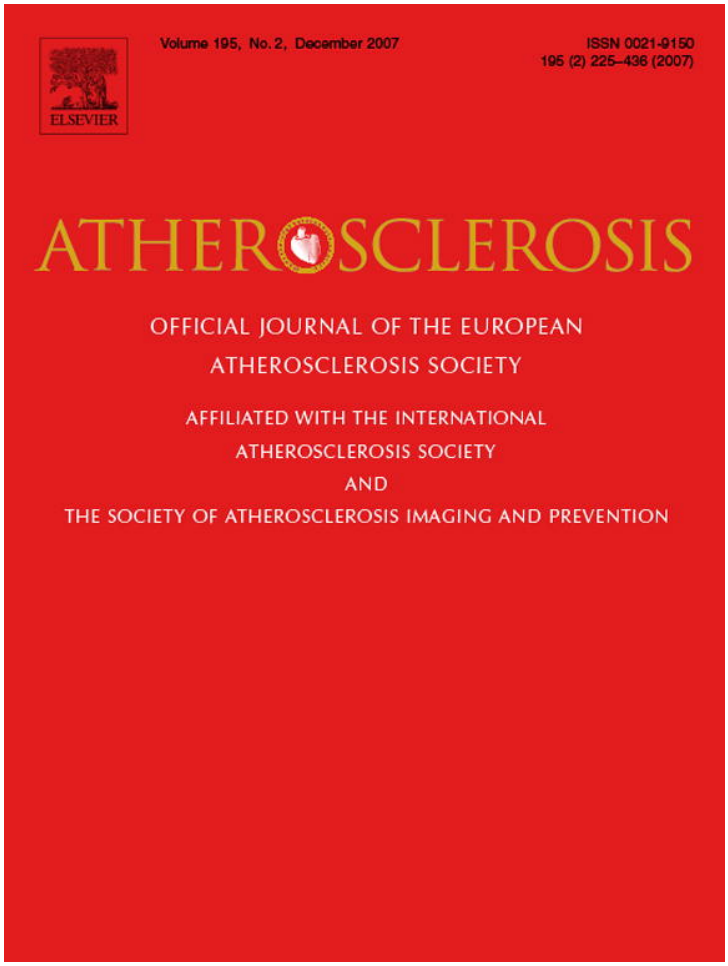

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Fish intake and *LPA* 93C>T polymorphism: Gene-environment interaction in modulating lipoprotein (a) concentrations

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Abstract

High plasma lipoprotein (a) [Lp(a)] concentrations are an independent risk factor for atherosclerotic diseases. To date, no effective intervention strategies on reducing Lp(a) concentrations have been reported. The aim of the study was to evaluate the possible modulation of two polymorphisms of *LPA* gene (*LPA* 93C>T and *LPA* 121G>A) and nutritional habits on Lp(a) concentrations. We studied 647 healthy Italian subjects (260 M; 387 F) with a median age of 48 years (range: 19–78) enrolled in an epidemiological study conducted in Florence, Italy. A linear regression analysis showed a significant negative influence of fish intake ($\beta = -0.174 \pm 0.084$; $p = 0.04$) on Lp(a) concentrations, after adjustment for smoking habit, C-reactive protein serum concentrations, dietary habits and LDL-cholesterol concentrations. With regard to *LPA* polymorphisms, *LPA* 93C>T polymorphism resulted to significantly affect Lp(a) circulating concentrations in a dose-dependent manner, with lower concentrations shown by subjects carrying the T rare allele, whereas no significant influence of *LPA* 121G>A polymorphism on Lp(a) concentrations was observed. Moreover, by analyzing the possible interplay between *LPA* 93C>T and dietary fish intake, a significant interaction between these two determinants in lowering Lp(a) concentrations was reported. In addition, lower Lp(a) concentrations were observed in subjects carrying the T allele of the *LPA* 93C>T polymorphism and consuming a high intake of fish with respect to those being in the highest tertile of fish consumption but homozygotes for the common allele of the polymorphism. In conclusion, this study reported a significant interaction of daily fish intake and *LPA* 93C>T polymorphism in decreasing Lp(a) concentrations.

