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Full Length Article

Pleiotropic effects of n-6 and n-3 fatty acid-related genetic variants on circulating hemostatic variables

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ABSTRACT

Introduction: Data from epidemiological studies and clinical trials suggest an influence of dietary and circulating polyunsaturated fatty acids (PUFAs) on the hemostasis profile. Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) related to plasma PUFAs levels. We aimed to investigate whether the SNPs related to plasma PUFAs levels were also associated with plasma levels of hemostatic variables.

Materials and methods: We tested the associations between 9 PUFA-related SNPs and 6 hemostatic variables in 9035 European Americans (EAs) and 2702 African Americans (AAs) in the Atherosclerosis Risk in Communities (ARIC) Study. We then conducted a replication study by looking-up our novel observed associations in three published GWAS for hemostatic factors in different EA populations.

Results: We observed a novel linoleic acid-related locus at the *JMJD1C* region associated with factor VII activity (FVIIc): rs10740118 and rs1935, Beta (p) = -1.31 (1×10^{-3}) and 1.37 (5×10^{-4}) in EAs, respectively, and -1.24 (5×10^{-4}) and 1.28 (3×10^{-4}) in meta-analysis of EAs and AAs of ARIC. This novel association was replicated in two of three independent EA populations ($p = 0.01$ and 0.03 in meta-analyses). We confirmed previously reported associations at the docosapentaenoic acid-related *GCKR* locus with protein C and FVIIc and at *JMJD1C* with fibrinogen. Adjustment for plasma PUFAs did not abolish the associations between these loci and hemostatic variables.

Conclusions: Our study identified a novel association for FVIIc at *JMJD1C*, a histone demethylase that plays a role in DNA repair and possibly transcription regulation and RNA processing.

1. Introduction

Large epidemiological studies have reported associations of plasma hemostatic factors with both dietary intake and circulating levels of n-3 and n-6 polyunsaturated fatty acids (PUFAs) [1–4]. For example, in a cross-sectional study of 14571 participants in the Atherosclerosis Risk in Communities Study (ARIC), dietary intake of n-3 PUFAs was negatively associated with fibrinogen, factor VIII (FVIII), and von Willebrand factor (VWF; in blacks and whites) and positively associated with protein C (in whites only) [1]. Additionally, most circulating levels of individual or joint PUFAs were positively associated with factor VII coagulant activity (FVIIc) and factor VII (FVII) antigen, and inversely

Abbreviations: AA, African American; aPTT, activated partial thromboplastin time; ARIC, the Atherosclerosis Risk in Communities Study; CHS, the Cardiovascular Health Study; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EA, European ancestry; EPA, eicosapentaenoic acid; FHS, the Framingham Heart Study; FVII, factor VII; FVIIc, factor VII coagulant activity; FVIII, factor VIII; FVIIIc, factor VIII coagulant activity; GWAS, genome-wide association studies; LA, linoleic acid; LD, linkage disequilibrium; Ln-fibrinogen, natural log transformed fibrinogen; Ln-FVIIIc, natural log transformed factor VIII coagulant activity; Ln-VWF, natural log transformed von Willebrand factor; PUFA, polyunsaturated fatty acid; RS, the Rotterdam Study; SHBG, sex hormone-binding globulin; QC, quality control; VWF, von Willebrand factor

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associated with fibrinogen [2–4].

In some clinical trials or feeding studies, dietary supplements high in n-3 and/or n-6 PUFAs resulted in changes in hemostatic variables, including fibrinogen, protein C, FVII, and VWF [5–10]. For example, in a clinical trial of 19 healthy subjects, dietary supplementation of 20 g of seal oil containing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) for 42 days resulted in a 7% increase in protein C and 18% decrease in fibrinogen, while no changes were observed in other hemostatic variables [5]. Another dietary intervention study conducted in 16 young men found that both saturated and unsaturated fats that were tested in the study increased FVII activation within 8 h of food consumption [10]. Moreover, the pooled data of the unsaturated fats, including oleic, trans 18:1, and linoleic acid (LA), showed a higher postprandial FVII antigen and FVIIc compared to the pooled data of stearic, palmitic, and palmitic plus myristic acids [10].

Genetic loci associated with plasma phospholipid n-3 and n-6 PUFA have been reported in genome-wide association studies (GWAS) [11, 12]. Recently published studies suggested that some genetic variants that were associated with triglycerides also had pleiotropic effects on hemostatic variables [13, 14], However, to date, it is unclear whether a pleiotropic effect can be observed for plasma PUFAs and hemostatic variables.

In order to evaluate pleiotropic effects for plasma PUFAs and hemostatic variables, we investigated the associations between hemostatic variables and SNPs identified for plasma phospholipid PUFA levels in GWAS [11, 12]. We further evaluated whether any identified genetic associations were mediated by the corresponding plasma PUFA. Our primary analysis was conducted in the ARIC Study, followed by replication of new associations in 3 published GWAS from other studies for hemostatic factors [15].

