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Genetics of C-reactive protein and complement factor H have an epistatic effect on carotid artery compliance: The Cardiovascular Risk in Young Finns Study

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Summary

Atherosclerosis is characterized by a prominent inflammatory component and C-reactive protein (CRP) has been implicated to modulate the complement activity in atherosclerotic arteries via complement factor H (CFH) binding. In this study, we examined whether the gene-gene interactions between CRP haplotypes and CFH Tyr402His functional polymorphism exerted an effect on early atherosclerosis. Single nucleotide polymorphisms (SNPs) in CFH (Tyr402His) and CRP (−717A > G, −286C > T > A, +1059G > C, +1444C > T and +1846G > A) were genotyped in the participants of the Cardiovascular Risk in Young Finns Study ($n = 1698$, aged 24–39 years). The CRP SNPs were further constructed into haplotypes and their interactive effects with the CFH Tyr402His polymorphism on the early atherogenic vascular changes [i.e. carotid artery compliance (CAC) and intima-media thickness (IMT)] were examined. After risk factor adjustment, a significant gene-gene interaction ($P = 0{\cdot}007$) on CAC was observed between CRP haplotype ATGTG and CFH Tyr402His polymorphism in males. Furthermore, logistic regression analysis verified the risk-modifying interactive effect on CAC between these loci (OR 3·70, 95% CI 1·37–10·02, $P = 0{\cdot}010$). No effects on CAC were observed in females and no effects on IMT were detected in either sex. We conclude that the combined presence of CRP haplotype ATGTG and CFH 402His allele may be disadvantageous to carotid artery elasticity in males.

Keywords: atherosclerosis, CAC, CFH polymorphism, CRP haplotypes, gene-gene interaction, inflammation

Introduction

A growing amount of evidence implies that inflammation plays a crucial role in cardiovascular disease (CVD). Both the complement proteins and C-reactive protein (CRP) have been intimately linked with the disease aetiology and these factors have concomitantly been found on atherosclerotic arteries and fibrous plaques [1–3]. However, the functional roles of CRP and complement factors or their interplay in atherosclerosis are yet to be elucidated. Besides serving as a marker of systemic inflammation, CRP plays a pivotal immunological role in clearance of pathogens, apoptotic cells, lipoproteins and damaged tissues components and also in the initiation of repair functions. The phagocytotic uptake and disposal of CRP-bound targets is mediated by the complement system and CRP interacts with the complement regulatory factor H (CFH) to suppress the lytic cycle and the alternative pathway amplification loop [4]. In normal conditions, the complement system is under strict control; healthy arterial intima is devoid of complement activity and the pathological manifestations are generated only when the regulation fails.

The CFH Tyr402His functional polymorphism in its seventh consensus repeat region has been reported to alter its CRP-binding properties, leading to markedly reduced affinity of the 402His variant towards CRP [5]. This impaired binding leads to aberrant control of the complement cascade and ensuing unintended inflammatory reaction [5,6]. In addition to the strong association of the CFH 402His allele with age-related macular degeneration (AMD) [6,7], this polymorphism has also been attributed to CVD in recent studies [8–10], although contradictory reports also exist [11,12]. Likewise, the genetic variants of CRP have been independently associated with early vascular changes as well

as with atherothrombotic events [13–15]. However, owing to their biologically relevant interaction, combining the genetic profiles of CRP and CFH could hold a more prominent value when evaluating their contribution to cardiovascular traits.

While the clinical outcomes of CVD usually arise after middle age, atherosclerosis develops over a long time span, having its origins in childhood. Quantifying the early changes of vessel dysfunction, such as decrease in arterial elasticity, provides a validated tool to predict the risk of cardiovascular morbidity and mortality at later stages in life [16]. As it has previously been demonstrated in this cohort by Eklund *et al.* (2008) that CRP haplotypes influence the carotid artery compliance (CAC) without risk factor adjustment, we now aimed to expand the study by exploring the risk factor-adjusted effects of the gene-gene interactions between the CRP haplotypes and the CFH Tyr402His haplotype tagging single nucleotide polymorphism (SNP) on the early vascular changes in the Cardiovascular Risk in Young Finns Study population [17–20].