Keywords: Lipoprotein (a); Fish intake; Cardiovascular risk factors; Polymorphisms

1. Introduction

Lipoprotein (a) [Lp(a)] is a plasma particle composed of a LDL particle and a highly glycosylated apolipoprotein, apo(a), disulfide linked to the apo B-100 of the LDL [1]. As a result of its structural similarity to LDL, Lp(a) has atherogenic properties [2]. In addition, Lp(a) has antifibrinolytic and thrombosis-promoting properties that arise from the structural similarity of apo(a) to plasminogen [3]. Over the last years, a large number of clinical studies reported evidence for an association between high Lp(a) concentrations and an increased risk for atherosclerotic diseases, including coronary heart disease, and stroke [4–9]. However, despite much progress in the knowledge of the atherogenic role of Lp(a), no effective therapeutic strategies on lowering Lp(a)

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concentrations have been found [10]. Moreover, dietary habits seem not to significantly affect Lp(a) circulating concentrations [11]. Recently, some intervention studies based on the effect of fish oil rich in *n*-3 polyunsaturated fatty acids (PUFA) on Lp(a) concentrations have been conducted, but no clear indications have been obtained [12–15]. Lp(a) concentrations are thought to be strongly under genetic control at the concentration of biosynthesis of the apo(a) protein, which is encoded by the *LPA* locus, so allelic differences at *LPA* are responsible for the variations in Lp(a) phenotype [16].

Data from *in vitro* and *in vivo* studies demonstrated that polymorphisms in the 5′-flanking region of the *LPA* gene affect the efficiency of its expression, so contributing to the regulation of Lp(a) concentrations, [17,18] and influence the variation of the promoter transcription activity in HepG2 cells [19]. Suzuki et al. by exploring the mechanisms of the genetic control of Lp(a) concentrations, demonstrated a relationship between polymorphisms in the *LPA* 5′-flanking region and different Lp(a) concentrations [17]. In particular, the C→T transition at position +93 of the transcription start site creates a new translation start codon, leading to a negative regulation in the protein synthesis, while the change of G to A, at position +121, determine a positive regulation of gene expression [17].

Furthermore, a role for *LPA* 93C>T polymorphism in affecting both the promoter activity [19] and Lp(a) concentrations in Africans, but not in Caucasians, was demonstrated [18]. It has been recently demonstrated, indeed, that *LPA* 93C>T, but not *LPA* 121G>A polymorphism influences Lp(a) concentrations among a population from Czech Republic [20].

To the best of our knowledge, no study which investigated both genetic and nutritional determinants of Lp(a) concentrations among a clinically healthy population was performed.

Therefore, aims of the study were: (1) to investigate the role of nutritional habits on modulating Lp(a) concentrations, (2) to evaluate the weight of the *LPA* locus in influencing Lp(a) concentrations by analysing the 93C>T and 121G>A polymorphisms at *LPA* locus, (3) to establish the possible interplay existing between *LPA* locus and dietary habits in modulating Lp(a) concentrations.

2. Methods

2.1. Subjects

The study population includes subjects enrolled in an epidemiologic study "Alimentazione per la Salute e la Prevenzione di Malattia", which was conducted in Florence, Italy with the aim of evaluating dietary and lifestyle habits of a middle-aged clinically healthy population. Details of the study are described in details elsewhere [21]. Briefly, from January 2002 to January 2004, 932 healthy subjects randomly drawn from the population registers of Florence, Italy, which are updated regularly, were enrolled in the study. The study was designed to enrol 1000 clinically healthy subjects. However, at the time of enrolment 68 subjects were excluded from the study since they refused to participate or reported low energy intake/basal metabolic rate (<1.14), as classified by using Goldberg cutoff. Overall, the participation rate was over 80% and the final study population was still representative of the whole population of the district (about 10%). A physical examination, anthropometric and blood pressure measurements, laboratory tests, and dietary survey were conducted by physicians and dieticians by using standardized protocols.

Body mass index (BMI) was calculated as weight (kg)/height (m^2). Three consecutive measures of blood pressure were taken after the subject had been resting for 10 min, and the average of the last two were used to calculate systolic and diastolic blood pressure. The subjects were classified as having hypertension according to the guidelines of European society of hypertension/European society of cardiology [22] or if they reported taking antihypertensive medications, as verified by the interviewer. Diabetic subjects were defined in line with the American diabetes association [23] or on the basis of self-report data (if confirmed by medication or chart review). Dyslipidemia and the metabolic syndrome were defined by following the criteria of the ATP III expert panel of the US National cholesterol education program [24].

