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ORIGINAL INVESTIGATION

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Interaction between inflammation-related gene polymorphisms and cigarette smoking on the risk of myocardial infarction in the Physician's Health Study

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Abstract Inflammation is known to be a major component of atherosclerosis, and cigarette smoking is known to induce a systemic inflammatory response. We therefore, investigated possible gene–environment interactions between various inflammation-related gene polymorphisms and cigarette smoking on the risk of myocardial infarction (MI) in the Physician's Health Study (PHS), a cohort of initially healthy middle-aged men. We used a nested case-control design consisting of 522 MI cases and 2,089 controls derived from PHS. Eleven inflammatory polymorphisms were studied using logistic regression analysis: eotaxin (ala23thr), intercellular adhesion molecule 1 (gly241arg), interleukin-4 (582C > T), interleukin-4 receptor (ile75val, gln576arg), interleukin-6 (−174G > C), interleukin-10 (−571C > A), P-selectin (val640leu, thr756pro, ser330asn), and vascular cell adhesion molecule 1 (−1594T > C). Interactions of smoking with all the three modes of inheritance (additive, dominant, recessive) were tested. Statistically significant ($P < 0.05$) interaction terms were found for interleukin-4 receptor (ile75val), with odds ratios of 0.52 (95%CI:0.29–0.95) for Ile–Val and 0.34 (95%CI:0.14–0.83) for Val-Val, compared to the wildtype Ile-Ile; for interleukin-6 (−174G > C) with odds ratios of 2.16 (1.14–4.09) for GC and 0.81 (0.31–2.12) for CC, compared to the wildtype GG; and for P-selectin (ser330asn) with odds ratios of 0.48 (0.24–0.95) for Ser-Asn and 1.08 (0.29–3.93) for Asn-Asn, compared to the wildtype Ser-Ser, with these effects occurring only among the smokers. These data raise the possibility of interaction between the smoking status and certain inflammatory polymorphisms on the risk of MI in men. However, these results should be interpreted with caution due to the potential for false positive results that can arise from analyses with multiple comparisons.

Keywords Epidemiology · Inflammation · Myocardial infartion · Polymorphism · Smoking · Gene-environment interaction

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Introduction

The role of inflammation has been recognized as a major factor in the initiation, progression, and rupture of atherosclerotic plaques. The inflammatory pathway involves an array of molecules, including adhesion molecules, cytokines, and chemokines. Inflammatory markers have been shown to reflect the severity of systemic vascular inflammation and can predict future coronary events. Patients with coronary artery disease (CAD) have been shown to have higher levels of C-reactive protein (CRP) and interleukin-6 compared to healthy controls. (de Maat and Kluft 2002) Plasma levels of interleukin-6, interleukin-10, P-selectin, and intercellular adhesion molecule 1 have also been shown to predict future myocardial infarction (MI) in healthy individuals (Heeschen et al. 2003; Hwang et al. 1997; Ridker et al. 1998, 2000, 2001).

The levels of inflammatory markers can be influenced by environmental factors like cigarette smoking. Smokers have been shown to have higher plasma levels of CRP, interleukin-6, soluble intercellular adhesion molecule 1, and P-selectin than nonsmokers (Barbaux et al. 2001; Bermudez et al. 2002; Carter et al. 2003). Smokers with CAD have the highest levels of CRP and

interleukin-6 compared to nonsmokers with CAD and smokers without CAD (de Maat and Kluft 2002). Cigarette smoke produces nitric oxide and free radicals which can cause endothelial dysfunction, abnormal proliferation of vascular smooth muscle, and disturbances in lipid metabolism and platelet coagulability (Wang et al. 2003). These factors promote vascular inflammation and atherogenesis.

It has been suggested that the extent of damage caused by cigarette smoke depends on an individual's genetic makeup (Wang et al. 2003). The purpose of our analysis was to investigate possible gene–environment interactions between various polymorphisms in genes that code for inflammatory proteins and cigarette smoking on the risk of MI in a cohort of healthy middle-aged men. We chose genes for inflammatory proteins that were representative of the inflammation pathway. We studied genes for the cell adhesion molecules intercellular adhesion molecule 1 (*ICAM-1*), vascular cell adhesion molecule 1 (*VCAM-1*), P-selectin (*SELP*), the interleukin-based cytokines interleukin-4 (*IL-4*), interleukin-4 receptor (*IL-4R*), interleukin-6 (*IL-6*), interleukin-10 (*IL-10*), and the eosinophil chemokine eotaxin (*SCYA11*). We hypothesized that the smokers may have differential risk of MI depending on their genotype for various genes that code for the inflammatory proteins.

