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Pleiotropic Effects of n-6 and n-3 Fatty Acid-Related Genetic Variants on Circulating Hemostatic Variables

Lu-Chen Weng^{a,1}, Weihua Guan^b, Lyn M. Steffen^a, James S. Pankow^a, Nathan Pankratz^c, Ming-Huei Chen^d, Mary Cushman^e, Saonli Basu^b, Aaron R. Folsom^a, and Weihong Tang^{a,*}

^aDivision of Epidemiology and Community Health, School of Public Health, University of Minnesota, 1300 South Second Street, WBOB 300, Minneapolis, MN 55454, USA

^bDivision of Biostatistics, School of Public Health, University of Minnesota, 420 Delaware St SE, Minneapolis, MN 55455, USA

^cDepartment of Laboratory Medicine and Pathology, School of Medicine, University of Minnesota, Mayo Mail Code 609, 420 Delaware Street S.E. Minneapolis, MN 55455, USA

^dNational Heart, Lung and Blood Institute's The Framingham Heart Study, Population Sciences Branch, Division of Intramural Research, National Heart, Lung and Blood Institute, Framingham, MA, USA

^eDepartments of Medicine and Pathology, University of Vermont, Burlington, Vermont, USA360 South Park Dr., 206D, Colchester, VT 05446, USA

Abstract

Introduction: Data from epidemiological studies and clinical trials suggest an influence of dietary and circulating polyunsaturated fatty acids (PUFAs) on the hemostasis profile. Genomewide 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 9,035 European Americans (EAs) and 2,702 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})

¹**Present address:** Cardiovascular Research Center, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA.

*Corresponding author: Weihong Tang, MD, PhD, Division of Epidemiology and Community Health, University of Minnesota, 1300 South Second Street, WBOB 300, Minneapolis, MN 55454, tang0097@umn.edu, Phone (612) 626-9140, Fax (612) 624-0315.

Conflict of Interest

The authors declare that there is no conflict of interest.

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in EAs, respectively, and $-1.24 (5 \times 10^{-4})$ and $1.28 (3 \times 10^{-4})$ 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.

Keywords

hemostatic variables; polyunsaturated fatty acids; single nucleotide polymorphism; genetic pleiotropy

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 14,571 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 FVII activity (FVIIc) and FVII antigen, and inversely 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 hours 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].

Materials and Methods

Study Population

The study population of our primary analysis was the ARIC study [16]. A total of 15,792 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.

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-ASI*, 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 nonsynonymous plus loss of function mutations within a 250KB 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].

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. FVII and VIII coagulant activities (FVIIc and 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].

Measurements of Plasma PUFA levels

Plasma phospholipid PUFAs were measured in 3,793 EA subjects in the Minneapolis field center of ARIC; of them, 3,206 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].

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 logtransformation 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 Figure 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} ($= 0.05/(9 \text{ independent SNPs} \times 5 \text{ independent phenotypes})$). 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 3,206 EA. In order to see if any relations existed beyond the significant genetic association with hemostatic factors, we additionally tested the associations between hemostatic factors and the corresponding PUFA level (adjusting for age and sex) as well as the associations between the corresponding PUFA and genetic variants (adjusting for age, sex and 10 principal components) in the EA subset.

The analysis was conducted separately in EAs and AAs, with EAs being the primary analysis. The sample of AAs ($N=2,702$) was not large enough to replicate most of the

associations observed in EAs but their results were presented to evaluate the consistency of associations between the two ethnic groups. Instead of directly pooling the EA and AA data together, we performed a trans-ethnic meta-analysis to pool the association results from each ethnic group to account for the differences between populations. In addition, we conducted a separate replication study to look up significant associations for FVII and FVIII in three independent EA populations in the CHARGE Consortium: [15] the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS) and the Rotterdam Study (RS). Description of the study populations, laboratory measurement, QC, and data analysis in the CHARGE GWAS have been described elsewhere [15].

