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Apolipoprotein C-I genotype and serum levels of triglycerides, C-reactive protein and coronary heart disease

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Abstract

Apolipoprotein C-I (apoCI) is implicated in lipid metabolism and inflammatory response, both important risk factors for human heart disease. However, most findings come from in vitro or animal studies, whereas data on human apoCI are sparse. To elucidate the role of apoCI in human disease, we analyzed a functional polymorphism in the promoter region of the apoCI gene in relation to blood lipids, C-reactive protein (CRP), coronary artery disease (CAD), and myocardial infarction (MI). Rs11568822 is a 4–base pair insertion/deletion (Ins/Del) polymorphism, and the Ins allele leads to a higher transcription in vitro compared with the Del allele. This polymorphism was analyzed in the Intergene study, a case-control study for CAD (N = 1236), and the Stockholm Heart Epidemiology Program, a case-control study for MI (N = 2774). Subjects homozygous for the Ins genotype had significantly higher serum levels of triglycerides ($P = .01$ and $P = .006$) and lower serum levels of CRP ($P = .02$ and $P < .0001$) compared with all other subjects in both studies. Similar results were obtained when analyzing only the controls of both studies ($P = .002$ and $P = .0002$, triglycerides; $P = .002$ and $P < .0001$, CRP). However, apoCI was not associated with CAD or MI. In conclusion, our data show that apoCI genotype is associated with serum levels of triglycerides and CRP, confirming the role of apoCI in lipid metabolism and suggesting that it also influences inflammation.

1. Introduction

Apolipoprotein C-I (apoCI) is a 6.6-kd protein present on very low-density lipoprotein particles (VLDL), high density lipoprotein particles (HDL), and chylomicrons [1]. Apolipoprotein C-I has been shown to decrease the binding of VLDL to the remnant receptor, low-density lipoprotein (LDL) receptor–related protein [2,3], and to decrease the apoE-mediated binding of VLDL and LDL to the LDL receptor [4,5]. Apolipoprotein C-I is a weak activator of lecithin cholesterol acyl transferase [6,7] and an inhibitor of phospholipase A2 [8], cholesteryl ester transfer protein [9,10], hepatic lipase (HL) [11,12], and lipoprotein lipase (LPL) [13-15]. Furthermore, overexpression of apoCI in mice results in hypertriglyceridemia [16,17].

Rs11568822 is a 4–base pair (bp) CGTT Ins/Del polymorphism located 317 bp upstream of the transcription initiation site of apoCI on chromosome 19 [18]. The Ins allele results in higher transcription of the apoCI gene compared with the Del allele in vitro, possibly by disrupting the binding of an inhibitory transcription factor [19]. Shachter et al [20] found that the Ins allele of rs11568822 is associated with lower serum levels of apoCI. However, this association was not present in the study by Cohn et al [21], making the relationship between genotype and serum levels uncertain. Together, the in vitro and animal data suggest that apoCI is an important regulator of lipid metabolism. However, the evidence in humans is limited

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to association studies performed in very small cohorts showing that individuals homozygous for the Ins allele of rs11568822 have higher serum levels of triglycerides (TG) compared with all other individuals [19,20,22].

In addition to its role in lipid metabolism, recent observations suggest that apoCI also plays a role in the defense against acute infections. Overexpression of apoCI in mice reduces mortality caused by sepsis, and it was hypothesized that apoCI enhancement of the early inflammatory response explains this effect [23]. Indeed, there is some support for this idea in humans. Among patients that developed endotoxemia after elective cardiac surgery, those that had higher preoperative apoCI levels had increased levels of the inflammatory marker tumor necrosis factor–α [24]. Furthermore, another study showed that patients that survived sepsis had higher plasma levels of apoCI compared with nonsurvivors [25]. In addition, individuals with high serum levels of apoCI had less risk of mortality from infections and lower levels of CRP compared with individuals with low levels of apoCI in a study of 85-year-old Dutch subjects [26].

Because apoCI is implicated in lipid metabolism and inflammation, both known risk factors for atherosclerosis, it is possible that apoCI plays a role in coronary heart disease. Our aim was therefore to investigate if a polymorphism in the apoCI gene is associated with serum levels of cholesterol, TG, and the inflammation marker CRP and to test the hypothesis that apoCI genotype is linked to coronary artery disease (CAD) and myocardial infarction (MI). In this study, we have analyzed a functional polymorphism in the apoCI gene in relation to lipids, CRP, and heart disease in 2 case-control cohorts.

2. Materials and methods

The studies were approved by the regional ethics committees in Gothenburg and Stockholm, and all subjects gave informed consent. Blood samples were taken after an overnight fast.