Materials and methods

Study population

This investigation used prospectively collected DNA samples from the Physician's Health Study (PHS), a randomized trial of aspirin and beta-carotene that was initiated in 1982. PHS consists of 22,071 middle-aged predominantly Caucasian (>94%) US male physicians who were free of prior myocardial infarction, stroke, transient ischemic attack, and cancer at the start of follow-up. The average follow-up was 13.2 years and information is complete for all the deaths and 99.4 percent of repeated morbid events. Blood samples were provided by 14,916 (68%) men at baseline and were used for genetic analysis.

This analysis used a nested case-control design which was based on all subjects who provided a blood sample. The cases consisted of those who experienced a myocardial infarction during follow-up and each case was initially matched to two or three controls. Eligible controls consisted of those who provided a blood sample at baseline and remained free of cardiovascular disease at the time the index event occurred in the case subject. The controls were randomly selected and matched to the cases on age (±2 years), current smoking status, and time since study entry. In addition to the controls matched to the MI cases, controls who were similarly matched to stroke and venous thromboembolism cases were included in the analysis to provide more stable allele frequency estimates. Thus, a total of 522 MI cases and a pooled group of 2,089 controls were used in the analysis.

For all incident vascular events that occurred during the follow-up period, hospital records, death certificates, and autopsy reports were requested and reviewed by an end points committee using standardized diagnostic criteria. The diagnosis of MI was confirmed if it met the World Health Organization criteria, which include symptoms in the presence of either elevations of cardiac enzymes or diagnostic changes on the electrocardiogram. For fatal events, the diagnosis of MI was also accepted based on autopsy findings. The study was approved by the Brigham and Women's Hospital's Institutional Review Board for Human Subjects Research.

Genotyping

For all cases and controls, the whole blood collected and frozen at baseline was subjected to DNA extraction and was genotyped for the following inflammation-related gene polymorphisms: *SCYA11* (ala23thr), *ICAM-1* (lys56met, gly241arg), *IL-4* (582C > T), *IL-4R* (ile75val, gln576arg), *IL-6* (−174G > C), *IL-10* (−571C > A), *SELP* (val640leu, thr756pro, ser330asn), and *VCAM-1* (−1594T > C). The genotyping was done using multiplex polymerase chain reaction (PCR) and immobilized probe-based assays for markers of cardiovascular disease, immune response, and inflammation. To confirm genotype assignments, the PCR procedure was performed in replicate on all samples, and scoring was carried out by two independent observers who were blinded to case-control status. Disagreements (<1%) were resolved by an additional joint reading and, where necessary, by repeat genotyping reaction.

Statistical analysis

The baseline clinical characteristics of the cases and controls were computed. Differences between the case and control groups were tested by using a Chi-square test for dichotomous variables and a 2-sample *t* test for continuous variables. A *P* value of less than 0.05 was considered statistically significant. The genotype frequencies between cases and controls were evaluated using a series of unconditional logistic regression analyses, which were adjusted for current smoking, age at randomization, alcohol use, diabetes, exercise, history of high cholesterol (≥260 mg/dL), history of hypertension (systolic ≥160 mmHg, diastolic ≥95 mmHg), body mass index and randomized aspirin and beta-carotene assignment. All the analyses were performed assuming additive, dominant, and recessive modes of inheritance for each allele. For each polymorphism, the more frequent allele in the control population was designated as wildtype. Under the additive model, subjects with two wildtype alleles were coded as '0', those with one wildtype and one mutant allele were coded as '1', and those

with two mutant alleles were coded as '2'. Under the dominant model, subjects with two wildtype alleles were coded as '0' and those with one or more mutant alleles were coded as '1'. Under the recessive model, subjects with zero or one mutant alleles were coded as '0' and those with two mutant alleles were coded as '1'.