Methods

Subjects

The participants of the study were part of the population-based Cardiovascular Risk in Young Finns Study, an ongoing multi-centre follow-up survey of atherosclerosis risk factors and precursors in children, adolescents and adults. The participants were randomly selected from the national population register of the study centres (Helsinki, Turku, Tampere, Kuopio and Oulu and their rural surroundings, total of 3596 boys and girls) and they fell into six age cohorts in 1980 when the study was initiated. The study design has been described in more detail elsewhere [17]. The most recent follow-up, in which CAC and intima-media thickness (IMT) were measured, was carried out in 2001, when the participants had reached 24–39 years of age. Cardiovascular risk factors such as serum lipids, BMI, smoking habits, blood pressure values, alcohol consumption, physical inactivity, diabetes and plasma CRP concentrations were also recorded in this follow-up [20]. The present study ($n = 1698$, 704 males and 994 females) involves those individuals who participated in the latest follow-up in 2001 and from whom the genotyping was successful and information about the classic risk factors was available. The study design was approved by the local ethics committees and the survey followed the guidelines of the Declaration of Helsinki. All participants gave their informed consent.

Clinical characteristics and biochemical analyses

Height and weight were measured and BMI was calculated. Blood pressures were measured with a random zero sphygmomananometer (Hawksley & Sons Ltd, Lancin, UK) and the HDL-cholesterol, total cholesterol and triglycerides were quantified from blood samples, whereas LDL-cholesterol was calculated with the Friedewald formula. Fasting plasma CRP was also determined from blood samples using a high-sensitive latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany) with a detection limit of 0·06 mg/l. The coefficient of variation for repeated measurements was 3·3%. Physical inactivity, smoking habits, alcohol consumption and use of oral contraceptives (OCs) were obtained with a questionnaire. Details of biochemical analyses and physical examination have been published earlier [18,19].

Ultrasound measurements of CAC and IMT

Carotid artery compliance is a functional indicator of vascular elasticity, describing the ability of large arteries to expand under pulse pressure, whereas IMT serves as a structural marker of early atherosclerotic changes. Ultrasound measurements of CAC and IMT were performed by scanning the left carotid artery using Sequoia 512 ultrasound mainframes (Acuson, CA) with a 13·0-MHz linear-array transducer. To assess the arterial elasticity, the left carotid artery was scanned and several 5-s moving-image clips were taken at ~10 mm proximal to the bifurcation curve. Luminal carotid diameters were calculated with ultrasonic calipers from the selected B-mode 5-s clip images of best quality cardiac cycles. CAC was calculated according to the following formula: $CAC = ([D_s - D_d]/D_d)/(P_s - P_d)$, where D_s is the systolic diameter, D_d is the diastolic diameter, P_s is the systolic blood pressure and P_d is the diastolic blood pressure [18]. To derive the mean IMT value, the image was focused on the posterior wall of the left carotid artery at ~10 mm proximal to the bifurcation [19].

Genotyping

DNA was extracted from whole blood using a commercially available kit (Qiagen Inc., Hilden, Germany). Genotyping of the five CRP gene polymorphisms −717A > G (rs 2794521), −286C > T > A (rs3091244), +1059G > C (rs1800947), +1444C > T (rs1130864) +1846G > A (rs1205) and the CFH gene polymorphism Tyr402His (+1277T > C, rs1061170) was performed using the ABI Prism 7900HT Sequence Detection System for both PCR and allelic discrimination according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). For SNP 1059G > C, a commercial kit from Applied Biosystems was used (Assay ID C_177490_10). CRP −717A > G, +1444C > T, +1846G > A and CFH 1277T > C were genotyped using Assays By Design from Applied Biosystems under standard conditions. The triallelic tagging SNP, −286C > T > A was genotyped as previously described [21] except for the genotype calling, which was completed manually from the PCR run component tab. The distributions of genotype frequencies of all SNPs

were tested against those excepted by the Hardy–Weinberg equation.

Statistical analyses

The CRP haplotypes were constructed from the five SNPs (−717A > G, −286C > T > A, +1059G > C, +1444C > T and +1846G > A) and estimated with the PHASE v2·0·2 programme, which employs a Bayesian statistical method for reconstructing haplotypes from population genotype data and displays the most probable haplotype pairs for each subject [22]. Statistical analyses and estimation of their power were performed using SPSS version 15·0 (SPSS Inc., Chicago, IL, USA). One-way analyses of variance without (ANOVA) and with covariates (ANCOVA) were used to test the heterogeneity of different CRP haplotype or CFH genotype carrier groups in CAC and IMT. The gene-gene interactions (i.e. the epistasis between the CRP haplotypes and the CFH Tyr402His polymorphism) were also calculated without (two-way ANOVA) and with covariates (two-way ANCOVA). The applied covariates were the classic risk factors of atherosclerosis (i.e. BMI, age, daily smoking, physical inactivity, LDL cholesterol and plasma triglycerides). Additional adjustment with plasma CRP concentration, systolic blood pressure, alcohol consumption and OC usage (females) was used to estimate whether the gene-gene interactions were influenced by these measures. The covariates were selected because of their independent effect on CAC in this cohort and also based on the fact that they are known risk factors for vascular changes and atherosclerosis. Prior to modelling, logarithmic transformations were made for plasma CRP and triglycerides because of their skewed distributions. Finally, multi-nominal logistic regression analysis applied with the above-mentioned covariates was used to confirm the observed interactive effects. For this analysis, CAC values were divided into quartiles.