All subjects gave informed consent and the study was approved by the local ethic committee.

Trained dieticians collected data in order to assess the habitual consumption of 109 food items, by performing a food-frequency questionnaire previously validated in Italy, and updated in 2003 [25]. For each specific food item a commonly used portion size was specified and subjects were asked how often they had consumed that unit (never, daily, weekly, monthly) on average during the past year. Emphasis was laid to ensure that the answers were related to a year-long dietary pattern and not to last few months, especially in terms of seasonal changes of diet. Portion sizes were shown in color photographs and indicated by the letters A (small), B (medium), C (large), D (very large). All data were then converted into nutrient daily intake using a specific software (InDali®) and a computerized database derived from the Italian standard food composition tables. A reduction between 33 and 66% of the indicated portions has been applied for seasonal foods. Lp(a) circulating concentrations and *LPA* polymorphisms were obtained in 647 subjects (260 M; 387 F) with a median age of 48 years (range: 19–78).

All subjects gave informed consent and the study was approved by the local ethical committee.

2.2. Determination of Lp(a) and CRP

Venous blood samples were taken, in the morning, after an overnight fasting. To determine Lp(a) and C-reactive protein (CRP) concentrations, venous blood was put in tubes without anticoagulant, centrifuged at room temperature (2000 $g \times 15$ min) and stored at $-80\,^{\circ}C$ until assay. Lp(a) concentrations were determined by an ELISA method (Mercodia Apo(a), Mercodia AB, Uppsala, Sweden). High

sensitivity CRP was assessed by a BNII nephelometer (Dade Behring, Marburg, Germany).

2.3. Determination of LPA polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes by using the Flexigene DNA kit (Qiagen, GmbH, Germany). *LPA* 93C>T and 121G>A polymorphisms were evaluated through an electronic microchip (NanoChip® molecular biology workstation; nanogen, San Diego, CA, USA) as otherwise described [26,27].

3. Statistical analysis

Statistical analyses were performed using the SPSS software (Chicago, IL, USA) for Windows (Version 11.5). Variables were reported as mean and S.D. or median and range, as appropriate. To evaluate the influence of nutritional as well as demographic and anthropometric biomarkers on Lp(a) concentrations we divided our study population into tertiles of age (first tertile: <40 years; second tertile: 40–54 years; third tertile: >55 years) and of Lp(a) distribution (first tertile: <104.8 mg/L; second tertile: 104.8–177.6 mg/L; third tertile: >177.6 mg/L). Kruskall–Wallis test was performed to investigate for comparisons among groups. Chi-square test was used to test for proportions and for deviation of genotype distribution of *LPA* polymorphisms from the Hardy-Weinberg equilibrium. The interaction hypotheses between Lp(a), fish intake and age tertiles in the different genotypes were ascertained by the likelihood ratio testing. Because Lp(a) distribution was right-skewed, values were log-transformed in regression analyses and back transformed for data presentation. To evaluate the influence of fish intake on Lp(a) concentrations, a linear regression analysis after adjustment for age, gender, smoking habit, CRP concentrations, total energy intake, alcohol and lipid intake as well as LDL-cholesterol concentrations, was performed and results were expressed as regression coefficient (β) ± SE. In addition, general linear models with polynomial contrasts for linear trend of distribution were used to analyse Lp(a) concentrations according to tertiles of fish intake (first tertile: <23 g/day; second tertile: 23.1–45 g/day; third tertile: >45 g/day) and different genotypes of *LPA* 93C>T polymorphism and values were expressed as geometric mean and 95% confidence interval. *LPA* polymorphisms were evaluated under a dominant genetic model. The dominant genetic model compares individuals with one or more polymorphic alleles with a baseline group with no polymorphic alleles (e.g. *LPA* 93CT+TT *versus* 93CC). All the general linear models were performed after adjustment for age, gender, total energy intake, smoking habit, CRP concentrations, lipid and alcohol intake, and LDL-cholesterol concentrations. Finally, a logistic regression analysis, in order to evaluate the association between fish intake and concentrations of Lp(a) in the third tertile, after adjustment for age, gender, total energy intake, smoking habit, CRP concentrations, lipid and alcohol intake, and LDL-cholesterol concentrations, was performed. Odds ratios and 95% confidence intervals are presented. A p-value less than 0.05 was considered to indicate statistical significance.

