediterranean diet and C-reactive protein genetic polymorphisms towards inflammation in adolescents.

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ABSTRACT

Aim: From a nutrigenetics perspective, we aim to investigate the moderating role of the Mediterranean diet and each of its subgroups in the association between C-reactive protein (*CRP*) gene polymorphisms and CRP blood concentration in adolescents.

Methods: In 562 adolescents (13-17y) of the European HELENA study, data was available on circulating CRP levels as inflammatory biomarker, three *CRP* gene SNPs (rs3093068, rs1204, rs1130864), food intake determined by a self-administered computerized 24h-dietary recall for 2 days, and body composition. A 9-point Mediterranean diet score and each food subgroup were tested as moderator via SNP*diet interaction. Analyzes were adjusted for age, sex, puberty, adiposity and socioeconomic status.

Results: The minor allele frequencies of rs3093068 and rs1130864 SNPs (GG and TT, respectively) were associated with higher CRP concentrations, while rs1205 (CT/TT) was associated with lower CRP concentrations. There were significant interactions between rs3093068 and Mediterranean diet (B= -0.1139, p=0.011), or the fish food subgroup (B= -0.0090, p=0.022), so that those with the highest genetic CRP risk underwent the highest CRP attenuation by a healthier diet. Although the effect of diet and SNP was substantial, the explained variance by interaction was only 1%.

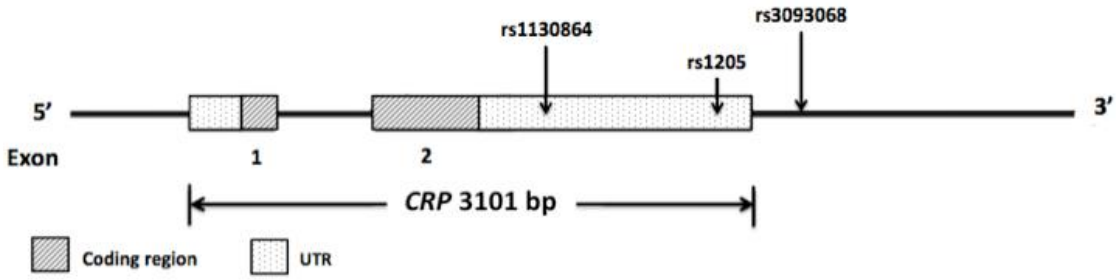
Conclusion: Greater adherence to the Mediterranean diet and particularly its fish component was associated with a lower CRP blood concentrations especially in those at highest genetic risk due to the rs3093068 SNP.

Key words: C-reactive protein, Single Nucleotide Polymorphism, Mediterranean diet, inflammation, interaction, nutrigenomics.

1. Introduction

Inflammation is a major health risk linked with worldwide mortality, as it is present in most non-communicable chronic diseases. As an example, the onset and progression of atherosclerosis as a pathological process towards cardiovascular diseases (CVD) is based on the establishment of an inflammatory process [1]. Changes in inflammation can be assessed by measuring inflammatory biomarkers in the blood; C-reactive protein (CRP) is herein one of the most studied biomarkers [2]. CRP is not only associated with acute inflammation, but also with other metabolic abnormalities [3] in which minor CRP elevation is considered a marker of low-grade inflammation [4]. Although data are limited, associations between CRP and CVD risk factors in children and adolescents are similar to those reported in adults [5–7]. CRP concentrations in childhood are increased in the settings of obesity and are strongly associated with waist circumference, body mass index, and adiposity [5,8–10].

Genetics play an important role in CRP concentrations. Extensive evidence shows that some single nucleotide polymorphisms (SNPs) within the *CRP* gene at the 1q21-q23 region of the proximal long arm of chromosome 1 (Figure 1, adapted from Lee *et al*. [11]) affect the amount of CRP produced with a heritability around 30% to 40% [6]. Individual SNPs in the *CRP* gene might explain up to 10% of the variance in blood CRP levels, e.g. the three *CRP* SNPs rs3093068, rs1205 and rs1130864 are shown to be important influencers of CRP levels [12–14]. Genetic variation in genes involved in the etiology of inflammation may interact with environmental exposures such as diet, to modulate susceptibility to inflammation [15].



Adapted from Lee *et al.* [11]

Figure 1. The *CRP* gene region (chromosome 1q21-q23) and the locations of the three SNPs tested in our study. UTR, untranslated region.