The null hypothesis underlying the statistical analysis was that the effects of CRP haplotypes and CFH Tyr402His polymorphism on early atherosclerotic markers are not modulated by each other. Conservative Bonferroni corrections were used to control Type I errors because of multiple testing. All the statistical analyses were conducted separately on males and females because the CRP haplotypes exhibit sex by haplotype interactions on CAC.

Results

The characteristics of the study population are presented in Table 1. The genotype frequencies of all CRP SNPs as well as the CFH + 1277T > C polymorphism followed the Hardy-Weinberg equilibrium. The most common CRP haplotypes (frequency > 0·05), which were used in further analyses, are presented in Table 1. Complete list of haplotypes, haplotype pairs and their frequencies in this cohort has been published elsewhere [15].

Table 1. Characteristics of the study population, 1698 young adults (aged 24–39).

Variable	Females ($n = 994$)	Males ($n = 704$)
	Mean ± SD	Mean ± SD
Age, years	31·7 ± 5·0	31·7 ± 5·0
BMI, kg/m^2	24·2 ± 4·3	25·8 ± 3·76
Total cholesterol, mmol/l	5·07 ± 0·91	5·27 ± 1·07
HDL cholesterol, mmol/l	1·40 ± 0·31	1·16 ± 0·28
LDL cholesterol, mmol/l	3·13 ± 0·76	3·43 ± 0·95
Systolic blood pressure, mmHg	116 ± 12·2	129·4 ± 13·5
Diastolic blood pressure, mmHg	71·5 ± 8·6	75·2 ± 9·06
Alcohol (no. drinks per week)	3·7 ± 5·8	8·9 ± 15·5
Physical inactivity index	16·2 ± 14·8	15·8 ± 17·1
CAC, %/10 mmHg	2·30 ± 0·76	2·00 ± 0·66
IMT, mm	0·57 ± 0·08	0·59 ± 0·10
	Median (IQR)	Median (IQR)
CRP, mg/l	0·89 (2·05)	0·62 (1·13)
Triglycerides, mmol/l	1·00 (0·60)	1·30 (0·90)
	Frequency	Frequency
Smoking (daily)	0·19	0·29
CFH 402 genotype:		
Tyr/Tyr	0·320	0·338
Tyr/His	0·480	0·473
His/His	0·200	0·189
CRP† haplotype:		
1. ATGTG	0·351	0·350
2. ACGCA	0·301	0·303
3. GCGCG	0·202	0·215
4. ACCCA	0·070	0·055
5. AAGCG	0·061	0·060
Other	0·016	0·016

†CRP −717, −286, +1059, +1444 and +1846 SNPs. BMI, body mass index; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; CRP, C-reactive protein; CAC, carotid artery compliance; IMT, intima media thickness; CFH, complement factor H; IQR, inter quartile range.

Among the five CRP haplotypes, the haplotype ACGCA imposed independently a mild effect ($P = 0·034$) on CAC and the haplotype ATGTG had an effect of borderline significance ($P = 0·054$) in males after risk factor adjustment (ANCOVA). No independent effects on CAC were observed for the remaining three CRP haplotypes or with the CFH Tyr402His polymorphism after risk factor adjustment (ANCOVA, data not shown). None of the five CRP haplotypes or the CFH Tyr402His polymorphism had an independent effect on CAC in females or on IMT in either sex (ANCOVA, data not shown).