4. Results

4.1. Nutritional determinants of Lp(a) concentrations

Median value of Lp(a) in our study population was 107 with a range of 3–1670 mg/L. Females showed significantly higher Lp(a) concentrations with respect to males [111 (3–1670) mg/L *versus* 105 (6–1500) mg/L; $p = 0.02$]. In particular, the subgroup of 137 menopausal women reported significantly ($p = 0.006$) higher Lp(a) concentrations than nonmenopausal women [129 (6–1390) mg/L *versus* 105 (3–1670) mg/L, respectively].

Demographic, anthropometric, laboratory, and nutritional parameters according to different tertiles of Lp(a) concentrations are described in Table 1. Higher LDL-cholesterol and C-reactive protein levels resulted to be significantly associated to the highest tertiles of Lp(a) levels, while no significant association for tryglicerides was observed. With regard to nutritional habits, a higher fish intake was reported to be significantly associated with lower Lp(a) concentrations, whereas no significant association for all the other nutritional parameters was observed. A linear regression analysis showed a significant influence of fish intake on Lp(a) concentrations, after adjustment for other possible confounders of Lp(a) concentrations, such as age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake as well as LDL-cholesterol concentrations ($\beta = -0.174 \pm 0.084$; $p = 0.04$).

To evaluate the possible influence of fish intake on Lp(a) concentrations we divided our study population into tertiles of fish intake, and a significant trend of decrease for Lp(a) concentrations according to the highest tertiles of fish consumption was observed (Fig. 1). Moreover, a logistic regression analysis showed a significant lower risk of having Lp(a) concentrations in the highest tertile for subjects being in the highest tertile of fish intake (OR: 0.54 95%C.I. 0.32–0.89; $p = 0.04$), after adjustment for age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, and LDL-cholesterol concentrations.

4.2. LPA polymorphisms and Lp(a) concentrations

Genotype distribution and allele frequencies of *LPA* 93C>T and *LPA* 121G>A polymorphisms were in agreement with those predicted by Hardy-Weinberg equilibrium and are reported in Table 2. A significant difference in genotype distribution for *LPA* 93C>T, but not *LPA* 121G>A polymorphism among tertiles of Lp(a) concentrations, was observed ($p = 0.03$ and $p = 0.4$, respectively).

Table 1
Demographic, anthropometric and nutritional characteristics according to tertiles of Lp(a) concentrations