We estimated the power using a formula for genetic analysis [23]. With 8,000 individuals and $\alpha = 0.0011$ (i.e., our statistical significant threshold after adjustment for multiple testing), we have 80% power to detect variants that explain at least 0.22% variance of phenotype distribution across different minor allele frequencies (0.05–0.50) under an additive genetic model. All analyses were conducted using ProbABEL 0.1–3, SAS v9.2 (SAS Institute, Inc., Cary, NC, USA), and R version 3.3.3.

Results

Table 1 shows the distributions of demographic and hemostatic variables in the 9,035 EAs and PUFAs in the subset of 3,206 EAs in ARIC. Supplemental Table 2 shows the distribution of hemostatic variables in the subset of 3,206 EAs with PUFA measurements.

Three of the seven plasma PUFA-related loci were significantly associated with multiple hemostatic variables in the EAs of ARIC, including *GCKR* (rs780094 and rs1260326) with FVIIc and protein C, *JMJD1C* (rs10740118 and rs1935) with FVIIc and Ln-fibrinogen, and *PTT2* (rs3134950) with aPTT (all $p < 0.0011$, Table 2). The associations in AAs were weaker compared to EAs but were in the same direction as in EAs. In the meta-analysis of ARIC EA and AA data, the associations remained statistically significant (Table 2). Since VWF serves as a carrier for FVIII in the circulation, in a secondary analysis we additionally adjusted the analysis of FVIIIc for VWF. The additional adjustment did not materially change the null findings for FVIIIc (data not shown).

The *GCKR* locus was previously reported in association with DPA in the *n-3* PUFA GWAS.¹¹ At this locus, both the DPA index variant, rs780094 (intronic to *GCKR*), and a nonsynonymous variant rs1260326 showed association with levels of FVIIc and protein C (Table 2). These two SNPs were in high LD ($r^2 = 0.91$). Each copy of the C allele of rs1260326 was associated with a 2.78% lower FVIIc and a 0.07 $\mu\text{g/mL}$ lower protein C in the meta-analysis of EA and AA data. In the subset of 3,206 EAs who had plasma DPA measurements, adjustment for DPA did not appreciably change the association between the two SNPs and FVIIc or protein C ($< 10\%$ change in the beta; Supplemental Table 3). For example, the betas for the association of rs1260326 with FVIIc and protein C were -1.62% and $-0.063 \mu\text{g/mL}$, respectively, before the adjustment, and -1.61% and $-0.058 \mu\text{g/mL}$ after the adjustment. The pattern was similar for rs780094 (Supplemental Table 3). In the ARIC EA subset, we observed significant associations of the *GCKR* variants with DPA level (both

$p < 1 \times 10^{-5}$), and DPA level with protein C ($p < 3.9 \times 10^{-5}$), while the association between DPA and FVIIc was not significant ($p = 0.75$).

The index variant related to adrenic acid (AdrA) level in the *n-6* PUFA GWAS,¹⁰ rs3134950 in *PPT2*, was associated with aPTT ($p = 0.0003$ in the ARIC meta-analysis of EA and AA data) (Table 2). Each A allele of rs3134950 was associated with a 0.14 second shorter aPTT. Adjustment for plasma AdrA level had little influence on the association between rs3134950 and aPTT in the EA subset with AdrA measurement ($\beta = -0.15$ seconds and -0.16 seconds before and after the adjustment, respectively, $< 10\%$ change in β ; Supplemental Table 3). Rs3134950 was associated with AdrA ($p = 2.2 \times 10^{-6}$), but AdrA was not associated with aPTT ($p = 0.15$).

The *JMJD1C* locus was previously associated with LA in the *n-6* PUFA GWAS.¹⁰ At this locus, the LA-index variant rs10740118 and a nonsynonymous variant rs1935 showed significant associations with FVIIc and Ln-fibrinogen (Table 2). These two SNPs were high LD in EAs ($r^2 = 0.87$). The C allele for rs1935 was associated with higher levels of FVIIc ($\beta = 1.28\%$, $p = 3 \times 10^{-4}$ in the meta-analysis of EA and AA data) and Ln-fibrinogen ($\beta = 0.01$, $p = 4 \times 10^{-5}$) (Table 2). Adjustment for plasma LA modestly changed the associations between the two SNPs and the 2 coagulation measurements in the EA subset with LA measurement (31%–45% greater in FVIIc associations and 14%–15% greater in Ln-fibrinogen associations; Supplemental Table 3). LA was associated with both FVIIc and Ln-fibrinogen at $p < 0.001$, and the *JMJD1C* variants were associated with LA at $p < 0.05$ (data not shown).