Four of the five most common CRP haplotypes were included in the interaction analyses, because the fifth haplotype group AAGCG contained an inadequate number of individuals when divided into CFH 402 allele carrier groups. A strong interactive effect ($P = 0·007$) on CAC was discovered between CRP haplotype ATGTG and CFH Tyr402His polymorphism in males in which the carriers of the CRP hATGTG

and the CFH 402His variant had by far the lowest CAC values (two-way ANCOVA, Table 2). The main effects of the CRP hATGTG and the CFH Tyr402His SNP appeared negligible in this model, because their effects were nested in the interaction (Table 2). Logistic regression analysis with risk factor adjustment confirmed the epistatic effect: the allele combination of CRP hATGTG and CFH 402His was found to be more abundant in the quartile of lowest CAC values compared with the highest (68·3% *versus* 47·0%; OR 3·70, 95% CI 1·37–10·02, $P = 0{\cdot}010$; Table 3). Additional adjustment with plasma CRP levels, systolic blood pressure or alcohol consumption did not change these results (data not shown). None of the remaining CRP haplotypes displayed an interactive effect on CAC with the CFH Tyr402His SNP (Table 2). No interactive effects on IMT were observed between CRP haplotypes and CFH Tyr402His polymorphism in males or on CAC or IMT in females (data not shown).

The power for the interaction analyses on CAC in males is presented in Table 2. In females, the power for the interaction analyses on CAC ranged from 5% to 23%, whereas for both sexes the power to detect differences in IMT ranged from 6·5% to 24%. The Bonferroni corrections were made by dividing the traditional significance level $\alpha = 0{\cdot}05$ by 10 (number of analyses performed on CAC), resulting in a highly conservative significance level of $\alpha = 0{\cdot}005$ and leaving our result for the interaction between hATGTG and CFH Tyr402His ($P = 0{\cdot}007$) of borderline significance.

Discussion

The results of this population-based study show that the combined presence of CRP haplotype ATGTG and CFH 402His allele has an epistatic effect on preclinical atherosclerosis indicator, CAC, in male subjects. The gene-gene interaction ($P = 0{\cdot}007$) between these loci was verified through a logistic regression analysis (OR 3·70, 95% CI 1·37–10·02, $P = 0{\cdot}010$), which revealed that carriers of this allele combination were enriched in the quartile of lowest CAC values.

Limitations of the study include the reduction of population size when dividing males and females into subgroups and the lack of power to detect interactive effects on CAC in females or on IMT in either sex. However, given that the current cohort consists of individuals younger than 40 years, lack of association on IMT may also reflect the fact that functional alterations in vasculature arise earlier than structural ones, whereas the lack of association in females with CAC might relate to the observation that when CAC values are stratified by age, the vasculature in females is approximately 10 years 'younger' than that in males [18]. In addition, when applying the conservative Bonferroni corrections ($\alpha = 0{\cdot}005$) the observed interaction between CRP hATGTG and CFH Tyr402His ($P = 0{\cdot}007$) remains of borderline significance, albeit these corrections might be too conservative for genetic analyses, because the allelic variants are not totally independent of each other.

Table 2. Main and interactive effects of CFH + 1277 polymorphism and CRP haplotypes on carotid artery compliance (CAC) in males.

		CAC (%/10 mmHg)					Main effects of:		Interaction	Observed
	CFH 402His carr+			CFH 402His carr–			CRP†	CFH‡	between loci	power§
Carriage of CRP haplotype	*n*	Mean (SE)	95% CI	*n*	Mean (SE)	95% CI	*P*¶	*P*¶	*P*¶	%
ATGTG+	268	1·925 (0·037)	1·853–1·998	134	2·034 (0·052)	1·931–2·136	0·339	0·620	**0·007**	77·5
ATGTG–	197	2·104 (0·043)	2·020–2·189	105	1·948 (0·059)	1·832–2·064			(0·037)	
ACGCA+	252	2·041 (0·038)	1·965–2·115	114	2·057 (0·057)	1·948–2·171	0·033	0·978	**0·718**	6·5
ACGCA–	213	1·959 (0·042)	1·873–2·036	125	1·939 (0·054)	1·832–2·045			(0·200)	
GCGCG+	171	2·037 (0·046)	1·943–2·124	106	2·074 (0·059)	1·960–2·192	0·047	0·874	**0·369**	15·4
GCGCG–	291	1·983 (0·036)	1·912–2·052	133	1·931 (0·053)	1·830–2·036			(0·500)	
ACCCA+	49	2·060 (0·130)	1·896–2·237	22	1·985 (0·087)	1·737–2·236	0·747	0·639	**0·650**	8·1
ACCCA–	413	1·997 (0·041)	1·935–2·052	217	1·995 (0·039)	1·916–2·079			(0·646)	

†Haplotype carriage. ‡Allele carriage. §Computed using $\alpha = 0{\cdot}05$. ¶Adjustment with BMI, age, daily smoking, physical inactivity, LDL cholesterol and log triglycerides. Values are presented after adjustment. Unadjusted *P*-values are presented in parentheses.