Nutrigenetics is a raising research area which examines how the interactions between genetic markers and phenotypic factors influence health [16]. Thus, the progression from a healthy phenotype to a chronic disease phenotype occurs by changes in gene expression or enzyme and protein activities which can be regulated by nutrients and bioactive dietary compounds [15]. Since certain foods can interact with the transcription process of pro- and anti-inflammatory factor, a diet pattern might attenuate/exacerbate the impact of genetic predisposition towards inflammation [17]. A healthy dietary intake, which is characterized by the presence of essential nutrients, antioxidant vitamins, polyunsaturated (omega 3) and monounsaturated fatty acids (as characterized by the Mediterranean diet) could attenuate low-grade inflammation by improving postprandial dysmetabolism, and blunt the post-meal increase in glucose, triglycerides, and oxidative stress [18]. In contrast, an energy-dense nutrient-low diet with non-essential fat (e.g. trans-fat) and refined carbohydrates can immediately induce oxidative stress after a meal. This triggers the increase of free radicals and atherogenic changes (including endothelial dysfunction, hypercoagulability, sympathetic hyperactivity), contributing to low-grade inflammation and a future cardiovascular event [19].

Indeed, Calder et al. [20] call for further research in this area, noting that the role of gene polymorphisms in the effect of nutrients and dietary patterns on inflammation requires much greater exploration. Testing this hypothesis in youth has the advantage of less confounding by existing chronic disease or medication and can emphasize the importance of early prevention. Thus, this study aims (1) to confirm whether genetic variation in three *CRP* SNPs (rs3093068, rs1204, rs1130864) is associated with higher CRP blood concentrations already during adolescence, and (2) to explore whether a higher adherence to the Mediterranean diet and each of its food subgroups could modulate this genetic susceptibility. After all, the Mediterranean diet score has already been associated with lower inflammation in this young study population [21].

2. Methods

2.1 Study design and participants

Data was derived from the HELENA Cross Sectional Study in 2006-2007 containing a sample of adolescents from 10 European cities located in separated geographical points in Europe: Austria, Belgium, France, Germany, Greece (two cities), Hungary, Italy, Spain and Sweden. The selected towns were equivalent and comparable between countries. Their size was sufficiently large to ensure a diverse population [22]. The main objective of the HELENA study was to obtain comparable data of a large sample of European adolescents on nutrition and health related parameters by a standardized procedure. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. All participants and their parents signed an informed consent.

The total HELENA sample was 3,528 adolescents in which a one-third subset (n=1,155) was selected for blood sampling. A-priori it was decided based on sample size calculation that only around 1000 participants were needed for immunological parameters and school classes were thus randomly chosen for blood collection. In the present study, 987 adolescents had data on both CRP blood concentration and *CRP* SNPs (rs3093068, rs1205, and rs1130864). Specific inclusion criteria such as data availability from the 24-

h dietary recall, anthropometry (measures on skinfold thickness, waist circumference), and data on socioeconomic status, were defined in the present study for the interaction analyses, resulting in 562 adolescents (263 male, 299 female), aged 13.0–16.99 years (Figure 2). This further selection from 987 to 562 adolescents did not introduce a significant difference in CRP levels or genotypes (*p* between 0.107 and 0.947).

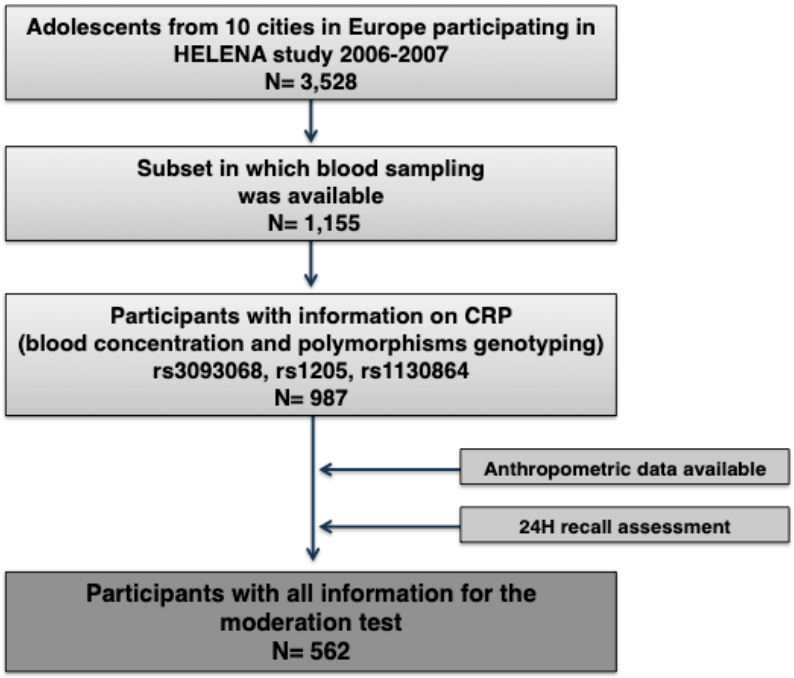


Figure 2. Sampling procedure scheme.

