A common variant highly associated with plasma VEGFA levels also contributes to the variation of both LDL-C and HDL-C

Maria G. Stathopoulou,1,2 Amélie Bonnefond,1,2 Ndeye Coumba Ndiaye,1,2 Mohsen Azimi-Nezhad,2 Said El Shamieh,2 Abdel salam Saleh,2 Marc Rancier,2 Gerard Siest,* John Lamont,† Peter Fitzgerald,† and Sophie Visvikis-Siest2,3

Université de Lorraine,* “Génétique Cardio-vasculaire,” EA-4373, Nancy, F-54000, France; and Randox Laboratories, Ltd., † Crumlin, Antrim, United Kingdom

Abstract  Vascular endothelial growth factor A (VEGFA) is among the most significant stimulators of angiogenesis. Its effect on cardiovascular diseases and on the variation of related risk factors such as lipid parameters is considered important, although as yet unclear. Recently, we identified four common variants (rs6921438, rs4416670, rs6993770, and rs10738760) that explain up to 50% of the heritability of plasma VEGFA levels. In the present study, we aimed at assessing the contribution of these variants to the variation of blood lipid levels (including apoE, triglycerides, total cholesterol, low- and high-density lipoprotein cholesterol levels (LDL-C and HDL-C)) in healthy subjects. The effect of these single-nucleotide polymorphisms (SNPs) on lipid levels was assessed using linear regression in discovery and replication samples (n = 1,006 and n = 1,145; respectively), followed by a meta-analysis. Their gene-gene and gene-environment interactions were also assessed. SNP rs6921438 was associated with HDL-C (β = −0.08 mmol/l, Poverall = 1.2 × 10−7) and LDL-C (β = 0.13 mmol/l, Poverall = 1.5 × 10−5). We also identified a significant association between the interaction rs4416670×hypertension and apoE variation (Poverall = 1.7 × 10−5). Therefore, our present study shows a common genetic regulation between VEGFA and cholesterol homeostasis molecules. The SNP rs6921438 is in linkage disequilibrium with variants located in an enhancer- and promoter-associated histone mark region and could have a regulatory effect in the expression of surrounding genes, including VEGFA.—Stathopoulou, M. G., A. Bonnefond, N. C. Ndiaye, M. Azimi-Nezhad, S. El Shamieh, A. Saleh, M. Rancier, G. Siest, J. Lamont, P. Fitzgerald, and S. Visvikis-Siest. A common variant highly associated with plasma VEGFA levels also contributes to the variation of both LDL-C and HDL-C. J. Lipid Res. 2013. 54: 535–541.

The vascular endothelial growth factor (VEGF) family is one of the most important regulators of vascular biology. VEGFA, in particular, stimulates angiogenesis in a wide range of normal and pathological processes (1). Owing to this marked role in blood vessel homeostasis, the contribution of VEGFA to cardiovascular diseases (CVDs) and atherosclerosis has been studied in recent years (2). However, the effect of VEGFA on CVDs is still unclear.

The use of anti-VEGF agents in cancer therapy has shown cardiovascular side effects such as hypertension, cardiomyopathy, and hemorrhagic events (3). Furthermore, VEGFA therapies have been tested in randomized clinical trials, particularly in patients with coronary artery disease, with results showing low clinical importance (2). Many studies have identified higher VEGFA levels in patients with vascular diseases; nevertheless, it is not clear whether the upregulation of VEGFA is an adaptation to ischemia or if it is, rather, causal in the onset of these diseases (4–11). Concerning the involvement of VEGFA in atherosclerosis, results seem to be conflicting. A possible neovascularization and proinflammatory effect of VEGFA, which leads to progression of atherosclerosis and plaque instability, in particular (12–17), has been identified in animal models and in vitro studies. In contrast, clinical trials using VEGFA in humans (2) and gene therapy in animal studies (18, 19) do not support a positive effect of VEGFA on atherosclerosis progression. The complexity of CVDs, combined with the pleiotropic effects of VEGFA, could partially explain the differences between studies.