Table 3. The effect of C-reactive protein (CRP) haplotype ATGTG and CFH Tyr402His allele carrier status in males in the lowest quartile of CAC and in the highest quartile of CAC.

	Lowest quartile of CAC		Highest quartile of CAC				
	CFH 402His carr+	CFH 402His carr–	CFH 402His carr+	CFH 402His carr–			
CRP hATGTG	*n* (%)	*n* (%)	*n* (%)	*n* (%)	$P^{\dagger}$	OR	95% CI
ATGTG+	84 (68·3)	39 (61·9)	54 (47·0)	35 (58·8)	0·010	3·70	1·37–10·02
ATGTG–	39 (31·7)	24 (38·1)	61 (53·0)	25 (41·7)			

[†]Adjustment with BMI, age, daily smoking, physical inactivity, LDL cholesterol and log triglycerides. Values are presented after risk factor adjustment. Nagelkerke R^2 is 22·1% for the analysis.

A deleterious effect of the CFH 402His allele on CVD has recently been reported in three population-based studies [8–10], whereas in some case-control studies, no association between the CFH Tyr402His polymorphism and atherothrombotic events was detected [11,12,23]. To the best of our knowledge, the current study is the second one to assess the interplay between CRP and CFH genetics on cardiovascular traits. The first implication was recently reported by Kardys *et al.* (2007), who observed an increased risk for myocardial infarction (MI) in individuals carrying both the CFH 402His variant and a certain CRP haplotype [24]. Unfortunately, our CRP haplotypes consist partly of discrete SNPs, rendering haplotype comparison unfeasible. However, one common allele, the +1846G (rs1205), and one distinct, the +1444C/T (rs1130864), are embedded in both risk-associated CRP haplotypes. One reason for this divergence could be that the risk-haplotype of Kardys *et al.* (2007) was detected for both men and women above 55 years of age, whereas ours concerned only men below 40 years of age. Our risk-bearing CRP haplotype ATGTG differs from the other haplotypes by its –286 and +1444 SNPs, which bear T alleles only in this haplotype (ATGTG) and both of these T alleles have been previously associated with increased baseline and stimulated CRP expression [6,25,26]. However, as additional adjustment with CRP plasma levels did not modulate the observed epistasis, the systemic plasma CRP is less likely to account for the decreased arterial elasticity. Instead, a potential theory could be a locally operating production of CRP and complement proteins and their retention in the inflamed arterial intima, which would not induce a marked elevation in systemic CRP. Indeed, local generation of CRP and complement factors in atherosclerotic arteries has been demonstrated [2,3,27].

The complement cascade in arterial intima can be activated by the initiators of classical pathway (C1q) and alternative pathway (hydrolyzed C3) when they deposit on activating surfaces, such as immunocomplexes, CRP and oxidized LDL particles. When bound to CRP and cell surface polyanions, CFH down-regulates the complement and protects the endothelium from membrane attack complex (MAC). The CFH 402His polymorphism creates a functional variant with a markedly reduced CRP binding capability [5]. Consecutively, this leads to aberrant regulation of the complement cascade and excessive inflammation and tissue damage because of the MAC formation – a phenomenon already characterized as pertaining to AMD [6,7]. The cell type to be attacked by the MAC in atherosclerotic arteries has turned out to be the smooth muscle cells (SMCs) [3] and several studies have demonstrated the localization of CRP, CFH and MAC in early lesions as well as in advanced plaques [1–3]. Instead of killing the cells, sublytic amounts of MAC induce SMC proliferation and expression of adhesion molecules and cytokines [28]. Moreover, the release of anaphylatoxins C3a and C5a in the course of MAC generation further enhances the inflammation by inducing IL-6, IL-1β, TNF-α and MMP-9 synthesis [29–31]. Of these factors, MMP-9 degenerates and modifies vascular collagen and elastin and thus impedes vascular compliance [32], whereas the cytokines up-regulate CRP synthesis, which in turn maintains the complement activity.

According to a recent implication by Mooijaart *et al.* (2007) the underlying complement-mediated inflammatory mechanism is causal to both CVD and AMD, because the diseases could share similar aetiology and both of them have been associated with the CFH 402His polymorphism [9]. Although the present study does not contain experimental data – a point calling for further research – the vascular remodelling activity of complement has been recognized. In conclusion, our results assert the risk-modifying interactive effect between CFH 402His and the commonest CRP haplotype ATGTG in male carriers. We suggest that the combined presence of these allelic variants may be disadvantageous to male carotid artery elasticity by means of low-grade inflammation in the arterial wall.

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