2.2 Dietary intake assessment

Eating habits were determined from a self-administered computerized validated 24-h dietary recall called the HELENA-Dietary Assessment Tool (HELENA-DIAT) [23,24], a tool validated in Flemish adolescents. Participants completed the HELENA-DIAT on two non-consecutive days within a time span of 2 weeks. Based on these data, the Mediterranean diet score was calculated by a balance of low (score 0 to 4) versus high (score 5 to 9) adherence to the traditional Mediterranean diet.

2.2.1 Mediterranean diet score

The Mediterranean diet score comprises nine single components: the ratio of monounsaturated/ saturated fatty acids, legumes, fruits and nuts, vegetables, meat, cereals, alcohol, dairy and fish. A scale indicating the degree of adhesion to the traditional Mediterranean diet was constructed [25], and later revised to include fish intake [26]. The adherence to the traditional Mediterranean diet was assessed by a score ranging from 0 to 9, with higher scores indicating greater adherence [27]. In this study, six components in the Mediterranean diet score were considered as positive: 1) high ratio of monounsaturated to saturated dietary lipids (mainly olive oil), 2) high consumption of vegetables, 3) high consumption of fruits and nuts, 4) high consumption of fish, 5) high consumption of cereals, and 6) high consumption of pulses; while three components were considered as negative: 7) high consumption of meat and meat products, 8) high consumption of milk and dairy products, and 9) any consumption of alcohol. The alcohol consumption (from alcoholic beverages) is presented as grams per day, for 25 consuming boys (from 263 subjects) and 66 consuming girls (from 299 subjects), and was considered negative because of our focus on an adolescent population.

2.2.2 Mediterranean diet food-subgroups' description

The *Vegetables* food-subgroup consists of all types of vegetables excluding potatoes. The *Fruits & Nuts* food-subgroup consists of all types of fruits and nuts (including nut- and seed spreads). The *Pulses* food-subgroup includes peas, beans, lentils, chickpeas, and fava beans (fresh peas and sweet corn were excluded). The *Cereal & Roots* food-subgroup is comprised by starch roots, potatoes and cereals such as rice and wheat. The *Monounsaturated/Saturated fat ratio* food-subgroup is a ratio of mono-unsaturated fats versus saturated fats. The *Dairy* food-subgroup is composed by all dairy products, such as: cow milk, yoghurt, cheese, sour cream, and other milk products. The *Fish* food-subgroup is composed by all types of fish and fish products. The *Meat* food-subgroup is composed by all types of meat, such as: chicken, pork, cow, turkey, and veal meat and processed meat (such as ham, sausage, salami and mortadella).

2.3 Adiposity assessment

To measure central adiposity, waist circumference was used. To reflect overall adiposity, the sum of six skinfolds was used: skinfold thickness was measured to the nearest 0.2 mm in triplicate on the left side at biceps, triceps, subscapular, suprailiac, thigh, and medial calf with a Holtain Caliper (Crymmych, UK). Fieldworkers followed a central training and followed the same manual. Intraobserver reliability was above 95%, while interobserver reliability was above 90% [28]. Body Mass Index (BMI) was categorized following the IOTF cut-offs [29]. The anthropometric methods in the HELENA study have been reported in detail elsewhere [28,30].

2.4 Biochemical analyzes

2.4.1 Blood sample collection and CRP assessment

In a randomly selected one-third subset of the total HELENA study population, blood samples were collected in overnight fasting state. We used a standardized methodology for blood collection, transport and analysis by a certified laboratory [31]. The quality control was within recommended levels and transport had no influence. Detection limits (sensitivity) for serum CRP by immunoturbidimetry (AU2700 biochemistry analyzer, Olympus, Watford, UK) were 0.007 mg/L and 0.05 pg/mL with intra-assay CV of 1.9%. Nobody took nonsteroidal anti-inflammatory drugs or had an active inflammatory disease. None of the girls had Polycystic Ovarian Syndrome or was in the menstrual period. Six individuals (only male) presented CRP levels higher than 10 mg/L. Only 8.4% smoked regularly.