Interaction with current smoking status was assessed by including an interaction term for current smoking by genotype (indicated by the term "smoking*genotype") in a logistic regression model. The significance of an interaction term was assessed by using a Wald Chi-square test with one degree of freedom. Odds ratios and 95% confidence intervals were calculated for each genotype-smoking status group with the referent group being those with the wildtype genotype. Since several different polymorphisms were examined, the *P*-values for the interaction terms were adjusted for multiple comparisons using the false discovery rate (Benjamini and Hochberg 1995). Additionally, pairwise linkage disequilibrium (LD) was examined as described by Devlin and Risch (1995). Haplotype frequencies were estimated from genotype data using Expectation–Maximization algorithm. The haplotype distribution between cases and controls was compared by using a likelihood ratio test. Furthermore, the relationship between haplotypes of *ICAM-1*, *SELP*, and *IL-4R* polymorphisms and MI was examined by logistic regression analysis using a baseline-haplotype parameterization, adjusting for potential confounders (Wallenstein et al. 1998). All the statistical analyses were done using SAS/Genetics v.9.

Results

The baseline characteristics for the study population are presented in Table 1. The MI cases and controls differed significantly on the traditional risk factors for heart disease: alcohol use, exercise, diabetes, high cholesterol, hypertension, body mass index, and aspirin and beta-carotene assignment. The cases and controls did not differ on the matching factors of current smoking and age at randomization.

Table 2 lists the 12 polymorphisms that were examined and the frequencies of each genotype. The more common allele was defined as wildtype. Due to the low allele frequency for *ICAM-1* lys56met (0 cases, 2 controls), this polymorphism was not considered in further regression analyses of interaction, but was used in haplotype-based analyses. All of the polymorphisms have been previously demonstrated to be in Hardy–Weinberg equilibrium (Zee et al. 2004b). As previously reported, only *SCYA11* ala23thr (recessive) was a significant predictor of MI risk after adjustment for current smoking, age at randomization, alcohol use, exercise, diabetes, high cholesterol, hypertension, body mass index, and aspirin and beta-carotene assignment (Zee et al. 2004a). The adjusted odds ratio comparing men with two 'Thr' alleles versus those with zero or one 'Thr' alleles was 1.83 (95% CI:1.08–3.09; *P* value = 0.02).

The results of the interaction analysis are presented in Table 3. The interaction between inflammatory polymorphisms and current smoking was assessed by the significance of the product term in a multivariate logistic regression model. *P* values for the product term for the dominant [DOM], recessive [REC], and additive [ADD] models are listed in Table 3. The following polymorphisms showed a significant interaction with smoking: *SELP* ser330asn ($P = 0.04$ [DOM]); *IL-4R* ile75val ($P = 0.01$ [DOM], $P = 0.01$ [ADD]); and *IL-6* −174G > C ($P = 0.03$ [REC])).

In addition to the interaction terms, odds ratios are presented for each of the six smoking status-genotype subgroups. There were no significant effects of the genotypes among the nonsmokers. Among the polymorphisms that had significant interaction terms, all exhibited significant *P*-values for at least one mode of transmission among the smokers. For *SELP* ser330asn, the odds ratio comparing smokers with one 'Asn' allele

Table 1 Baseline demographic characteristics of the case and control groups

Characteristics	MI cases ($n = 522$)		Controls ($n = 2089$)		*P* value
	n	%	*n*	%	
Current smoker	79	15.1	319	15.3	MF
Daily alcohol use	118	22.7	604	29.1	0.004
Exercise (at least 1x/week)	337	65.2	1521	73.4	0.0002
Diabetes	29	5.6	57	2.7	0.001
High cholesterol	63	13.3	156	8.3	0.0008
Hypertension	130	25.1	316	15.2	< 0.0001
Random aspirin assignment	222	42.5	1035	49.6	0.004
Random beta carotene assignment	240	46.0	1103	52.8	0.005
	Mean	SD	Mean	SD	
Age at randomization (years)	58.7	8.6	58.8	8.5	MF
Body mass index (kg/m∧2)	25.5	3.3	25.0	3.0	0.0006