Of the significant associations identified, the associations between the *JMJD1C* variants and FVIIc were not previously reported.

Replication study for JMJD1C variants with FVII

The CHARGE Consortium published a GWAS for hemostatic factors [15], FVIIc was available in CHS (N=3,266, median=123%, interquartile range (IQR)=107–143%), FVII antigen in FHS (N=2,801, median= 99 mg/dL, IQR=89–110 mg/dL), and FVIIc in RS (N=1,473, median=106%, IQR=93 – 120%). The associations of rs1935 and rs10740118 with FVIIc were replicated in CHS and RS with a similar direction of association as ARIC and was statistically significant ($p < 0.05$) when p-values and β -coefficients from the two studies were pooled using a fixed-effects, inverse-variance weighted meta-analysis approach (Table 3). The association in FHS was weaker for both SNPs compared to CHS and RS, and for rs10740118 was in the opposite direction to that of ARIC, CHS, and RS, indicating that these associations were specific to FVIIc, but not FVII antigen.

Discussion

Our study identified a novel association between SNPs on the *JMJD1C* gene and FVIIc in ARIC and replicated the association in a meta-analysis of two independent EA populations. We also corroborated previously reported associations of the *GCKR* locus with FVIIc and protein C in EAs [15, 24, 25] and AAs [18, 25], and the *JMJD1C* locus with fibrinogen in EAs and AAs [26], where all of the prior studies included ARIC but did not address the pleiotropic effects on PUFAs and hemostatic variables. In the subset of 3,206 ARIC EA

individuals who had plasma PUFA measurements, adjustment for the corresponding plasma PUFAs did not appreciably change the associations of the *GCKR* variants with FVIIc or protein C or the association of the *PPT2* variant with aPTT. However, adjustment for plasma LA modestly changed the associations of the *JMJD1C* SNPs with FVIIc and Ln-fibrinogen.

The protein encoded by *JMJD1C*, named Jumonji Domain Containing 1C, which was initially found to interact with thyroid and androgen receptors [27, 28], is a histone demethylase that demethylates lysine 9 of histone H3 and plays a role in DNA repair [29, 30]. Emerging evidence indicates that histone methylation is involved in transcription regulation and RNA processing [31]. The novel association of the *JMJD1C* locus with FVIIc observed in this study adds to the body of evidence on the pleiotropic effects of the *JMJD1C* region. Specifically, in addition to plasma LA [11] and fibrinogen (rs7896783, $r^2 = 0.84$ in EAs) [26], variants in moderate to high LD with *JMJD1C* rs10740118 reported in our study have been linked to a variety of biomarkers in GWAS and candidate gene studies, including circulating levels of triglycerides (rs10761731; $r^2 = 0.96$ in EAs) [32], platelet count (rs7896518; $r^2 = 1$) [33], mean platelet volume (rs2393967; $r^2 = 0.65$) [34], platelet reactivity (rs2893923, $r^2 = 0.67$) [35], white blood cell count (rs1935) [36], liver enzyme alkaline phosphatase (rs12355784 and rs7923609, $r^2 = 0.84$ – 0.85) [37, 38], androgens (rs10822184, $r^2 = 0.75$) [39], sex hormone-binding globulin (SHBG) (rs7910927, $r^2 = 0.87$) [40], and vascular endothelial growth factor (rs10761741, $r^2 = 1.0$) [41]. Additional adjustment for triglycerides level slightly weakened but did not abolish the associations between rs10740118, FVIIc, and fibrinogen ($\beta = -1.09$, $p = 0.003$ for FVIIc, and $\beta = -0.01$, $p = 9.5 \times 10^{-5}$ for Ln-fibrinogen), suggesting that these associations are largely independent of the influence of triglyceride level. To the best of our knowledge, the *JMJD1C* region has not been previously reported to be related to plasma FVIIc level. A few prospective epidemiological studies, while not a majority, reported higher FVIIc to be a biomarker for stroke and CHD [42–44]. Therefore, the link between this gene and FVIIc suggests that *JMJD1C* might be a potential candidate for identification of new pathways and drug targets for atherothrombotic conditions.