2.4.2 Single Nucleotide Polymorphisms genotyping

Blood for DNA extraction was collected in EDTA K3 tubes and stored at IEL Bonn, and sent to the Laboratoire d'Analyse Génomique Centre de Ressources Biologiques (LAG-CRB) - BB-0033-00071 Institut Pasteur de Lille, F-59000 Lille, France. DNA was extracted from white blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) and stored at -20°C. Although several SNPs were genotyped in this study, only three of these SNPs were located on the *CRP* gene: the rs3093068, rs1205, and

rs1130864. The genotyping was done by an Illumina system (Illumina, Inc, San Diego, California) using the Golden- Gate technology (GoldenGate Software, Inc, San Francisco, California). The genotyping success rate was 100% for rs3093068 and rs1205, and was 99.9% for rs1130864. The genotype distributions respected the Hardy-Weinberg equilibrium (HWE) for the *CRP* rs3093068 (χ^2= 1.15, p= 0.28), rs1205 (χ^2= 1.08, p= 0.30) and rs1130864 (χ^2= 0.77, p= 0.38) SNPs. Genotypes were coded as follows: CC, homozygous major alleles; CG, heterozygotes; and GG, homozygous minor alleles for rs3093068, while CC; CT; and TT, respectively, for rs1205 and rs1130864. The values for the linkage disequilibrium between the three investigated SNPs was above 95 for D' and was between 3 and 20 for r^2.

2.5 Socioeconomic status (SES) and parental education

As indicator of material affluence, the family affluence scale ranging from 0 (low) to 8 (high) was based on family car ownership, having an own bedroom, Internet availability and computer ownership [30]. In addition, parental educational level was categorized into three score groups: primary education and lower secondary education (score 1), higher secondary education (score 2), and tertiary education (score 3). A detailed description of SES and parental education level can be found elsewhere [23].

2.6 Data analyses

Statistics were performed using SPSS (IBM SPSS Statistics, version 24.0) and moderation effects were obtained by testing interactions [32,33]. The statistical significance was set at two-sided p<0.05. As CRP levels were not normally distributed, the Mann-Whitney test was performed in comparing CRP levels between groups and CRP was log-transformed for the regression analyses. The moderation effect of the diet in the SNPs-CRP relation was tested by adding the interaction term “diet*SNP”. In the case of a significant interaction, the SNP relation was tested for 3 representative groups: those at the mean, at 1 SD below the mean and 1 SD above the mean of the dietary index. Six individuals had high CRP levels (>10 mg/L) but were not excluded from the analyzes as it did not change any result.

3. Results

3.1 Characteristics of the study sample

The participants' characteristics are shown in Table 1. Based on BMI, 13.7% of the adolescents (38 male; 39 female) were overweight, while 4.6% (17 male; 9 female) were obese.

Table 1. Characteristics of the study sample.

	N=562 (263 males; 299 females)	
	Median [P25; P75] or %	
C-reactive protein (mg/L)	0.37 [0.16 – 0.88]	
	Mean	**Std. Deviation**
Age	14.81	1.22
Socio-economic characteristics		
High socioeconomic status (%)[a]	80.07%	
High paternal education (%)[b]	65.30%	
High maternal education (%)[b]	69.39%	
Anthropometric characteristics		
Skinfold thickness (sum) (mm)	88.64	39.75
Body Mass Index (z-score)	0.37	1.08
Waist circumference (cm)	72.10	8.25
Diet-related characteristics		
Mediterranean diet score (0 – 9)	4.15	1.45
Vegetables (g/day)	93.96	56.61
Fruits and Nuts (g/day)	129.38	100.05
Pulses (g/day)	10.56	34.09

Cereal and roots (g/day)	297.46	99.95
Monounsaturated/Saturated fat (ratio)	0.89	0.17
Dairy (g/day)	220.35	39.49
Fish (g/day)	18.48	19.42
Meat (g/day)	147.44	78.02
Alcohol consumption (g/d)	0.89	2.34
Energy (kcal)	2199.76	795.18
Total carbohydrate (g/day)	240.78	154.98
Total protein (g/day)	87.95	31.78
Total fat (g/day)	81.64	34.36
Cholesterol (mg/day)	248.81	16.16
Saturated fat (g/day)	44.94	18.22
Monounsaturated fat (g/day)	44.38	17.57
Polyunsaturated fat (g/day)	40.18	18.66
Eicosapentaenoic acid (mg/day)	17.11	2.87
Docosahexaenoic acid (mg/day)	20.89	2.99
Total fiber (g/day)	12.41	1.60

Data displayed for 562 adolescents (263 males; 299 females) who had all information for the moderation test: data on 24-h dietary recall, anthropometry, and SES. Values are presented as median or mean and standard deviation for continuous variables, and as % (Chi square) for categorical variables. P25, 25th percentile; P75, 75th percentile; SD, standard deviation.

a Socioeconomic status was based on the family affluence scale (family car ownership, having an own bedroom, internet availability and computer ownership); a sum score from 4 to 8 represents medium to high socio-economic status.

b High parental education represents higher secondary education and tertiary education. Some variables were log-transformed as they were not normal distributed but backtransformed numbers are shown: saturated, monounsaturated and polyunsaturated fat; eicosapentaenoic and docosahexaenoic acid; total fiber.