2.1. Intergene study

The Intergene study is a population-based case-control study including 618 subjects with CAD (MI or unstable angina) and 618 individually age- and sex-matched controls selected from the INTERGENE cohort. The cohort comprises 3610 randomly sampled individuals from a source population aged 25 to 74 years at the time of sampling. Samplings and measurements took place between 2001 and 2004 as previously described [27].

2.2. Stockholm Heart Epidemiology Program

Stockholm Heart Epidemiology Program (SHEEP) is a population-based case-control study including 2246 patients aged 45 to 70 years who suffered a first MI. For the present sub study, 1213 patients who survived at least 28 days after their MI were included along with 1561 controls matched to the cases for age, sex, and hospital catchment area. In the cases, blood sampling took place approximately 3 months after the MI. The subjects were Swedish citizens residing in the Stockholm County and recruited during 1992 to 1994. Measurements were performed as previously described [28,29].

2.3. Genotyping

The apoCI polymorphism (rs11568822), a 4-bp CGTT insertion/deletion (Ins/Del), was genotyped by the allelic discrimination method on the ABI Prism 7900HT Sequence Detection System using an assay-by-design and Universal Taqman master mix from Applied Biosystems (Foster City, CA).

2.4. Statistics

Hardy-Weinberg equilibrium (HWE) was tested using exact test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and procedures from STATA v10 (hwsnp, genhwcci; StataCorp, College Station, TX).

Multiple linear regression was used to test the association between fasting serum levels of TG, total cholesterol, HDL cholesterol (HDL-C), CRP, and LDL cholesterol (LDL-C) and rs11568822 genotype, adjusting for age, sex, waist-hip ratio (WHR), current smoking, cholesterol-lowering treatment, diabetes (diagnosis of diabetes or fasting glucose levels >6.7 mmol/L), and hospital catchment area (SHEEP study). Because the distributions of all lipids and CRP, with the exception of LDL-C, were skewed to the right, we used their $\log_e$-transformed values to meet the requirement of normality required for linear regression. For all outcome variables, we tested an additive model describing the effect of increasing the insertion allele (Ins) by 1. As a result, the regression coefficient β describes the average change in millimoles per liter (LDL-C) or log(units) (TG, total-C, HDL-C, CRP) by the increase of 1 Ins allele. For log-transformed outcome variables, $\exp(\beta)*100$ may be interpreted as the percentage change of the outcome variable by the increase of 1 insertion allele. We also tested a dominant and a recessive model for the Ins allele. To investigate if the associations between lipids and CRP and genotype were influenced by CAD status, we repeated all analyses in the control groups alone.

Associations between rs11568822 genotype and CAD and MI were investigated using logistic regression, adjusting for age, sex, and diabetes, which were the only background variables associated with the apoCI genotype. In the Intergene study, we used conditional logistic regression adjusting for diabetes, taking advantage of the matching between cases and controls with respect to age and sex. The results are given as odds ratio (OR) by the increase of 1 Ins allele, together with the 95% confidence interval (CI).

3. Results

3.1. Analysis of rs11568822 genotype in relation to lipids, CRP, and CAD in the Intergene study

We examined the effect of the rs11568822 polymorphism in the Intergene study. Genotyping was successful in 1194 (96.6%) of 1236 individuals, and there was no deviation from HWE (P = .24). Genotypes in relation to blood lipids and CRP are shown in Table 1. When adjusting for sex, age, diabetes, current smoking, WHR, and cholesterol-lowering treatment, individuals homozygous for Ins had significantly higher serum TG levels compared with all others (recessive model; Table 1 and Fig. 1A-B). Furthermore, subjects homozygous for Del had higher LDL-C, HDL-C, and CRP levels compared with non-Del/Del subjects (dominant model; Table 1 and Fig. 1A-B). There were additive associations between apoCI genotype and TG, LDL-C, and CRP levels (Table 1 and Fig. 1A-B). However, there were no associations between rs11568822 and total cholesterol or CAD (OR, 0.91 per Ins allele; 95% CI, 0.74-1.12; P = .4).

3.2. Analysis of rs11568822 genotype in relation to lipids, CRP, and MI in the SHEEP

In the SHEEP study, genotyping was successful in 2553 (92.0%) of 2774 individuals; and the polymorphism was in HWE (P = .10). Again, we found that subjects homozygous for Ins had higher TG levels compared with all others and that subjects homozygous for Del had higher CRP levels compared with non-Del/Del subjects (Table 1 and Fig. 1A-B). Additive associations between rs11568822 genotype and TG and CRP were also present, confirming the associations observed in the Intergene study (Table 1 and Fig. 1A-B). However, no associations between rs11568822 genotype and total cholesterol, LDL-C, HDL-C, or MI (OR, 0.94 per Ins allele; 95% CI, 0.83-1.07; P = .4) were observed.