Supplementary key words vascular endothelial growth factor A polymorphism • lipid metabolism • cardiovascular disease • high density lipoprotein-C • low density lipoprotein-C • epistatic interaction • gene-environment interaction

This project was realized thanks to the Bio-Intelligence project. The authors also thank the “Institut national de la santé et de la recherche médicale” (INSERM), the “Région Lorraine,” the “communauté Urbaine de Grand Nancy,” and the “Université de Lorraine” for supporting this work.

Manuscript received 17 July 2012 and in revised form 21 November 2012.

Published, JLR Papers in Press, December 2, 2012

DOI 10.1194/jlr.R030551

This online version of this article (available at http://www.jlr.org) contains supplementary data in the form of three tables.

Abbreviations: BMI, body mass index; BRC IGE-PCV, Biological Resources Bank "Interactions Gène-Environnement en Physiopathologie Cardio Vasculaire"; CVD, cardiovascular disease; GWAS, genome-wide association study; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; TC, total cholesterol; VEGF, vascular endothelial growth factor.

1 M. G. Stathopoulou, A. Bonnefond, and N. C. Ndiaye contributed equally to this work.

2 To whom correspondence should be addressed.

e-mail: Sophie.Visvikis-Siest@insERM.fr


cite journal {
  title = {A common variant highly associated with plasma VEGFA levels also contributes to the variation of both LDL-C and HDL-C},
  journal = {J. Lipid Res.},
  year = {2013},
  volume = {54},
  pages = {535–541},
  doi = {10.1194/jlr.R030551},
  publisher = {American Society for Biochemistry and Molecular Biology},
  url = {http://www.jlr.org},
}

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An interesting point would be the assessment of the effect of VEGFA (which is expressed in almost all vascularized tissues, including the heart, the liver, and the kidney) on known cardiovascular risk factors in supposedly healthy populations, such as lipid parameters (20), inasmuch as this could partially explain the implications of VEGFA in the physiopathology of CVDs. In particular, high-density and low-density lipoprotein cholesterol levels (HDL-C, LDL-C, respectively) are considered independent risk factors for the development of CVD (20). Increased levels of circulating VEGFA have been found in subjects with uncomplicated hyperlipidemia in a small-sample-size study (21), and a similar finding has been shown in a pilot study in patients with hypercholesterolemia (22). Significant associations were found between HDL-C and VEGFA levels in a supposedly healthy population from Japan (23), whereas in a supposedly healthy population in the SAPHIR study, VEGFA was negatively correlated with LDL-C, total cholesterol (TC), and apoB only in women (24). Although the relation between lipid profiles and VEGFA levels is not yet clearly defined, these observations suggest an eventual implication of this molecule in lipid metabolism.

Genetic studies can be highly useful in the identification of strong associations between distinct metabolic pathways of risk factors involved in a common pathophysiology. Via a recent genome-wide association study (GWAS), we identified four single-nucleotide polymorphisms (SNPs) that explained up to 50% of the heritability of VEGFA circulating levels (25). The investigation of the effect of these new genetic variants, as well as the interactions between them and with environmental factors, on blood lipids levels might give some insight into the relation between VEGFA and blood lipids. Therefore, the aim of the present study is the assessment of genetic determinants of blood lipid levels using these four novel VEGFA-related SNPs in supposedly healthy discovery and replication populations.

METHODS

Subjects

Discovery and replication population. Discovery (n = 1,006) and replication (n = 1,143) samples belong to two independent and nonoverlapping populations extracted from the Biological Resources Bank (BRC) “Interactions Gène-Environnement en Physiopathologie CardioVasculaire” (IGE-PCV) in Nancy, in northeast France. They consist of supposedly healthy, unrelated adults of European origin (discovery population: Portugal, France; and replication population: Ireland, Greece). Individuals with chronic disorders (cardiovascular or cancer) or having a personal history of CVD were not included. Subjects taking blood lipid-lowering drugs or medications having an effect on cardiovascular function (including isotropic agents, β blockers, calcium-channel blockers, organic nitrates, anti-arrhythmics, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, diuretics, clot busters, anti-coagulants, anti-platelet drugs, anti-diabetic drugs, and insulin) were also excluded. The study protocols were approved by the local ethics committee of each recruitment center, and all subjects gave written informed consent for their participation in the study.