Missing data: Alcohol use (10 controls, 2 cases), Diabetes (5 controls, 2 cases), Exercise (17 controls, 5 cases), History of High Cholesterol (207 controls, 47 cases), History of Hypertension (12 controls, 3 cases)
MF matching factor

Table 2 Gene frequencies in the case and control groups

Polymorphism	Genotype	MI cases ($n = 522$)		Controls ($n = 2089$)	
		n	%	*n*	%
ICAM-1 lys56met [rs5491]	Lys-Lys (ref)	516	98.9	2065	98.9
	Lys-Met	6	1.2	22	1.1
	Met-Met	0	0.0	2	0.1
ICAM-1 gly241arg [rs1799969]	Gly-Gly (ref)	422	80.8	1703	81.5
	Gly-Arg	93	17.8	367	17.6
	Arg-Arg	7	1.3	19	0.9
VCAM-1 −1594T > C [rs1041163]	TT (ref)	330	63.2	1409	67.5
	CT	172	33.0	596	28.5
	CC	20	3.8	84	4.0
SELP val640leu [rs6133]	Val-Val (ref)	413	79.1	1660	79.5
	Leu-Val	101	19.4	396	19.0
	Leu-Leu	8	1.5	33	1.6
SELP thr756pro [rs6136]	Thr-Thr (ref)	423	81.0	1688	80.8
	Thr-Pro	96	18.4	380	18.2
	Pro-Pro	3	0.6	21	1.0
SELP ser330asn [rs6131]	Ser-Ser (ref)	340	65.1	1327	63.5
	Ser-Asn	158	30.3	672	32.2
	Asn-Asn	24	4.6	90	4.3
IL-4 582C > T [rs2243250]	CC (ref)	363	69.5	1439	68.9
	CT	140	26.8	578	27.7
	TT	19	3.6	72	3.5
IL-4R ile75val [rs1805010]	Ile-Ile (ref)	174	33.3	657	31.5
	Ile-Val	247	47.3	986	47.2
	Val-Val	101	19.4	446	21.4
IL-4R gln576arg [rs1801275]	Gln-Gln (ref)	325	62.3	1313	62.9
	Gln-Arg	179	34.3	699	33.5
	Arg-Arg	18	3.5	77	3.7
IL-6 −174G > C [rs1800795]	GG (ref)	204	39.1	822	39.4
	GC	233	44.6	973	46.6
	CC	85	16.3	294	14.1
IL-10 −571C > A [rs1800872]	CC (ref)	309	59.2	1233	59.0
	CA	176	33.7	720	34.5
	AA	37	7.1	136	6.5
SCYA11 ala23thr [rs3744508]	Ala-Ala (ref)	352	67.4	1399	67.0
	Ala-Thr	145	27.8	635	30.4
	Thr-Thr	25	4.8	55	2.6

to those with no 'Asn' alleles (wildtype) was 0.48 (95% CI:0.24–0.95). The odds ratio comparing smokers with two 'Asn' alleles to wildtype smokers was not significant. For *IL-4R*, there appeared to be a decreasing risk of myocardial infarction with increasing numbers of the mutant allele ('Val') among smokers only. The odds ratio comparing smokers with one 'Val' allele to those with no 'Val' alleles (wildtype) was 0.52 (95% CI:0.29–0.95), while the odds ratio comparing smokers with two 'Val' alleles to those with no 'Val' alleles was 0.34 (95% CI:0.14–0.83). For *IL-6*, the odds ratio comparing smokers with one mutant allele 'C' to smokers with the wildtype genotype (GG) was 2.16 (95% CI:1.14–4.09), but the odds ratio comparing those with two 'C' alleles to wildtype smokers was not significant and in the opposite direction. Furthermore, we analyzed the combined effect of having the mutant allele for *SELP* ser330asn ('Asn') together with the mutant allele for *IL-4R* ile75val ('Val') under a dominant model. Among smokers, the odds ratio were 0.39 (95%CI: 0.20–0.73) for those with one mutant allele ('Asn' or 'Val') and 0.26 (95%CI: 0.11–0.62) for those with both mutant alleles ('Asn' and 'Val'), as compared to those with no mutant alleles. Among nonsmokers, the corresponding odds ratios were 1.13 (95%CI: 0.84–1.53) and 1.03 (95%CI: 0.73–1.45), respectively.