Lp(a)	First tertile (<104.8 mg/L)	Second tertile (104.8–177.6 mg/L)	Third tertile (>177.6 mg/L)	*p*
Age, years[a]	45 (20–74)	50 (19–78)	49 (25–76)	0.02[b]
Females, *n* (%)	126 (58.6)	124 (57.4)	135 (62.5)	0.5[c]
Waist circumference (cm)	85.6 ± 14.4	87.1 ± 14.1	87.1 ± 14	0.5[b]
BMI (kg/m^2)	24.9 ± 4.6	24.8 ± 4.4	25.0 ± 4.3	0.5[b]
Smoking habit, *n* (%)	109 (50.7)	122 (56.5)	114 (52.8)	0.5[c]
Hypertension, *n* (%)	29 (13.5)	35 (16.2)	28 (12.9)	0.6[c]
Dyslipidemia, *n* (%)	25 (11.6)	30 (13.9)	28 (12.9)	0.8[c]
Diabetes, *n* (%)	6 (2.8)	8 (3.7)	7 (3.2)	0.9[c]
Tryglicerides, mg/dL	118.2 ± 101.7	114.5 ± 83.7	118.6 ± 102.7	0.5[b]
LDL-cholesterol, mg/dL	108.4 ± 34.2	115.5 ± 31.1	120.8 ± 32.3	0.001[b]
C-reactive protein, mg/L	2.3 ± 1.5	2.4 ± 1.5	2.7 ± 2.4	0.04[b]
Dietary pattern				
Total energy intake (Kcal/day)	2061.1 ± 605.7	2108.5 ± 590.8	2053.7 ± 559.4	0.6[b]
Protein intake (% Kcal)	17.3 ± 3.5	17.2 ± 2.9	17.0 ± 2.9	0.5[b]
Carbohydrates (% Kcal)	51.2 ± 7.7	50.7 ± 7.2	51.7 ± 7.1	0.3[b]
Total fats (% Kcal)	33.9 ± 7.5	33.4 ± 6.6	33.2 ± 6.1	0.7[b]
SFA (% Kcal)	9.8 ± 2.5	9.6 ± 2.5	9.6 ± 2.5	0.8[b]
MUFA (% Kcal)	12.7 ± 3.9	12.9 ± 4.1	12.5 ± 3.5	0.9[b]
PUFA (% Kcal)	3.0 ± 0.9	3.1 ± 1.0	2.9 ± 0.8	0.2[b]
Fibre (g/day)	21.2 ± 6.1	22.2 ± 7.9	22.2 ± 6.4	0.3[b]
Cholesterol (mg/day)	195.1 ± 86.1	199 ± 76.1	194.6 ± 92.6	0.3[b]
Alcohol intake (g/day)	7.5 ± 4.4	8.5 ± 3.9	8.6 ± 3.5	0.3[b]
Vitamin E (mg/day)	10.4 ± 5	10.5 ± 5	11 ± 5	0.7[b]
Vitamin C (mg/day)	135 ± 58.2	131.1 ± 52.5	141.9 ± 62	0.7[b]
Folic acid (mg/day)	310.7 ± 137.3	311.6 ± 129.3	318.6 ± 130.4	0.2[b]
Vitamin B6 (mg/day)	1.78 ± 0.6	1.78 ± 0.65	1.80 ± 0.62	0.9[b]
Food groups				
Dairy products (g/day)	244.2 ± 157.6	255.9 ± 166.7	254.9 ± 128.4	0.5[b]
Cereals (g/day)	342.2 ± 186.2	376.9 ± 194.6	385.7 ± 303.2	0.1[b]
Fish intake (g/day)	38.6 ± 27.8	31.7 ± 21.3	32.7 ± 22.4	0.04[b]
Meat (g/day)	95.6 ± 75.9	82.1 ± 51.1	91.5 ± 70.9	0.1[b]
Vegetables (g/day)	132.1 ± 41.8	132.8 ± 42.9	146.1 ± 59.7	0.9[b]
Nuts (g/day)	12.9 ± 11.2	14.9 ± 13.6	10.3 ± 9.5	0.6[b]
Fruits (g/day)	84.7 ± 50.6	83.2 ± 47.6	78.5 ± 48.1	0.7[b]
Legumes (g/day)	37.8 ± 30	41.3 ± 34.7	37.7 ± 31.1	0.7[b]
Olive oil (g/day)	42 ± 34.3	43.9 ± 32.7	39.9 ± 37.8	0.3[b]

Values are expressed as mean ± S.D.
BMI, body mass index; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.
[a] Median and (range).
[b] Kruskall–Wallis test.
[c] Chi-square test.

Table 2
Genotype distributions of *LPA* polymorphisms in the whole population and according to tertiles of Lp(a)

Genotype distribution (%)	Allele frequency	Study population (*n* = 647)	Lp(a) tertiles		
			First tertile (<104.8 mg/L)	Second tertile (104.8–177.6 mg/L)	Third tertile (>177.6 mg/L)
*LPA*93CC, *n*		458 (70.8)	145 (67.4)	146 (67.6)	167 (77.3)
*LPA*93CT, *n*		176 (27.2)	66 (30.7)	62 (28.7)	48 (22.2)
*LPA*93TT, *n*		13 (2.0)	4 (1.9)	8 (3.7)	1 (0.5)
	*LPA*93T	0.16			
*LPA*121GG, *n*		524 (81.0)	180 (83.7)	175 (81)	169 (78.2)
*LPA*121GA, *n*		120 (18.5)	34 (15.8)	41 (19)	45 (20.8)
*LPA*121AA, *n*		3 (0.5)	1 (0.5)	–	2 (0.9)
	LP121A	0.10			

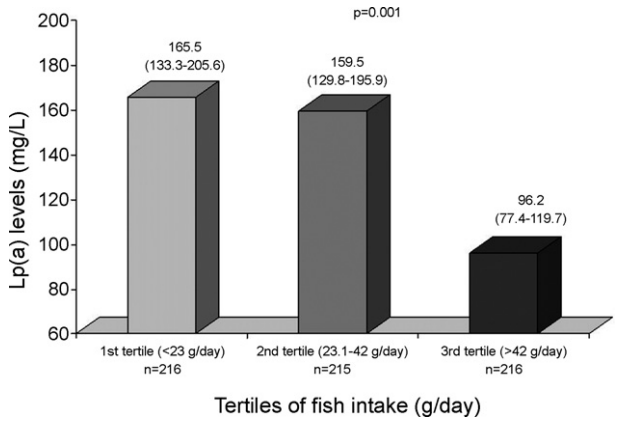

Fig. 1. Lp(a) concentrations according to tertiles of fish intake. Values are shown as geometric mean (95% C.I.). General linear model adjusted for age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, LDL-cholesterol concentrations.