3.2 Associations between the allele frequencies of the investigated *CRP* genotypes (rs3093068, rs1205 and rs1130864) and CRP blood concentrations.

Table 2 shows the patterns in median CRP differences (without logarithmic transformation) related to each genotyped SNP. Consistent with previous studies, the minor alleles of rs3093068 and rs1130864

SNPs were associated with higher CRP concentrations (+0.15 mg/L, p=0.040; and +0.09 mg/L, p=0.004, respectively). In contrast, the minor allele of rs1205 was associated with lower CRP concentrations (-0.07 mg/L, p=0.012).

Table 2. Associations between the allele frequencies of the investigated *CRP* genotypes (rs3093068, rs1205 and rs1130864) and CRP blood concentrations in the HELENA study.

***CRP* SNPs**		**C-reactive protein (mg/L)**			
	N	**Median**	**Min-Max**	**Mean rank**	***P* value**
rs3093068					
CC	834	0.37	0.01 - 75.99	280.59	**0.042**
CG or GG	153	0.54	0.01 - 66.26	286.73	
C allele	1813	0.39	0.01 - 75.99	561.92	**0.043**
G allele	161	0.54	0.01 – 66.26	569.52	
rs1205					
CC	468	0.43	0.01 - 75.99	512.85	**0.049**
CT or TT	519	0.36	0.01 - 25.10	477.00	
C allele	1370	0.42	0.01 - 75.99	1008.91	**0.012**
T allele	604	0.35	0.01 – 25.10	938.94	
rs1130864					
CC	458	0.34	0.01 - 66.26	467.60	**0.008**
CT or TT	528	0.44	0.01 - 75.99	515.97	
C allele	1350	0.37	0.01 - 75.99	961.60	**0.004**
T allele	622	0.46	0.01 - 75.99	1040.53	

Data displayed for 987 adolescents who had information on both CRP blood concentration and *CRP* polymorphisms genotyping (rs3093068, rs1205, and rs1130864). SNPs: Single

Nucleotide Polymorphisms. Min-Max: Minimum and Maximum. C, cytosine; G, guanine; T, thymine. Bold: statistical significance when $p < 0.05$.

3.3 Moderating effect of diet on the relation between *CRP* gene SNPs (rs3093068, rs1205, rs1130864) and CRP blood concentrations

Table 3 presents the interaction effects of diet on the relation between CRP concentrations and *CRP* SNPs (rs3093068, rs1205, rs1130864, homozygous major allele carriers versus other genotypes) in 562 adolescents from the HELENA study. Significant interaction was detected for rs3093068 using the Mediterranean diet score and the fish food subgroup (B= -0.1139, p=0.0110; B= -0.0090, p=0.0220; respectively). As can be seen in Figure 3, higher adherence to the Mediterranean diet (panel A) or higher fish intake (panel B) was associated with a slight attenuation of the CRP levels in those adolescents carrying the genetic risk of rs3093068 SNP (G allele carriers).

238 **Table 3.** Moderating effects of diet on the relation between *CRP* gene SNPs (rs3093068, rs1205, rs1130864) and CRP blood concentrations in 562
239 adolescents from the HELENA study.
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Food groups	**rs3093068 (CC *vs* CG+GG)**			**rs1205 (CC *vs* CT+TT)**			**rs1130864 (CC *vs* CT+TT)**		
	B	**95%CI**	***p***	**B**	**95%CI**	***p***	**B**	**95%CI**	***p***
Mediterranean diet score	-0.1139	-0.2016 – -0.0262	**0.01**	-0.0077	-0.0699 – 0.0545	0.80	0.0245	-0.0374 – 0.0864	0.43
Vegetables	-0.0014	-0.0033 – 0.0004	0.13	1.21×10^{-5}	-0.0016 – 0.0016	0.98	-0.0002	-0.0018 – 0.0014	0.82
Fruits & Nuts	-0.0004	-0.0015 – 0.0008	0.53	0.0004	-0.0006 – 0.0013	0.44	0.0002	-0.0007 – 0.0011	0.67
Pulses	-0.0010	-0.0056 – 0.0036	0.67	-0.0016	-0.0043 – 0.0011	0.24	-0.0001	-0.0030 – 0.0028	0.95
Cereal & Roots	-0.0002	-0.0014 – 0.0011	0.79	-0.0006	-0.0015 – 0.0003	0.19	0.0001	-0.0008 – 0.0010	0.82
Monouns./Saturated fat ratio	-0.4483	-1.1845 – 0.2879	0.23	0.3199	-0.2071 – 0.8469	0.23	0.0521	-0.4811 – 0.5853	0.84
Dairy	0.0002	-0.0005 – 0.0008	0.61	-0.0003	-0.0007 – 0.0001	0.19	-0.0001	-0.0005 – 0.0003	0.55
Fish	-0.0090	-0.0166 – -0.0013	**0.02**	0.0013	-0.0034 – 0.0061	0.58	-0.0015	-0.0062 – 0.0032	0.53
Meat	0.0002	-0.0015 – 0.0020	0.79	0.0001	-0.0011 – 0.0012	0.92	-0.0001	-0.0012 – 0.0011	0.90
Alcohol	-0.0090	-0.0600 – 0.0420	0.72	-0.0118	-0.0580 – 0.0344	0.61	0.0059	-0.0417 – 0.0534	0.80