Data collection

For both populations, biological and clinical measurements and health and lifestyle information were collected using appropriate validated questionnaires and procedures as described previously (26, 27). Hypertension was defined as systolic blood pressure ≥ 140 mm/Hg, diastolic blood pressure ≥ 90 mm/Hg; and smokers were identified based on current smoking status. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Obesity was defined as BMI ≥ 30 kg/m². ApoE serum levels were measured using a turbidimetric immunoassay method (28), and triglycerides, TC, and LDL-C plasma levels were measured as previously described (26, 27). In particular, TC was measured using a cholesterol oxidase-paraminophenazine method, triglycerides using a glycerophosphate oxidase/paraminophenone alanylglycine glycine method, and HDL-C levels using a phoshpotungstate method. LDL-C levels were calculated using the Friedewald formula (29). VEGFA plasma levels were measured in a subsample of 403 individuals from the discovery population by Randox, Ltd. (Crumlin, UK) using a biochip array analyzer (Evidence®) (25). Blood collection was performed after overnight fasting.

Genotyping

DNA was extracted from all participants, and relative biobanks have been constructed in the BRC-IGE-PCV. The SNPs rs6921438, rs4116670, rs6993770, and rs10738760 were genotyped by Genoscreen® (http://genoscreen.fr) using a Sequenom® iPLEX Gold assay (Medium Throughput Genotyping Technology) (30) and in Khbioscience (http://www.kbioscience.co.uk) using the competitive allele-specific PCR (KASP) chemistry coupled with a FRET-based genotyping system (http://www.kbioscience.co.uk/reagents/KASP/KASP.html) in the replication population. For each SNP, 192 duplicate samples were used, and a concordance of 100% was found.

Statistical analysis

Continuous variables are presented as mean value ± standard deviation, and categorical variables are given in percentages. Hardy-Weinberg equilibrium was tested using the χ² test. VEGFA concentrations were natural log-transformed to normalize their distribution in a subsample of the discovery population. Correlations were evaluated by calculating the Pearson coefficient (r). Linear regression models adjusted for age, gender, and BMI were used to test possible associations between VEGFA plasma levels and the levels of the assessed lipid traits. Significance was assessed at a two-tailed P = 0.05 level.

Genetic analyses were performed under the assumption of an additive model. For the discovery population and the replication populations, linear regression models adjusted for age, gender, and BMI were used for the assessment of the effect of each SNP (independent variable) in blood lipid concentrations (dependent variables). Further adjustments were performed in both populations for smoking and hypertension. Significance was assessed at a two-tailed P = 0.0125 level (adjustment for multiple testing). In a case in which more than one SNP is associated with one trait, a conditional analysis assessing the main effect of all significant SNPs in the same model of linear regression (adjusted for age, gender, and BMI) was performed to clarify the independent determinants of the trait.

Concerning the use of BMI as a covariate in the regression models and before performing the analysis on SNP associations with lipids traits, we assessed the existence of direct effects of the SNPs on BMI, using linear regression models adjusted for age and gender. The results were not statistically significant (data not shown), thus allowing the use of BMI in the analyses models.
The environmental factors used for the gene-environment interactions assessment were BMI, smoking, or hypertension. We assessed the contribution of these interactions using linear regression models adjusted for age, gender, BMI, the environmental factor, and the additional interaction term (SNP×environmental factors). Significant results were considered those with P ≤ 0.004. For the significant SNPs implicated in gene-environment interactions, separate regression models using the environmental factor as the dependent variable were performed to control for a direct association between the SNPs and the factor.

The assessment of gene-gene interactions was tested using all possible pairwise combinations between the four SNPs in both discovery and replication populations. In the regression models adjusted for age, gender, and BMI, two SNPs and their interaction term were added. In order to adjust for multiple testing, significance was set at P = 0.008.

All analyses were performed using PLINK 1.07 software (http://pngu.mgh.harvard.edu/~purcell/plink) (31) and the SPSS statistical software version 16.0 (SPSS, Inc.; Chicago, IL).

Meta-analysis for each quantitative trait was performed using a weighted inverse normal method via the function “metagen,” with a fixed effect, in the “META” R 2.15.1 package.