Moreover, as Lp(a) concentrations among the different genotypes of *LPA* polymorphisms were evaluated, we observed a statistically significant trend of decrease of Lp(a) concentrations according to the increasing number of *LPA* 93T allele, after adjustment for possible confounders (age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, as well as LDL-cholesterol concentrations) (Fig. 2). Conversely, no significant differences for Lp(a) concentrations among *LPA* 121G>A genotypes were found ($p = 0.3$).

Since increasing age was significantly related to higher tertiles of Lp(a) concentrations, we searched for a possible influence of age and *LPA* 93C>T polymorphism on Lp(a) concentrations. At a general linear model, after adjustment for possible confounding factors we observed that subjects in the highest tertile of age and carrying the rare 93T allele had significantly lower Lp(a) concentrations than subjects belonging to the same tertile of age but homozygotes for the wild type 93C allele [93CT+TT: 83.8 (46.7–150.2) mg/L *versus* 93CC: 193.8 (146.9–255.4) mg/L; $p = 0.001$].

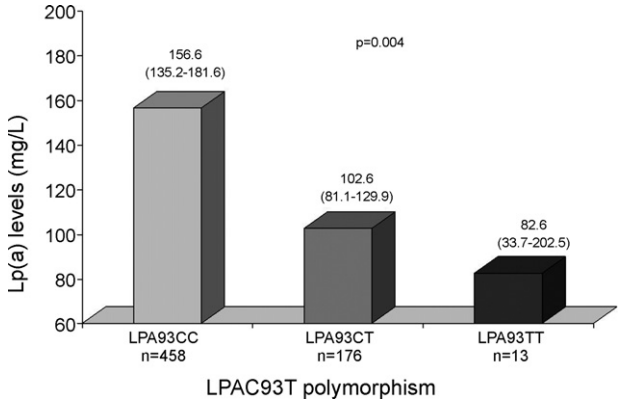

Fig. 2. Lp(a) concentrations according to *LPA*C93T polymorphism. Values are shown as geometric mean (95% C.I.). General linear model adjusted for age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, LDL-cholesterol concentrations.

4.3. Relationship between fish intake and LPA 93C>T polymorphism on Lp(a) concentrations

In order to investigate the possible interplay between fish intake and *LPA* polymorphisms in influencing Lp(a) concentrations, we evaluated the effect of daily fish consumption on Lp(a) concentrations among the different genotypes of *LPA* 93C>T polymorphism. Fish intake was found to significantly affect Lp(a) concentrations in subjects carrying the 93T allele (TC+TT genotypes) ($\beta = -0.591 \pm 0.167$; $p = 0.001$), but not in homozygotes for the *LPA* 93C allele (CC genotype) ($\beta = 0.038 \pm 0.092$; $p = 0.7$), at a linear regression model after adjustment for age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, and LDL-cholesterol concentrations.

Hence, we investigated Lp(a) concentrations according to *LPA* 93C>T polymorphism and tertiles of fish intake, by observing that a significant interaction between *LPA* 93T allele and fish intake in reducing Lp(a) concentrations was present (Fig. 3). Indeed, significantly lower Lp(a) concentrations were observed in subjects carrying at least one *LPA* 93T allele and being in the third tertile of fish intake (likelihood ratio test: $p = 0.001$), with respect of subjects who were in the third tertile of fish intake but not carrying the *LPA* 93T allele (likelihood ratio test: $p = 0.3$) (Fig. 3). This result remained significant even after menopausal women were excluded from the analysis.