Data displayed for 562 adolescents (263 males; 299 females) who had all information for the moderation test (data on 24-h dietary recall, anthropometry, and SES). Moderation was tested using interaction (by Process macro for SPSS) based on including the diet**CRP* polymorphism term (CC *vs* CG+GG for rs3093068, and CC *vs* CT+TT for rs1205 and rs1130864). Analyzes were adjusted for age, sex, socioeconomic status, overall adiposity (sum of six skinfold thickness) and waist circumference adjusted by pubertal status, in the prediction of CRP concentrations. B= unstandardized coefficient. Bold: statistical significance when $p < 0.05$.

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242 **Figure 3.** Moderation by diet on the relation between *CRP* rs3093068 SNP and CRP blood concentrations

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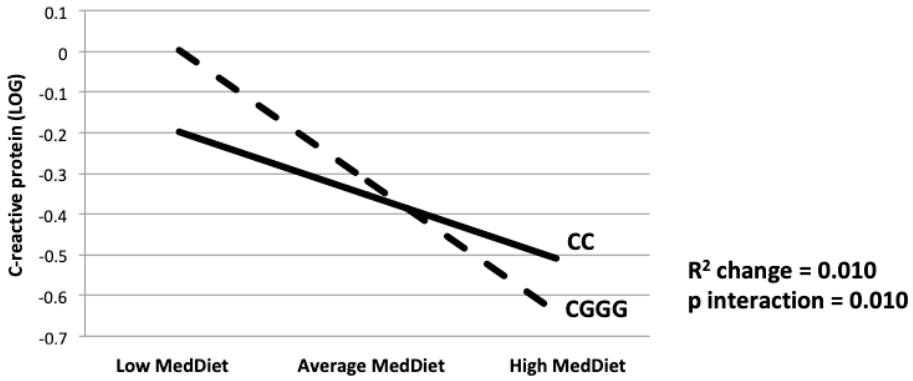
(A) Moderating effect of the adherence to the Mediterranean diet on CRP blood concentrations among adolescents with and without the CC genotype of rs3093068.



C-reactive protein level log transformed. Black straight line: CC, wild homozygous; black dotted line: CG, heterozygous, and GG, variant homozygous of the rs3093068 SNP. Analyzes were adjusted for age, sex, socioeconomic status, overall and central adiposity (skinfold thickness and waist circumference, respectively). In the case of a significant moderation, the CRP-SNP relation was tested for 3 representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (higher adherence) of the dietary index.

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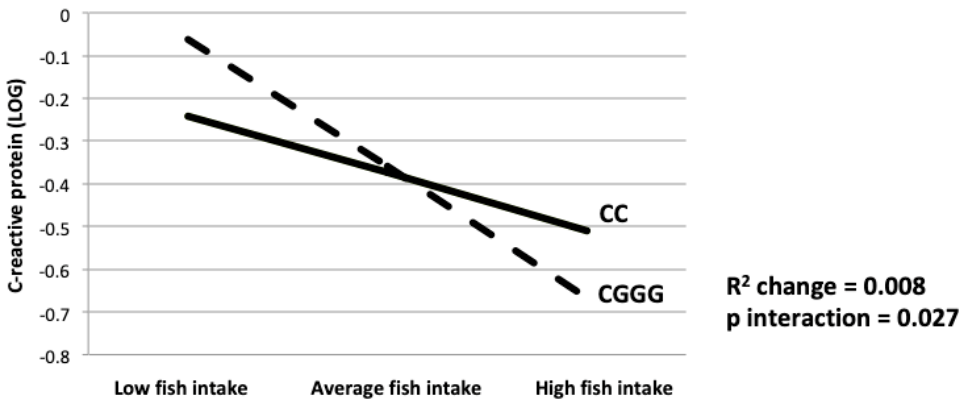
(B) Moderating effect of the fish subgroup intake on CRP blood concentrations among adolescents with and without the CC genotype of rs3093068.