3.3. Separate analysis of rs11568822 in the controls from Intergene and SHEEP studies

To ensure that the observed associations also were present in the general population and not caused by the inclusion of the subjects with CAD or MI, we reanalyzed all models for lipids and CRP separately in the control groups. For investigated lipids, the associations reported in Table 1 were strengthened despite the reduced power, for example, log(TG): β = 0.07 units per Ins allele (P = .025, Intergene) and β = 0.08 units per Ins allele (P < .0001, SHEEP; Fig. 1C). For log(CRP), we observed a stronger effect in the control group of the Intergene study (β = −0.19 units per Ins allele, P = .002), whereas the effect in the SHEEP study was largely similar in the controls (β = −0.16, P = .002; Fig. 1D).

4. Discussion

We have examined a polymorphism in the apoCI gene in relation to blood lipids and CRP in 2 separate study populations. Subjects homozygous for the Ins genotype had significantly higher serum levels of TG and lower serum levels of CRP compared with all other subjects in both studies. However, we found no association to either CAD or MI.

Apolipoprotein C-I has been shown to inhibit both HL and LPL in vitro [12,14,30]. Hepatic lipase is an enzyme with diverse effects, including hydrolyzation of TG; and deficiency of HL leads to hypertriglyceridemia [31]. Lipoprotein lipase hydrolyzes TG from VLDL and chylomicrons and thereby supplies peripheral tissues with fatty acids [32]. Deficiency of LPL also leads to hypertriglyceridemia [33]. The hypertriglyceridemia in mice overexpressing apoCI further supports the inhibitory effect of apoCI on HL and LPL [13,15-17]. Besides these in vitro and animal data, very little is known about human apoCI in vivo. A few small genetic studies, consisting of 50 to 250 individuals,

Table 1
Serum levels of lipids and CRP in relation to apoCI genotype

	n	Del/Del	Ins/Del	Ins/Ins	P recessive model	P dominant model	P additive model
Intergene	1194	708 (59%)	413 (35%)	73 (6%)			
TG (mmol/L)	1192	1.57 (1.03)	1.60 (1.15)	1.70 (0.76)	.01 (β = 0.15)	NS	.05 (β = 0.05)
CRP (mg/L)	1186	4.17 (9.81)	3.17 (6.45)	3.24 (5.59)	NS	.007 (β = −0.17)	.02 (β = −0.12)
Total C (mmol/L)	1192	5.14 (1.19)	5.14 (1.20)	5.14 (1.11)	NS	NS	NS
LDL-C (mmol/L)	1114	3.02 (1.01)	2.93 (1.01)	2.94 (1.01)	NS	.03 (β = −0.12)	.04 (β = −0.09)
HDL-C (mmol/L)	1169	1.44 (0.41)	1.51 (0.48)	1.44 (0.37)	NS	.02 (β = 0.04)	NS
SHEEP	2553	1501 (59%)	893 (35%)	159 (6%)			
TG (mmol/L)	2550	1.70 (1.20)	1.74 (1.12)	1.98 (1.59)	.006 (β = 0.11)	.004 (β = 0.06)	.0006 (β = 0.06)
CRP (mg/L)	1887	3.43 (6.54)	2.80 (4.73)	2.01 (2.68)	.0002 (β = −0.4)	<.0001 (β = −0.21)	<.0001 (β = −0.20)
Total C (mmol/L)	2549	6.03 (1.09)	6.02 (1.12)	6.15 (1.27)	NS	NS	NS
LDL-C (mmol/L)	2503	4.07 (0.97)	4.05 (0.99)	4.09 (1.11)	NS	NS	NS
HDL-C (mmol/L)	2529	1.20 (0.35)	1.20 (0.37)	1.20 (0.38)	NS	NS	NS

Serum levels of lipids and CRP (mean ± SD) as well as P values for the effect of apoCI Ins genotype on lipids and CRP as obtained from multiple linear regression adjusting for age, sex, WHR, current smoking, cholesterol-lowering treatment, diabetes status, and hospital catchment area (SHEEP study). β gives the effect of Ins on LDL and logarithmic values of TG, HDL-C, and CRP. NS indicates not significant.