For the haplotype-based logistic regression analysis, neither *ICAM-1* haplotypes nor *SELP* haplotypes showed any significant association with a risk of MI. In a multivariate model, controlling for the variables in Table 1, the *IL-4R* haplotypes were significantly associated with the risk of MI among smokers only (likelihood ratio Chi-sq = 10.88, $df = 3$, P-value = 0.01). Compared to the baseline haplotype Ile-Gln (1–1 in Table 4), the RR of MI was 1.90 (95%CI: 0.41–8.87) for the Ile-Arg haplotype (1–2 in Table 4), 0.56 (95%CI: 0.20–1.55) for the Val-Gln haplotype (2–1 in Table 4), and 0.10 (95%CI: 0.02–0.56; P-value = 0.01) for the Val-Arg haplotype (2–2 in Table 4). There was no association between *IL-4R* haplotypes and the risk of MI among nonsmokers.

In summary, there appears to be a differential risk of myocardial infarction according to genotype only among the smokers for *SELP* ser330asn, *IL-4R* ile75val, and *IL-6* −174G > C. The P values for the significant interaction terms became nonsignificant, however, after

Table 3 Odds ratios for MI stratified by genotype and smoking status

Polymorphism	Genotype	Nonsmoker ($n = 2213$)			Current Smoker ($n = 398$)			Interaction *P*-value*
		OR	95% CI	*P*-value	OR	95%CI	*P*-value	
ICAM-1 gly241arg	Gly-Gly	1.00 (ref)	–	–	1.00 (ref)	–	–	0.85 (DOM)
	Gly-Arg	0.96	0.71–1.31	0.81	1.01	0.52–1.95	0.98	[a](REC)
	Arg-Arg	1.66	0.57–4.82	0.35	[a]	[a]	[a]	0.72 (ADD)
VCAM-1 −1594T > C	TT	1.00 (ref)	–	–	1.00 (ref)	–	–	0.70 (DOM)
	CT	1.27	0.99–1.62	0.06	1.07	0.57–2.01	0.83	0.86 (REC)
	CC	1.02	0.58–1.79	0.95	0.99	0.23–4.28	0.99	0.80 (ADD)
SELP val640leu	Val-Val	1.00 (ref)	–	–	1.00 (ref)	–	–	0.98 (DOM)
	Leu-Val	0.99	0.74–1.32	0.93	1.02	0.50–2.11	0.95	0.73 (REC)
	Leu-Leu	0.77	0.29–2.07	0.61	0.93	0.10–8.24	0.95	0.94 (ADD)
SELP thr756pro	Thr-Thr	1.00 (ref)	–	–	1.00 (ref)	–	–	0.49 (DOM)
	Thr-Pro	1.10	0.82–1.48	0.52	0.94	0.47–1.90	0.87	[a](REC)
	Pro-Pro	0.76	0.22–2.68	0.67	[a]	[a]	[a]	0.45 (ADD)
SELP ser330asn	Ser-Ser	1.00 (ref)	–	–	1.00 (ref)	–	–	**0.04** (DOM)
	Ser-Asn	0.99	0.78–1.27	0.96	0.48	0.24–0.95	**0.04**	1.00 (REC)
	Asn-Asn	1.06	0.62–1.83	0.82	1.08	0.29–3.93	0.91	0.08 (ADD)
IL-4 582C > T	CC	1.00 (ref)	–	–	1.00 (ref)	–	–	0.49 (DOM)
	CT	0.92	0.71–1.19	0.53	1.15	0.63–2.10	0.65	0.88 (REC)
	TT	0.86	0.47–1.57	0.61	1.04	0.20–5.31	0.96	0.52 (ADD)
IL-4R ile75val	Ile-Ile	1.00 (ref)	–	–	1.00 (ref)	–	–	**0.01** (DOM)
	Ile-Val	1.03	0.80–1.34	0.80	0.52	0.29–0.95	**0.03**	0.20 (REC)
	Val-Val	0.95	0.70–1.31	0.77	0.34	0.14–0.83	**0.02**	**0.01** (ADD)
IL-4R gln576arg	Gln-Gln	1.00 (ref)	–	–	1.00 (ref)	–	–	0.15 (DOM)
	Gln-Arg	1.11	0.88–1.41	0.38	0.58	0.31–1.08	0.09	0.43 (REC)
	Arg-Arg	0.97	0.53–1.81	0.93	1.31	0.38–4.58	0.67	0.35 (ADD)
IL-6 −174G > C	GG	1.00 (ref)	–	–	1.00 (ref)	–	–	0.12 (DOM)
	GC	0.85	0.67–1.09	0.21	2.16	1.14–4.09	**0.02**	**0.03** (REC)
	CC	1.26	0.90–1.76	0.18	0.81	0.31–2.12	0.67	0.87 (ADD)
IL-10 −571C > A	CC	1.00 (ref)	–	–	1.00 (ref)	–	–	0.39 (DOM)
	CA	0.96	0.75–1.22	0.73	0.80	0.44–1.43	0.44	0.15 (REC)
	AA	1.23	0.80–1.91	0.35	0.40	0.11–1.46	0.17	0.19 (ADD)
SCYA11 ala23thr	Ala-Ala	1.00 (ref)	–	–	1.00 (ref)	–	–	0.28 (DOM)
	Ala-Thr	0.92	0.71–1.18	0.49	0.52	0.26–1.04	0.07	0.14 (REC)
	Thr-Thr	1.52	0.85–2.74	0.16	3.23	0.86–12.1	0.08	0.69 (ADD)