The locus *PPT2* that we found associated with aPTT is in close proximity to the locus *C6orf10* that ARIC previously associated with aPTT, but this finding was not replicated in the other cohorts of the published GWAS for aPTT [45]. The index variant in *C6orf10*, rs2050190, is about 211 KB from the *PPT2* top variant rs3134950 and in LD with it ($r^2 = 0.16$ in EA samples of HapMap II). Therefore, the association at *PPT2* may reflect the same signal at *C6orf10*. Further studies of additional populations are needed to confirm or reject the association of the *C6orf10/PPT2* locus with aPTT.

Interestingly, in the subset of 3,206 participants from the Minneapolis field center who had plasma PUFA measurements, adjustment for DPA scarcely changed the associations of the *GCKR* variants with FVIIc or protein C, while adjustment for LA modestly changed the associations of the *JMJD1C* SNPs with FVIIc and fibrinogen. Caution is required when interpreting the adjustment results because this subset may not be representative of the whole EA population of ARIC. While the distribution of the hemostatic variables and allele frequencies of these SNPs were similar between the subset and the whole EA population (data not shown), in the subset sample the betas for the genetic associations of FVIIc and

fibrinogen (before adjusting for PUFAs) were weaker compared to those obtained from the entire EA population of ARIC. It is unknown if the difference was due to random variation or un-identified, special characteristics of the subset sample. On the other hand, if we can assume that the data in the subset represent the entire EA population of ARIC, the adjustment results suggest the possibility that these loci perhaps contribute to the hemostatic variables via multiple pathways, with DPA playing little or no role in the *GCKR* associations with FVIIc or protein C, whereas LA is probably a mediator in the *JMJDIC* associations with FVIIc and fibrinogen. This is possible given the pleiotropic effects of both *GCKR* and *JMJDIC* variants on a variety of physiological processes, some of which are associated with hemostatic variables. For example, both the *GCKR* and *JMJDIC* variants have been associated with triglycerides [32, 46] which may causally influence protein C based on results of a recent Mendelian randomization study [14]. Triglycerides are also associated positively with FVIIc [47, 48]. But additional adjustment for triglycerides level did not abolish the associations between the *JMJDIC* variants and FVIIc in our study. Moreover, the *JMJDIC* variants have been associated with circulating levels of androgens [39] and SHBG [40], both of which have been implicated in the regulation of FVII [49–51]. More importantly, the *JMJDIC* variant (rs10740118) showed the same direction of effect in its association with FVIIc and LA, so did the *GCKR* variant (rs780094) with DPA and protein C. The patterns of associations for the hemostasis variables and PUFAs are consistent to what are expected based on the prior clinical trials [5, 10], further supporting the existence of shared underlying genetic mechanisms between plasma PUFA and hemostatic factors (Table 2). Future studies including functional follow-up are needed to elucidate the biological relationship between these loci, the hemostatic measurements, and the other physiological traits.

A major limitation of this study is the availability of PUFA measurements in only the subset of 3,206 EA subjects, thus limiting our ability to evaluate whether plasma PUFAs mediated the associations between the PUFA SNPs and the hemostatic variables. In addition, the approach of adjustment for PUFAs may not be the best way to evaluate whether the genetic effects on hemostatic variables were indeed mediated through PUFA levels. But formal mediation analysis, which should yield more accurate information, requires larger sample size (i.e., ideally in the full cohort). Another limitation is that the study population consisted of predominantly EA individuals, even though the results in the small sample of AAs were consistent with those of EAs. Moreover, the identified loci only explained 0.1–0.7% of the variation of the hemostatic variables, which may indicate limited clinical application. However, our findings still contribute to the science by suggesting potentially new mechanisms/pathways in the regulation of those traits. Finally, our study does not provide data to clarify the health effects of dietary supplementation with PUFAs, which requires data from well-designed clinical trials.