The significant results of this study were compared with previous findings in the literature (previous GWAS). SNPs with a correlation coefficient ≥80% were considered in linkage disequilibrium (LD). The GWAS investigator of HuGENavigator engine (32) and the NHGRI catalog of published GWAS (http://www.genome.gov/gwastudies) (33) were used to assess previous GWAS concerning blood lipid levels.

RESULTS

Participants’ data are presented in Table 1. The characteristics of the genotyped SNPs are shown in Table 2. All SNPs in both populations are in agreement with the Hardy-Weinberg equilibrium.

In the discovery population, significant associations were observed between rs6921438 and rs6993770, and the levels of HDL-C (β = −0.09 mmol/l, P = 1.2 × 10−4 and β = −0.01 mmol/l, P = 8.3 × 10−3, respectively; Table 3) and between rs6921438 with plasma LDL-C concentrations (β = 0.14 mmol/l, P = 6.7 × 10−3, Table 3). Specifically, the minor allele A of rs6921438 is associated with decreased HDL-C and increased LDL-C values, whereas the minor allele T of rs6993770 is associated with increased levels of HDL-C. These significant associations were only confirmed for SNP rs6993770 in the replication population (β = −0.07 mmol/l, P = 2.7 × 10−4 and β = 0.12 mmol/l, P = 8.4 × 10−3 for HDL-C and LDL-C, respectively; Table 3). Conditional analysis including both rs6993770 and rs6921438 revealed that rs6921438 was the only SNP with significant direct effect on HDL-C levels in the discovery and replication populations (β = −0.09 mmol/l, P = 1.2 × 10−4 and β = −0.07 mmol/l, P = 2.7 × 10−4, respectively; Table 3). Of note, these results remained significant in both populations after adjustments for both smoking and hypertension (data not shown). SNP rs6921438 explained 1% of the variability of HDL-C and 0.2% for LDL-C in both populations. Meta-analysis of the results of both populations gave highly significant associations between rs6921438 and both HDL-C and LDL-C levels (β = −0.08 mmol/l, P = 1.2 × 10−7 and β = 0.13 mmol/l, P = 1.5 × 10−4 for HDL-C and LDL-C, respectively; Table 3). SNP rs6993770 was not significantly associated with HDL-C levels in joint analysis of the two populations. Furthermore, meta-analysis of the conditional analysis results including rs6993770 and rs6921438 verified that rs6921438 is the only independent determinant of HDL-C levels (β = −0.08 mmol/l, P = 1.2 × 10−7; Table 3).

The nonsignificant associations between the four assessed SNPs and the other lipid traits are presented in supplementary Table I for the discovery and replication populations, as well as the results of the meta-analyses.

Among 10 previously published GWAS concerning lipid levels, rs6921438 (and SNPs in LD with rs6921438) was not reported in the lists of statistically significant SNPs (34–43). Supplementary Table II presents the nonsignificant associations of the four SNPs with the assessed lipid traits in a large GWAS meta-analysis (43). Therefore, rs6921438 is a novel SNP associated with both HDL-C and LDL-C levels.

A significant association between the interaction rs4416670×hypertension and apoE variation was also identified (P = 3.5 × 10−3, 1.6 × 10−3, and 1.7 × 10−5, respectively, in the discovery and replication populations and meta-analysis; Table 4). The minor allele of the polymorphism was associated with lower levels of apoE in hypertensive participants (β = −0.71 mg/l, −0.75 mg/l, and −0.73 mg/l, respectively; Table 4). To test for a possible direct association of rs4416670 in hypertension, further analyses were performed in both populations. No significant associations were observed between the same SNP and hypertension (P = 0.576 for discovery and P = 0.592 for replication population).

Regarding epistatic interactions, in the discovery set, we found that the SNP rs6921438 interacted with rs6993770 for HDL-C levels (β = 0.05 mmol/l, P = 2.4 × 10−3, Table 4). Although this finding was not significant in the replication population (β = 0.02 mmol/l, P = 0.035, Table 4), it was significant in the meta-analysis of the results (β = 0.03 mmol/l, P = 2.6 × 10−3, Table 4).