Adjusted for current smoking (yes vs. no), age at randomization (years), alcohol use (daily vs. weekly/monthly/rarely), diabetes (yes vs. no), exercise (at least 1x/week vs. < 1x/week), history of high cholesterol (yes vs. no), history of hypertension (yes vs. no), body mass index (kg/m∧2), randomized aspirin assignment (yes vs. no), randomized beta carotene assignment (yes vs. no)*DOM* dominant model, *REC* recessive model, *ADD* additive model**P* value for the product term for ''polymorphism*current smoking''

[a]Insufficient sample size for calculation

Table 4 Haplotype frequency for the total study population and haplotype-based adjusted logistic regression with baseline-haplotype parameterization among smokers for *IL-4R*

Haplotype	Controls	Cases	χ^2_{3df}	*p*
	n (Frequency)	*n* (Frequency)	6.39	0.09
1-1	1922 (0.46)	543 (0.52)		
1-2	376 (0.09)	136 (0.13)		
2-1	1337 (0.32)	282 (0.27)		
2-2	543 (0.13)	73 (0.07)		
Haplotype	Odds Ratio	95%CI	*P*	$*\chi^2_{3df}$, *P*
1-1	Reference	–	–	10.88, 0.01
1-2	1.90	0.41–8.87	0.42	
2-1	0.56	0.20–1.55	0.26	
2-2	0.10	0.02–0.56	0.01	

Adjusted for current smoking (yes vs. no), age at randomization (years), alcohol use (daily vs. weekly/monthly/rarely), diabetes (yes vs. no), exercise (at least 1x/week vs. < 1x/week), history of high cholesterol (yes vs. no), history of hypertension (yes vs. no), body mass index (kg/m∧2), randomized aspirin assignment (yes vs. no), randomized beta-carotene assignment (yes vs. no)

1 denotes the more-frequent allele, and 2 denotes the less-frequent allele at each polymorphic site.

1-1 = Ile-Gln

1-2 = Ile-Arg

2-1 = Val-Gln

2-2 = Val-Arg

*χ^2 - and *P* values for likelihood ratio test comparing models with genetic data to models without

adjustment for multiple comparisons using the false discovery rate. Using the dominant model, the following adjusted *P* values were obtained: *SELP* (adjusted $P = 0.22$), *IL-4R* (adjusted $P = 0.11$), *IL-6* (adjusted $P = 0.41$). Although, there does appear to be a significant association between *IL-4R* haplotypes and a risk of MI among smokers.