Conclusions

In summary, we identified and replicated new, pleiotropic effects of the *JMJDIC* locus on plasma levels of FVIIc, in addition to confirming previously reported associations of *GCKR* with protein C and FVIIc and of *JMJDIC* with fibrinogen. Adjustment for corresponding PUFAs did not abolish the associations between these loci and hemostatic variables. Future

studies of larger size with different ethnic populations and functional follow-up are needed to evaluate inter-ethnic transferability of our findings and clarify the biological relationship between these loci, PUFAs, and the hemostatic variables.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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
FVIIc	FVII 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
QC	quality control
VWF	von Willebrand factor

Reference

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HIGHLIGHTS

- We identified a novel genetic association for factor VII activity at *JMJD1C*
- This association was replicated in independent populations
- We also replicated the association of *JMJD1C* gene with fibrinogen
- The *JMJD1C* gene has been reported to show pleiotropic effects on other phenotypes

Table 1.

Baseline Characteristics of European American and African American Participants in ARIC, 1987–89

	European Americans		African Americans	
	N	Mean (SD)	N	Mean (SD)
Age, years	9035	54.3 (5.7)	2702	53.3 (5.8)
Male, %	9035	47.04	2702	37.31
<i>Hemostatic variables</i>				
aPTT, in seconds	9016	29.0 (2.8)	2699	29.1 (3.0)
Factor VIIc, %	8830	119 (27.3)	2637	118 (29)
Factor VIIIc, %	9028	125 (33.9)	2702	147 (47.0)
Ln-FVIIIc	9028	4.79 (0.27)	2702	4.94 (0.30)
Fibrinogen, mg/dL	9033	296 (60.9)	2700	319 (72.0)
Ln-Fibrinogen	9033	5.67 (0.20)	2700	5.74 (0.22)
Protein C, µg/mL	9031	3.2 (0.6)	2701	3.1 (0.6)
VWF, %	9034	112 (42.5)	2702	133 (55.7)
Ln-VWF	9034	4.65 (0.37)	2702	4.81 (0.41)
<i>Plasma Phospholipid, % of total fatty acid</i>				
Linoleic acid (LA)	3206	22.0 (2.7)	NA	NA
α-Linolenic acid (ALA)	3206	0.14 (0.05)	NA	NA
γ-linolenic acid (GLA)	3206	0.11 (0.06)	NA	NA
Dihomo-γ-linolenic acid (DGLA)	3206	3.33 (0.77)	NA	NA
Arachidonic acid (AA)	3206	11.5 (1.9)	NA	NA
Eicosapentaenoic acid (EPA)	3206	0.56 (0.3)	NA	NA
Adrenic acid (AdRA)	3206	0.52 (0.1)	NA	NA
Docosapentaenoic acid (DPA)	3206	0.9 (0.17)	NA	NA
Docosahexaenoic acid (DHA)	3206	2.8 (0.9)	NA	NA

Data listed as mean (standard deviation, SD) unless otherwise specified; N: sample size; NA: not available.

Table 2.

Significant Associations between PUFA-related SNPs and Hemostatic Variables in ARIC European American (EA) and African American (AA) Participants^a (p 1.1×10⁻³)