In a subsample of the discovery population (n = 403) with VEGFA plasma level measurements, no correlation was found between VEGFA plasma levels and any of the assessed lipid traits (R = 0.022, P = 0.657 for HDL-C, R = −0.010, P = 0.845 for LDL-C, R = 0.017, P = 0.741 for TC, R = 0.069, P = 0.164 for triglycerides and R = 0.066, P = 0.184 for apoE). Furthermore, VEGFA levels were not associated with lipid levels in regression models (P < 0.05) (see supplementary Table III).

DISCUSSION

The present study assessed the effect of VEGFA-related SNPs on blood lipid traits and found significant associations and gene-environment interaction for HDL-C, LDL-C, and apoE levels in two independent populations of healthy unrelated adults of European origin.

In particular, SNP rs6921438 was robustly associated with both HDL-C and LDL-C plasma levels. This SNP is an
intergenic genetic variant located on chromosome 6p21.1, at 171 kb downstream of the VEGFA gene. More precisely, this SNP is located between LOC100132354 (a noncoding RNA) and the C6orf223 gene (encoding an uncharacterized protein). To our knowledge, no expression data are available for the C6orf223 gene. SNP rs6921438 is in linkage disequilibrium with a few SNPs close to it (<6 kb): rs513773, rs7767396, and rs9472159 ( r² > 0.7; D’ > 0.9; in the HapMap CEU population), all located between LOC100132354 and C6orf223. SNP rs9472159 is located in an enhancer- and promoter-associated histone mark region according to ENCODE (http://genome.ucsc.edu/ENCODE/index.html). Therefore, it could regulate the expression of surrounding genes, including VEGFA. In a recent GWAS, we showed that the minor allele of rs6921438 strongly contributed to decreased VEGFA circulating levels (with an explained variance of VEGFA levels of 41%) (25). Subsequently, it appears that rs6921438 could have a negative effect in the cardiovascular system through a decrease in HDL-C levels, an increase in LDL-C levels, and a decrease in VEGFA levels.

It should be mentioned that to our knowledge, this is the first study that investigates the effects of VEGFA-related SNPs with blood lipid traits. Also, rs6921438 has not been identified in any previous GWAS concerning HDL-C and LDL-C levels (34–43). The existing GWASs have managed to explain a small percentage of the blood lipid trait variance (e.g., for HDL-C, it ranges from 0.6% to 10%); thus, other genetic variants remain to be found. Nevertheless, in the present study, rs6921438 explained 1% of HDL-C variability and 0.2% of LDL-C variability (in both populations). A large meta-analysis of GWAS including more than 100,000 participants did not identify the genetic variants assessed in the present study as significant determinants of blood lipid levels (see supplementary Table II) (43). The reason for this discrepancy may be due to differences in the studied populations. Teslovich et al. (43) included in their study more than 45 populations of European descent corresponding also to family-based or case-control studies. These populations may be different from our healthy unrelated European adult populations.

In the present study, a candidate gene approach was used based on SNPs identified from a GWAS concerning VEGFA-level heritability. The use of GWAS-identified SNPs as candidate loci for other trait variations could help in the elucidation of genetic relationships between phenotypes and new biological mechanisms associated with pathologies. Hence, the present study, which suggests a common genetic regulation of blood lipid traits and VEGFA, could support this methodology.

To further assess the effect of this common regulation, we tested the association between VEGFA and blood lipid levels, although the results were nonsignificant. Therefore, plasmatic levels of VEGFA may have an indirect implication in lipid metabolism, at least in supposedly healthy individuals. However, Blann et al. (21) demonstrated that

### TABLE 2. Characteristics of the four studied genetic variants

| Chr | SNP    | Minor allele | Direction of effect on plasma VEGFA | Variance of plasma VEGFA explained (%) | Function | Closest genes     | Discovery | Replication |
|-----|--------|--------------|-------------------------------------|----------------------------------------|----------|-------------------|-----------|-------------|
| 6   | rs6921438 | A            | −0.72                               | 41.2                                   | Intergenic | LOC100132354/C6orf223 | 0.42      | 0.40        |
| 6   | rs4416670 | C            | −0.13                               | 1.5                                    | Intergenic | LOC100132354/C6orf223 | 0.47      | 0.45        |
| 8   | rs6993770 | T            | −0.17                               | 2.0                                    | Intronic  | ZFP2              | 0.30      | 0.31        |
| 9   | rs10738760 | G           | −0.28                               | 5.0                                    | Intergenic | KCV2/ VLDLR       | 0.48      | 0.45        |

*According to Ref. 25, effect size in the discovery cohort (VEGFA values in ng/l, log-transformed).