C-reactive protein level log transformed. Black straight line: CC, wild homozygous; black dotted line: CG, heterozygous, and GG, variant homozygous of the rs3093068 SNP. Analyzes were adjusted for age, sex, socioeconomic status, overall and central adiposity (skinfold

thickness and waist circumference, respectively). In the case of a significant moderation, the CRP-SNP relation was tested for 3 representative groups: those at the mean (average fish intake), at 1 SD below the mean (lower fish intake), and 1 SD above the mean (higher fish intake).

4. Discussion

To our knowledge, this is the first study that investigates the capacity of the Mediterranean diet and its food sub-groups in influencing inflammatory status (here measured with CRP concentrations) according to some selected *CRP* gene polymorphisms in European adolescents. The cross-sectional results showed that a higher Mediterranean diet adherence and fish intake seemed to attenuate a genetic risk (rs3093068) towards CRP-related inflammation. This was not the case for the two other SNPs.

Our first observation was that the genetic variants in the *CRP* gene were substantially associated with CRP concentrations, in agreement with other studies [34]. The minor G or T alleles of rs3093068 and rs1130864 were significantly associated with higher CRP concentrations as demonstrated by some studies [13,14] while minor allele T carriers of rs1205 associated with lower levels of CRP [11].

Second, the interaction between diet and SNPs regarding CRP blood concentrations was tested. Although slight, a higher Mediterranean diet adherence and fish intake attenuated the CRP blood concentrations for homozygous major alleles carriers (CC) but even more pronounced in heterozygotes and homozygous minor alleles carriers (CG/GG) that are genetically at higher inflammatory risk. The effect was small as the interaction term only explained around 1% additional variance in CRP concentrations. The CRP attenuation in the high-risk genetic group was double the size (around 0.6 mg/L) compared to the attenuation in the low-risk genetic group (around 0.3mg/L).

This is in the expected direction as the Mediterranean diet has been associated with better metabolic and inflammatory profiles in some clinical trials [35,36], and fish has anti-inflammatory potential [37]. Indeed, studies show that a lower adherence to the Mediterranean diet leads to a worse profile of inflammatory markers in adults and adolescents [38], while greater adherence brings significant improvements in lipid profile [39] and reduction in risk of developing type 2 diabetes [40]. This can be explained mainly by specific food groups present in the

Mediterranean diet such as vegetables, fruits, nuts, legumes, fish, and a high proportion of monounsaturated fat, which all provide essential nutrients, antioxidant vitamins and minerals capable of interacting with genes [41]. As an example, green dark vegetables are rich in folate, which is crucial for DNA methylation status and thus epigenetics [42]. Regarding the fish subgroup, several of its nutrients might contribute to lower CRP: mainly long chain polyunsaturated fatty acids omega-3, but it also contains protein, iron, zinc, iodine, vitamin B12, vitamin A (especially oily fish), and vitamin D [43]. In fact, these essential nutrients may lead to a better cardiovascular health, hence the calls for higher fish consumption [44]. Using an overall dietary pattern score like our Mediterranean diet score has the advantage of considering the potential synergy between multiple dietary factors.

Several studies have shown genotype-nutrient interactions, including interactions with SNPs that affect inflammation [15,45]. Nutrients, micronutrients and phytochemicals found in food are the most influential environmental stimuli that govern the expression of genetic information. From a preventive perspective, genetic predisposition conferring health disadvantages like inflammation, could possibly be attenuated with diet modifications [46]. A fish oil supplementation for 12 weeks in healthy young men showed a decrease in TNF-α production depending on the genotype [47]. A cross-sectional study in 2,010 South African adults also tested such SNP*diet interaction using 12 *CRP*-related SNPs [48]. A high ratio of omega-6 to omega-3 intake was associated with higher CRP concentrations in those carrying the major alleles at SNPs rs3093058 and rs3093062, compared to those carrying the minor allele [48]. Yet, increased cholesterol intake led to increased CRP levels in those individuals carrying the minor allele in rs3093058 or rs3093062, while those homozygous for the major allele were more protected and were thus "low responders" to cholesterol intake [48]. These cholesterol and omega interactions were not found for the three SNPs we considered [48]. For none of the 12 SNPs, interactions with the diet quality score, fruits and vegetables, pulses/nuts/seeds, carbohydrates, added sugar, total fat, monounsaturated/saturated fat ratio, alcohol, fiber, vitamin C, vitamin E, zinc or magnesium were significant [48]. Interactions were thus SNP and dietary factor specific. Although there is absolutely no consensus, our findings together with previous ones highlight fish or related omega-3 fatty acid balance as a potential important dietary factor.