Finally, we conducted a multiple regression analysis to estimate the risk of having Lp(a) concentrations in the highest tertile according to tertiles of fish intake and *LPA* 93C>T polymorphism and a significant protection against being in the third tertile of Lp(a) for subjects consuming a higher amount of daily fish was observed only when the 93T allele was present (OR: 0.12; 95%C.I. 0.03–0.46; $p = 0.02$) after adjustment for age, gender, smoking habit, CRP concen-

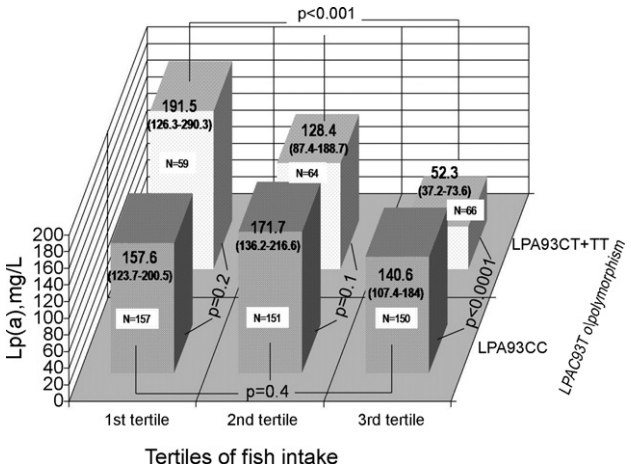

Fig. 3. Lp(a) concentrations according to fish intake and *LPA*C93T polymorphism. Values are expressed as geometric mean (95% confidence interval). General linear model adjusted for age, gender, smoking habit, CRP concentrations, total energy intake, lipid and alcohol intake, LDL-cholesterol concentrations.

trations, total energy intake, lipid and alcohol intake, and LDL-cholesterol concentrations.

5. Discussion

This study observed a significant interaction between a dietary component, namely fish intake and a genetic determinant, the *LPA* 93C>T polymorphism, in modulating circulating Lp(a) concentrations. Indeed, significantly lower Lp(a) concentrations in subjects carrying the *LPA* 93T allele of the *LPA* 93C>T polymorphism and who were in the highest tertile of fish intake, as compared to subjects who consume a high intake of fish from diet but who carry the *LPA* 93C wild-type allele, were observed. To the best of our knowledge, this is the first report of a gene–diet interaction in modulating Lp(a) circulating concentrations. To date, other examples of gene–environment interaction in modulating circulating lipoproteins, in previous studies, have been reported, indicating that diet may influence circulating lipoproteins in genetically predisposed individuals [28,29]. In particular, a diet rich in *n*-3 PUFA of fish origin has been shown to determine a beneficial change in lipid profile only among subjects predisposed for polymorphic variants of the gene encoding for components of the lipid particles [29].

A large number of clinical studies, during the last decades, have provided strong evidence for an association between high Lp(a) concentrations and increased risk for atherosclerotic diseases [4–9]. However, despite intense scientific efforts, effective interventions for lowering Lp(a) concentrations in clinical practice are scarce and limited [10]. Lp(a) concentrations are strongly influenced by genetic factors and seems not to be influenced by diet, exercise, environmental conditions and drugs [11]. In our study population we demonstrated a significant influence of fish intake on Lp(a) concentrations. This is the first evidence of an influence, albeit modest, of fish consumption on this cardiovascular risk marker, in an industrialized population. In 1999, a report from the Lugalawa study reported that a population of Bantu fishermen from Tanzania had significantly lower Lp(a) concentrations as compared to a genetically similar population of vegetarians [30]. The authors accounted this difference of Lp(a) concentrations to the large amount of fish daily consumed by the fishermen. On the other hand, this study was conducted in two populations far removed from a western lifestyle, consuming a high intake of daily fish from diet, thus probably not fully reflecting the "real world" of the western diet. Our population study, instead, is a cohort of subjects living in a Mediterranean region and mostly consuming a Mediterranean-like diet. With regard to fish consumption, our population did not report a high mean value of daily fish intake from diet. Nevertheless, by dividing the study population into tertiles of fish consumption we observed an inverse association between the highest intake of fish from diet and Lp(a) circulating concentrations, even after adjustment for the other dietary variables and for LDL-cholesterol.

Actually, the mechanisms linking high fish intake and lower Lp(a) concentrations are not yet well established. However, some hypotheses can be made. Lp(a) has been reported to play a role in the inflammatory process, and it has been evidenced an association between Lp(a) and inflammation reactants such as interleukin-6 and CRP [31]. Conversely, *n*-3 PUFA endow anti-inflammatory properties, so significantly attenuating inflammatory parameters [32]. Hence, it may be postulated that a diet rich in *n*-3 PUFA is able to modulate Lp(a) concentrations by reducing inflammatory state. Furthermore, it can also be hypothesized that *n*-3 fatty acids may affect apo(a) metabolism, either by impairing its assembly with LDL or by enhancing its catabolism [33].