*According to Ref. 25.

*MAF, minor allele frequency.
subjects with hyperlipidemia have increased levels of VEGFA compared with healthy controls, even if the authors found no correlation of VEGFA with blood lipid levels. Additionally, similar results were observed in a small pilot study of hypercholesterolemia patients by Belgore, Lip, and Blann (22). Both of these studies were performed in small sample sizes, and they have included pathological populations in a case-control design. Thus, these results cannot be directly compared with the present study, where healthy populations have been used. In the study of Kimura et al. (23), serum VEGFA levels were negatively correlated with HDL-C levels in healthy adults; however, this correlation was observed only in male populations. Moreover, in this study, measurements were performed in serum samples, which demonstrate higher levels of VEGFA compared with plasma levels that were measured in the present study (23, 25). Finally, Sandhofer et al. (24) have shown that plasma VEGFA levels were negatively associated with TC and LDL-C in a healthy female sample. Although the population of this study is larger than our discovery cohort, the female sample is significantly older compared with ours. As previously observed, VEGFA levels increase with age, especially in women (44). In the abovementioned study, VEGFA plasma levels in the female sample are higher than the levels of our study, and this could probably explain the different results between them. It should be mentioned that in our sample, there were no significant differences between genders (data not shown). Taken together, it seems that in supposedly healthy populations, VEGFA is only marginally or not associated with blood lipid levels. Thus, although a common genetic background between VEGFA and blood lipids, especially HDL-C and subjects with hyperlipidemia have increased levels of VEGFA compared with healthy controls, even if the authors found no correlation of VEGFA with blood lipid levels. Additionally, similar results were observed in a small pilot study of hypercholesterolemia patients by Belgore, Lip, and Blann (22). Both of these studies were performed in small sample sizes, and they have included pathological populations in a case-control design. Thus, these results cannot be directly compared with the present study, where healthy populations have been used. In the study of Kimura et al. (23), serum VEGFA levels were negatively correlated with HDL-C levels in healthy adults; however, this correlation was observed only in male populations. Moreover, in this study, measurements were performed in serum samples, which demonstrate higher levels of VEGFA compared with plasma levels that were measured in the present study (23, 25). Finally, Sandhofer et al. (24) have shown that plasma VEGFA levels were negatively associated with TC and LDL-C in a healthy female sample. Although the population of this study is larger than our discovery cohort, the female sample is significantly older compared with ours. As previously observed, VEGFA levels increase with age, especially in women (44). In the abovementioned study, VEGFA plasma levels in the female sample are higher than the levels of our study, and this could probably explain the different results between them. It should be mentioned that in our sample, there were no significant differences between genders (data not shown). Taken together, it seems that in supposedly healthy populations, VEGFA is only marginally or not associated with blood lipid levels. Thus, although a common genetic background between VEGFA and blood lipids, especially HDL-C and

| SNP          | Traits/Study | Mean values level ±SD by genotype (mmol/l) | \( \beta \) [SE] \( ^{a} \) (mmol/l) | \( P \)  |
|--------------|--------------|---------------------------------------------|-------------------------------------|------|
|              | HDL-C/       | XX  1.97 ± 0.47  | −0.09 [0.02]  | 1.2 × 10^{-4} |
| rs6921438    | discovery    | XY  1.89 ± 0.49  |                  |      |
|              | replication  | YY  1.76 ± 0.54  |                  |      |
| X-allele = G | HDL-C/       | NA  | −0.08 [0.01]  | 1.2 × 10^{-7} |
| rs6993770    | meta-analysis| NA  |                  |      |
|              | HDL-C/       | NA  |                  |      |
| rs6921438    | meta-analysis| NA  |                  |      |
| Y-allele = A | LDL-C/       | NA  |                  |      |
| rs6993770    | discovery    | NA  |                  |      |
|              | replication  | NA  |                  |      |
| rs6921438    | meta-analysis| NA  |                  |      |
| X-allele = T | HDL-C/       | NA  | 0.01 [0.01]    | 0.298 |
| rs6993770    | meta-analysis| NA  |                  |      |
| rs6921438    | conditioned for rs6993770 | NA |                  |      |
| rs6993770    | conditioned for rs6921438 | NA |                  |      |