5. Strengths and Limitations

For the first time, we have shown that a Mediterranean diet could attenuate inflammation by gene-diet interaction in adolescents. A first strength is the multi-country design of this study. As this investigation involved the participation of adolescents from 10 European cities, this created the advantage of using a large database with relevant information on the diversity of dietary intake e.g. both Mediterranean and non-Mediterranean countries. Herein, we focus on an understudied population i.e. adolescents in the perspective of early treatment and prevention. A second asset is that we tested three SNPs and a holistic view of using an overall dietary index with sub-analyses on separate food groups allowing overall and specific public health recommendations. The analyses were adjusted for adiposity as an important source of chronic inflammation: the waist circumference was used to reflect central adiposity, while we applied the sum of six skinfolds for overall adiposity, since it is considered as a better predictor of body fat than BMI [49].

The present study has some limitations. First, a self-administered computerized 24-h dietary recall relies on the respondents' memory and their capabilities to interpret those questions on frequency and quantity of consumption in the last 24 hours. Despite the assessment on two non-consecutive days, this may not reflect their eating habits on weekends or holidays. Second, the cross-sectional nature of our study does not permit causality statements. Third, a single measurement of CRP does not imply that there has been long-term inflammation as CRP is a sensitive biomarker with high variability and can be influenced by interacting with e.g. metabolism. Fourth, assessing SNPs of only one inflammatory parameter (CRP) is not enough to understand the complex interactions that occur in inflammation, since inflammatory status is characterized by the production of a wide range of mediators working in a complex network. Fifth, a smaller sample size due to the specific criteria (participants with all information for the interaction test) limited the power to detect significant interactions for very small effects and can increase selection bias. For the main hypothesis, an effect size of $f^2=0.015$ as found for rs3093068*Mediterranean diet still resulted in a power of 0.82. Finally, this study is rather exploratory as no adjustment for multiple testing was implemented. Since the moderation effect of the Mediterranean diet score was tested thrice (for 3 SNP's), a p-value<0.017 could be used as significance level: even in that case the overall Mediterranean diet score was a significant moderator but the fish component would lose its significance. Indeed, the examination of the subgroups was rather a subhypothesis to detect which subgroups are responsible for the observed overall effect.

6. Conclusion

This cross-sectional study confirmed that *CRP* SNPs are related to higher blood CRP concentrations in European adolescents and also showed the moderating potential of the Mediterranean diet herein. Indeed, greater adherence to the Mediterranean diet and high fish intake were associated with a slight attenuation of the rs3093068 SNP genetic risk towards higher CRP levels. This was not the case for the two other SNPs. We encourage other investigations to reproduce this hypothesis. The results may have practical and clinical importance if causality is confirmed. As most people are far from meeting the basic dietary recommendations, a dietary lifestyle towards a more general Mediterranean diet with a high plant-based intake and sufficient omega-3 would be recommended. By advancing the knowledge of the interaction between genetics and nutrition, better prevention strategies can be developed with nutrition as a cornerstone.

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Research data

The authors do not have permission to share the entire dataset.

Conflicts of interest

The authors declare no competing interests.

Credit authorship contribution statement

Aline Arouca formulated the research question, has analyzed the data and wrote a draft of the paper. Nathalie Michels helped in formulating the research question, analyzing the data and did editing of the first draft. Nathalie Michels and Stefaan De Henauw are co-supervisor and supervisor of Aline Arouca; Luis Moreno was coordinator of the HELENA project. Aline Meirhaeghe and Jean Dallongeville were responsible for the DNA

extraction and genotyping of the SNPs. Gustavo Lourenço performed the statistical analyzes on SNPs. Marcela González-Gross was responsible for blood sampling and collection. Ascensión Marcos was responsible for the inflammatory parameter analyses. Inge Huybrechts developed the Mediterranean diet score. All authors have made substantial contributions to all of the following: (1) data collection, (2) interpretation of data, (3) revising the article critically for important intellectual content, and (4) final approval of the manuscript.

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Figure and Tables legends

Figure 1. The *CRP* gene region (chromosome 1q21-q23) and the locations of the three SNPs tested in our study.

Figure 2. Sampling procedure scheme.

Figure 3. Moderation by diet on the relation between *CRP* rs3093068 SNP and CRP blood concentrations.

(A) Moderating effect of the adherence to the Mediterranean diet on CRP levels among adolescents with and without the CC genotype of rs3093068.

(B) Moderating effect of the fish subgroup intake on CRP blood concentrations among adolescents with and without the CC genotype of rs3093068.

Table 1. Characteristics of the study sample.

Table 2. Associations between the allele frequencies of the investigated *CRP* genotypes (rs3093068, rs1205 and rs1130864) and CRP blood concentrations in the HELENA study.

Table 3. Moderating effects of diet on the relation between *CRP* gene SNPs (rs3093068, rs1205, rs1130864) and CRP blood concentrations in 562 adolescents from the HELENA study.