Currently, some intervention studies based on the effect of fish oil rich in PUFA *n*-3 on Lp(a) concentrations have been conducted but they did not report clear indications [12–15]. Some studies resulted in a significant reduction of Lp(a) concentrations [12–14], whereas a controlled study did not show any change in Lp(a) concentrations [15]. These differences may be related to the different genetic and nutritional background of the study populations, as well as by the different therapeutic interventions in terms of duration and dosage, among these studies.

Another finding of this study is the significant modulation of Lp(a) concentrations shown by *LPA* 93C>T polymorphism. In our study population we analyzed, together with Lp(a) circulating concentrations, two polymorphisms of gene encoding for apo(a), the *LPA* 93C>T and the *LPA* 121G>A polymorphisms. *LPA* 93C>T polymorphism resulted to significantly affect Lp(a) concentrations, as seen by significantly lower Lp(a) concentrations among subjects carrying T variant allele as compared to homozygotes for the common variant. This result is in keeping with data from an experimental study which demonstrated that nucleotide changes at apo(a) promoter region, such as *LPA* 93C>T may contribute to the variation of its transcription activity, thus influencing Lp(a) concentrations [19]. Our results are at variance with those from Kraft et al. [18], who found no evidence for an effect of the 93C>T polymorphism on Lp(a) concentrations in Caucasians. This discrepancy may be due either to the ethnic composition and homogeneity of the study populations, or to a potential masking on the biological effect of the 93C>T polymorphism, which may be in linkage disequilibrium with a repeat-allele.

With regard to the *LPA* 121G>A polymorphism, we found no evidence of its role in modulating Lp(a) concentrations. Our finding is in agreement with those from Zidkova et al. [20], who reported a not significant influence of this polymorphism on Lp(a) concentrations. Thus, one can hypothesize that this polymorphism does not have a functional role in influencing the promoter activity, possibly representing only a marker for another polymorphism that could affect gene regulation.

Limitations can be identified in this study: first of all, our food-frequency questionnaire has been validated only to assess food groups and not micro- and some specific types of macronutrients, thereby not being able to establish the type and quality of fatty acid intake consumed by our study population. Thus, the statistical value for the analyses of these parameters could be strongly affected by such an insufficient experimental accuracy. In addition, only two polymorphisms of the several known genetic determinants contributing to the great interindividual variability of Lp(a) concentrations, were investigated. On the other hand, the two polymorphisms studied in our study are of relevance since they are localized in the promoter region of the gene, so contributing to the variations of its transcription activity. Finally, because of the observational design this study is unable to determine if nutritional and genetic determinants as well as their interaction are causally related to Lp(a) concentrations. A prospective study would be the best way to confirm this finding.

However, several strengths are present: the high number of subjects analysed for nutritional, genetic determinants of Lp(a) and circulating concentrations of Lp(a) as well as the type of the study population investigated (i.e. healthy middle-aged population). In addition, data of our study were adjusted for several confounders which included, other than age, dietary and lifestyle habits, also inflammatory markers and menopause which are known to influence Lp(a) concentrations.

In conclusion, our findings, which demonstrated a role for *LPA* 93C>T polymorphism in modulating Lp(a) phenotype among a middle-aged healthy Caucasian population, on one hand support the observation that variations of Lp(a) concentrations are under genetic control, whereas on the other hand speculate on the gene–environment interaction in controlling such atherosclerotic marker. At present, Lp(a) is not a cardiovascular risk factor and there are no guidelines recommending intervention to lower its circulating concentrations. However, there is some consensus to indicate that Lp(a) lowering might be beneficial in atherosclerotic patients as a whole, and in some specific subgroups of patients [9]. Our study, performed in a healthy population with normal Lp(a) values does not give indications for lowering Lp(a) concentrations below the threshold risk for atherosclerotic disease, but only provides evidence for the possible modulation of Lp(a) concentrations among a general population. On the other hand, the possibility of modulating Lp(a) concentrations by dietary means is intriguing since no effective measures for lowering Lp(a) concentrations are, to date, available. Future studies tailored on vascular patients with abnormal Lp(a) concentrations will need to validate these results.

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