Trait	SNP	PUFA ^b	Chr/Gene	Func	A1/A2	AF1	Beta/SE ^d	EA (N=8,830-9,034)	P	AF1	Beta/SE ^d	AA (N=2,637-2,702)	P	Beta/SE ^d	Meta-analysis	P	Comment
FVIIc	rs780094 ^c	DPA (-)	2/GCKR	intron	C/T	0.60	-2.65/0.41		8.0×10 ⁻¹¹	0.82	-0.26/1.04		0.80	-2.33/0.38		1×10 ⁻⁹	Replication
FVIIc	rs1260326	DPA	2/GCKR	cns	C/T	0.59	-2.83/0.41		4.8×10 ⁻¹²	0.84	-2.40/1.12		0.03	-2.78/0.39		5×10 ⁻¹³	Replication
FVIIc	rs10740118 ^c	LA (-)	10/JMJD1C	intron	C/G	0.42	-1.31/0.40		0.0010	0.31	-0.93/0.83		0.26	-1.24/0.36		0.0005	New
FVIIc	rs1935	LA	10/JMJD1C	cns	C/G	0.52	1.37/0.39		0.0005	0.67	0.90/0.82		0.27	1.28/0.35		0.0003	New
Protein C	rs780094 ^c	DPA (-)	2/GCKR	intron	C/T	0.60	-0.07/0.01		2.6×10 ⁻¹⁶	0.82	-0.04/0.02		0.07	-0.07/0.01		9×10 ⁻¹⁷	Replication
Protein C	rs1260326	DPA	2/GCKR	cns	C/T	0.59	-0.08/0.01		9.4×10 ⁻¹⁷	0.84	-0.05/0.03		0.06	-0.07/0.01		3×10 ⁻¹⁸	Replication
aPTT	rs3134950 ^c	AdRA (-)	6/PPT2	intron	A/C	0.62	-0.17/0.04		0.0001	0.71	-0.03/0.10		0.79	-0.14/0.04		0.0003	Uncertain
Ln-fibrinogen	rs10740118 ^c	LA (-)	10/JMJD1C	intron	C/G	0.42	-0.01/0.003(-3.51/0.90)		6.0×10 ⁻⁵	0.31	-0.01/0.006(-1.96/2.05)		0.32	-0.01/0.003(-3.26/0.82)		4×10 ⁻⁵	Replication
Ln-fibrinogen	rs1935	LA	10/JMJD1C	cns	C/G	0.52	0.01/0.003(3.76/0.89)		2.3×10 ⁻⁵	0.67	0.01/0.006(1.83/2.02)		0.37	0.01/0.003(3.45/0.81)		4×10 ⁻⁵	Replication

^a Adjusted for age, gender, field centers, and the first 10 principal components

^b the specific type of PUFA that was associated with the SNP in the published PUFA GWASs (the direction of association for the A1 allele with the PUFA level in prior GWASs; lack of the direction sign indicates that the SNP was not the top variant in the GWASs but included in our study based on the SNP selection strategy (see SNP selection section))

^c the top SNPs identified in the PUFA GWASs

^d Numbers in parenthesis are shown in original unit obtained from repeating the analysis with the untransformed fibrinogen.

Chr: chromosome; Func: function of the SNP; A1: coded allele; A2: non-coded allele; AF1: allele frequency of A1; Beta: refers to the phenotype change per 1 copy increment of A1 allele; SE: standard error; DPA, docosapentaenoic acid; LA, linoleic acid; AdRA, adrenic acid; cns, coding nonsynonymous; Ln-fibrinogen: natural log transformed fibrinogen.

Table 3.

Replication of Associations between the *JMJD1C* SNPs and FVII in the EA participants of CHS, FHS, and RS studies

SNP	CHS (FVIIc, N=3,266)				FHS (FVIIc, N=2,801)				RS (FVIIc, N=1,473)				Meta-analysis	
	AF1	Beta	SE	P	AF1	Beta	SE	P	AF1	Beta	SE	P	Meta_p1	Meta_p2
A1/A2														
rs1935 [*]	C/G	0.50	1.18	0.61	0.05	0.53	0.15	0.43	0.73	0.51	1.55	0.97	0.11	0.03
rs10740118 [†]	C/G	0.46	-1.43	0.68	0.04	0.41	0.28	0.44	0.53	0.41	-0.89	0.99	0.37	0.17

^{*} imputation quality >0.97 in all three studies

[†] imputation quality =0.79 in CHS and >0.99 in FHS and RSM

A1: coded allele; A2: non-coded allele; AF1: allele frequency of A1; Beta: refers to the phenotype change per 1 copy of A1 allele; SE=standard error

Meta_p1= p-value from meta-analysis of CHS, FHS and RS data using p-value and direction of association weighted by sample size of each study; Meta_p2= p-value from meta-analysis of CHS and RS data using effect size (beta) and standard error of each study.