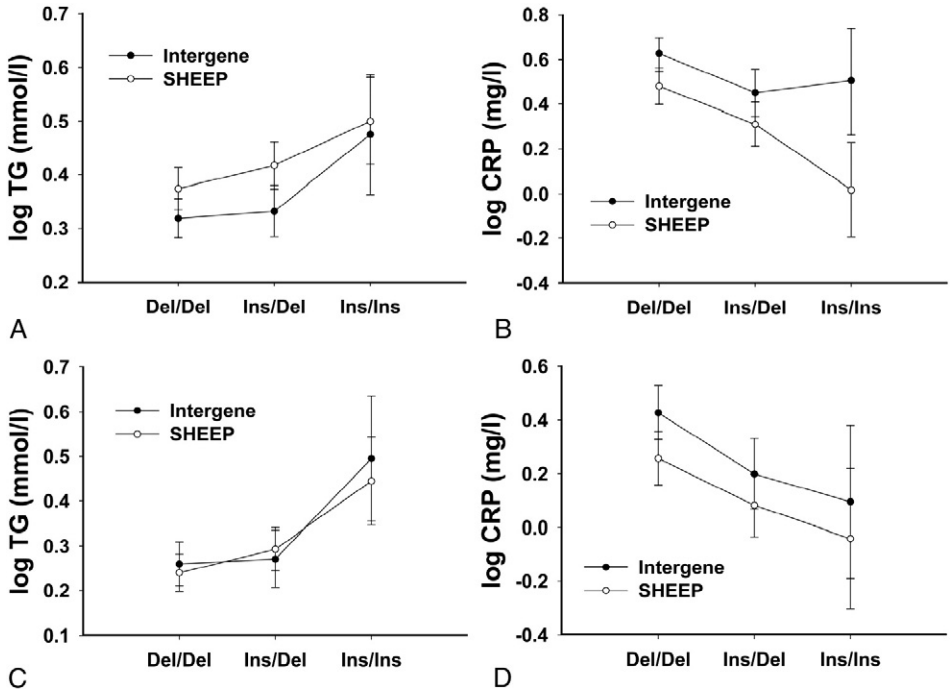

Fig. 1. Serum levels of TG and CRP according to apoCI genotype (rs11568822). Multiple linear regression adjusting for age, sex, WHR, current smoking, cholesterol-lowering treatment, diabetes (diagnosis of diabetes or fasting glucose levels >6.7 mmol/L), and hospital catchment area (SHEEP study). Mean values and 95% CI of log (A) TG (recessive model $P = .01$, Intergene and $P = .006$, SHEEP; additive model $P = .05$, Intergene and $P = .0006$, SHEEP) and (B) CRP (dominant model $P = .007$, Intergene and $P < .0001$, SHEEP; additive model $P = .02$, Intergene and $P < .0001$, SHEEP) in both cases and controls. Separate analyses in the controls of (C) TG (recessive model $P = .002$, Intergene and $P = .002$, SHEEP; additive model $P = .02$, Intergene and $P < .0001$, SHEEP) and (D) CRP (dominant model $P = .002$, Intergene and $P < .0001$, SHEEP; additive model $P = .002$, Intergene and $P < .0001$, SHEEP).

have shown that individuals homozygous for the Ins allele of rs11568822 have higher serum TG levels compared with all other individuals [19,20,22]. Our report based on more than 4000 individuals from 2 separate studies confirms these findings and provides conclusive evidence that subjects homozygous for the Ins allele have higher serum TG levels than non-Ins/Ins subjects.

C-reactive protein is an acute phase protein mainly produced in the liver, and the serum CRP concentration is routinely used as a general marker of inflammation. Our analysis of the relation between apoCI genotype and CRP levels was performed because of the protective effects of apoCI against sepsis in mice [23] and the observation that humans that survive sepsis have higher levels of apoCI compared with nonsurvivors [25]. In addition, Schippers et al [24] have shown that there may be a link between apoCI and tumor necrosis factor–α, another marker of inflammation, in man. Berbée et al [26] have shown that that there is a link between serum levels of apoCI and CRP, where subjects with the highest levels of apoCI have the lowest levels of CRP and less risk of dying from infections than subjects with low levels of apoCI. Our data demonstrating that subjects homozygous for the Ins allele have the lowest and subjects homozygous for the Del allele have the highest levels of CRP suggest a link between rs11568822 and CRP. Together, these data indicate that apoCI may play a role in general inflammation in man; but the mechanisms behind this are unknown. However, Berbée et al speculate that high levels of apoCI would lead to a better protection against pathogens, which would explain the low levels CRP.

Our finding that apoCI genotype was associated with TG and CRP is interesting because both apoCI and CRP are established risk factors for cardiovascular disease [34,35]. We therefore analyzed the effects of apoCI genotype on CAD and MI. However, there was no association between apoCI genotype and CAD in the Intergene study or MI in the SHEEP study. The lack of effect of apoCI genotype on coronary heart disease may be related to the observation that the apoCI genotype that was associated with the highest TG levels was also associated with the lowest levels of CRP and vice versa.

A concern when analyzing our case-control populations was that CAD is known to be linked to the risk factors that were analyzed. However, the associations were also present in the separate analysis of the controls from both studies, demonstrating that they were independent of the inclusion of subjects with CAD.

In conclusion, the polymorphism rs11568822, located in the promoter of the apoCI gene, is associated with circulating levels of TG and CRP in 2 separate studies. These findings suggest that apoCI plays a role in human hypertriglyceridemia and inflammatory response.

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