\( ^{a} \text{SD, standard deviation.} \)
\( ^{b} \beta, \text{effect size; SE, standard error.} \)
\( ^{c} \text{HDL-C, HDL-cholesterol.} \)
\( ^{d} \text{LDL-C, HDL-cholesterol.} \)
\( ^{e} \text{NA, not applicable.} \)

**TABLE 3.** Significant associations of SNPs with blood lipids

**TABLE 4.** Significant gene\texttimes;environment and gene\texttimes;gene interactions with blood lipids

| Interaction          | Traits/Study                     | \( \beta \) [SE] \( ^{a} \) | \( P \)  |
|----------------------|----------------------------------|-----------------------------|------|
| rs4416670 ×          | ApoE/ discovery                  | −0.71 [0.25]               | 3.5 × 10^{-3} |
| hypertension         |                                  | −0.75 [0.23]               | 1.6 × 10^{-3} |
| rs6921438 ×          | HDL-C/ discovery                 | 0.05 [0.01]                | 2.4 × 10^{-3} |
| rs6993770 ×          | HDL-C/ replication               | 0.02 [0.01]                | 0.035 |
| rs6921438 ×          | HDL-C/ meta-analysis              | 0.03 [0.01]                | 2.6 × 10^{-3} |

\( ^{a} \beta, \text{effect size; SE, standard error; units for } \beta \text{ coefficient are: mg/l for } \text{apoE} \text{ and mmol/l for HDL-cholesterol.} \)
\( ^{b} \text{ApoE, apolipoprotein E.} \)
\( ^{c} \text{HDL-C, HDL-cholesterol.} \)
LDL-C, may exist, a clinical manifestation cannot be detected in physiological situations. However, the functionality of these SNPs and the clarification of molecular pathways that are implicated should be determined in other functional studies, including transcriptomic analysis for the expression of key proteins.

We also analyzed the effect interactions of the four VEGFA-related SNPs between them and with environmental factors, on lipid parameters. It is currently widely accepted that gene×gene and gene×environmental interactions can explain a significant amount of genetic heritability and can help identify subgroups among populations with higher disease risk (45–47). Hypertension, BMI, and smoking were the “environmental factors” selected because these are known risk factors for CVD. Because these are factors commonly present in individuals with pathological blood lipid levels, the effect of their interactions with genetic variants could have significant clinical importance. We identified a significant effect of the interaction between rs4416670 and hypertension on decreased apoE levels. ApoE serum levels are known to be associated with the APO-E common polymorphism; in particular, we have previously shown that the APO-E 2/2 genotype contributed to high apoE levels and APO-E 4/4 with low apoE levels (48). Here, we have identified a new genetic variant that has an effect on apoE levels through an interaction with hypertension. The SNP×hypertension interaction that we demonstrated is not due to a direct relationship between the two components of the interaction. Hypertension is among the major risk factors for CVD (49). The presence of altered blood lipid levels and high blood pressure is a very common manifestation in subjects with CVD. Thus, the identification and explanation of these types of interactions between risk factors and genes could be important for the understanding of the complex mechanisms that define the phenotypes in CVD.

This study was the first designed to assess common genetic regulation between VEGFA and blood lipids. The selection of healthy populations is important for the understanding of these relationships because in pathological situations like CVD, the clinical profile of both blood lipids and VEGFA is affected by many disease-related factors which complicate the situation and do not allow the demonstration of comprehensive results. Another strong point of the study is the replication of the most-significant results in an independent population, as well as the use of meta-analyses, that ensured the validity of the findings. We acknowledge, however, the limited number of individuals with VEGFA plasma-level measurements.

In conclusion, we found a robust association between a common VEGFA-related genetic variant and both HDL-C and LDL-C. Furthermore, apoE variation was partly explained by interactions between another VEGFA-related SNP and hypertension, which is known to be related to CVD. The assessment of other CVD risk factor associations with these polymorphisms could assist in the understanding of the normal regulation and the pathophysiological mechanisms that underlie these complex diseases.

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