2. Materials and methods

2.1. Study population

The study population of our primary analysis was the ARIC study [16]. A total of 15792 subjects (55% women and 27% African American (AA)) aged 45–64 were enrolled at the baseline exam in 1987–1989 from four communities, Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Demographic and lifestyle information, including age, sex, smoking status, family disease history, medication usage, and dietary intake were collected by trained interviewers at baseline and follow-up exams. Fasting blood samples were collected for DNA extraction. Informed consent was provided by each participant, and the protocol was approved by Institutional review board at each field center.

2.2. SNP selection

Nine index variants of seven loci reported in the previous GWAS for PUFAs in individuals of European ancestry (EA) [11, 12] and judged to be independent, based on linkage disequilibrium (LD) $r^2 < 0.3$ in EAs, were selected for analysis (Supplemental Table 1, Bold text): rs780094 in *GCKR*, rs3734398 and rs12662634 in *ELOVL2-AS1*, rs174538 in *FEN1*, rs3134950 in *PPT2*, rs10740118 in *JMJD1C*, rs2727270 in *FADS2*, and rs16966952 and rs2280018 in *NTAN1*. All SNPs were either genotyped or imputed with imputation quality score ≥ 0.90 in ARIC (Supplemental Table 1). In order to capture functional variants for hemostatic factors in the same gene region that were not included in the published GWAS for fatty acids, we expanded our analysis to non-synonymous plus loss of function mutations within a 250 KB window from the index SNPs if significant associations were identified with the hemostatic variables in our primary analysis.

Studied SNPs were either genotyped by Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) or imputed by MACH v1.0.16 [17] to the CEU reference panel (for EA) or a combined CEU + YRI panel (for AA) from the HapMap phase II (release 22, build 36). The quality control (QC) filtering of the GWAS data included removal of first-degree relatives, genetic outliers, or not matching existing genotype data. Details of Affymetrix genotyping, QC filtering, and imputation methods were described in previous reports [11, 12, 18].

2.3. Measurements of hemostatic variables

Citrated fasting plasma samples were collected, processed, and frozen at −70 °C at each field center at baseline and then shipped on dry ice to the central Hemostasis Laboratory. FVIIc and FVIII coagulant activities (FVIIIc), fibrinogen, VWF, protein C, and activated partial thromboplastin time (aPTT) were assayed in plasma within two weeks of blood collection [19]. Lab methods can be found elsewhere [20]. In brief, aPTT measurement was tested by automated coagulometers following a standard protocol. Fibrinogen was measured by the thrombin-time titration method and compared with a concentration curve prepared from a calibrated reference. Levels of FVIIc and FVIIIc were measured by clotting assays. Protein C and VWF antigens were assayed by ELISA techniques. Reliability coefficients of repeated measurements from a sample of 39 subjects over several weeks were 0.92 for aPTT, 0.72 for fibrinogen, 0.78 for FVIIc, 0.86 for FVIIIc, 0.56 for protein C, and 0.68 for VWF [21].

2.4. Measurements of Plasma PUFA levels

Plasma phospholipid PUFAs were measured in 3793 EA subjects in the Minneapolis field center of ARIC; of them, 3206 individuals who had complete genetic data and principal components information (for genetic ancestry) were included in the sub-analysis of plasma PUFAs. Individual PUFAs were assessed by thin-layer chromatography [22] and the relative amount of fatty acid as a % of total fatty acids was calculated. Details of PUFA measurements are described elsewhere [11, 12].

2.5. Statistical analysis

To normalize the trait distributions, we first tried to remove outliers that were > 5 SD from the mean and if the exclusion did not correct the trait distribution, we then applied natural log-transformation to the trait. As a result, natural log-transformation was applied to FVIIIc (Ln-FVIIIc), VWF (Ln-VWF), and fibrinogen (Ln-fibrinogen), and outlier exclusion applied to the other hemostatic variables in both ethnic groups. The distributions of the ln-hemostatic variables were approximately normal: skewness and kurtosis of the traits were −0.14 to 0.69 and 0.03 to 1.33, respectively. Supplemental Fig. 1 shows the flowchart of data selection for the current study.

We evaluated the associations between dosage of each SNP and each hemostatic variable in a general linear regression assuming an additive genetic effect. The beta coefficient for the SNP in the model represented the difference in hemostatic variable level per increment of one coded allele (A1) increment. The analysis was adjusted for age, sex, field center, and 10 principal components (accounting for population stratification or genetic admixture). Several hemostatic variables were correlated at least moderately ($r = 0.20$ to 0.72 in ARIC), with the highest correlation at 0.72 between Ln-VWF and Ln-FVIIIc. Therefore, we treated the 6 phenotypes as 5 independent ones to adjust for multiple testing. Accounting for multiple testing, the significant p-value threshold was set at 1.1×10^{-3} (= 0.05 / (9 independent SNPs × 5 independent phenotypes)). When a significant association was identified, we adjusted for the corresponding plasma PUFA to evaluate whether this association was possibly mediated by the PUFA in the subset of 3206 EA. In order to see if any relations existed beyond the