Discussion

The purpose of our analysis was to determine if the effect of cigarette smoking on the risk of MI varied according to a person's genotype for polymorphisms in genes that code for inflammatory markers. Of the 11 polymorphisms investigated, 3 had statistically significant interaction terms: *SELP* ser330asn, *IL-4R* ile75val, and *IL-6* −174G > C. Interestingly, for all the three polymorphisms, the differential risk of MI according to genotype appeared to exist only among the smokers. Among nonsmokers, the risk of MI did not differ according to genotype. In the haplotype-based analysis, haplotypes for IL-4R were significantly associated with MI among smokers but not among nonsmokers. This is consistent with our initial hypothesis that the effect of cigarette smoking on the risk of MI varies according to an individual's genotype. After adjustment for multiple comparisons, though, none of the interaction terms were statistically significant. This finding raises the possibility that our results may either represent false positives or that our sample size was not large enough to produce the power required to detect an interaction in the presence of multiple comparisons.

The panel of polymorphisms used in this analysis was chosen specifically because of their involvement in the inflammatory pathway leading to myocardial infarction. We considered three groups of inflammatory markers: cell adhesion molecules, interleukin-based cytokines, and eosinophil chemokines. The cell adhesion molecules that were studied consisted of intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and P-selectin. Intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 are involved in the adhesion and transmigration of leukocytes through the vascular endothelium and have been found to be expressed in atherosclerotic plaques (Libby et al. 2002; Ridker et al. 1998). P-selectin mediates leukocyte rolling along the endothelial surface (Dong et al. 1998). *SELP* val640leu has previously been shown to predict ischemic stroke in the PHS cohort (Zee et al. 2004b).

The interleukin-based cytokines considered in the analysis consisted of interleukin-4, interleukin-4 receptor, interleukin-6, and interleukin-10. Interleukin-4 is a pleiotropic cytokine that is produced by activated T cells, mast cells, and basophils and mediates its biological effects by binding to the interleukin-4 receptor (Hackstein et al. 2001). The interleukin-4 receptor is composed of two subunits: the α subunit which binds interleukin-4 and the γc subunit (Hershey et al. 1997). Interleukin-4 appears to play a role in the pathogenesis of allergy and asthma. *IL-4* 582C > T has been shown to predict strokes in the PHS cohort (Zee et al. 2004b). Mice who are deficient in the interleukin-4 receptor α subunit lack IgE production and Th2 inflammatory reactions (Mitsuyasu et al. 1998). Interleukin-6 is a proinflammatory cytokine that regulates the hepatic synthesis of CRP and is an important mediator of the acute-phase inflammatory response (Ridker et al. 2000; Yudkin et al. 2000). Interleukin-6 is secreted by numerous molecules such as activated macrophages and lymphocytes and is also secreted by adipose tissue (Yudkin et al. 2000). Interleukin-10 is an antiinflammatory cytokine that inhibits the production of proinflammatory cytokines by macrophages (Niiro et al. 1997). Interleukin-10 is expressed in atherosclerotic plaques and is thought to have a protective effect (Donger et al. 2001).

The third category of inflammatory markers considered was the eosinophil chemokines, which consisted of eotaxin. Eotaxin is a chemotactic factor for eosinophils and the eotaxin protein has been found to be expressed in the smooth muscle cells in human atheroma (Haley et al. 2000). *SCYA11* ala23thr has been shown to predict the risk of MI in the PHS cohort (Zee et al. 2004a).

Of all the genes considered code for proteins that are involved in the inflammatory process, only three polymorphisms were found to interact with smoking status. An examination of the biology of these polymorphisms may provide a rationale for their interaction with smoking. No study has examined the interaction of the *SELP* ser290asn polymorphism with cigarette smoking. The association of this polymorphism with MI has been previously investigated in the Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) cohort; no association with MI was found which is consistent with our results (Herrmann et al. 1998). The effect of the polymorphism on soluble P-selectin levels is unknown.

The majority of the research involving interleukin-4 and interleukin-4 receptor has focused on allergy and asthma and only recently it has been studied in relation to other inflammatory diseases. No study has examined the association between the ile75val polymorphism and the risk of MI or its interaction with smoking. However, it has been shown that smokers have higher serum interleukin-4 and IgE levels than nonsmokers (Byron et al. 1994). Elevated serum IgE levels have been associated with increased risk of MI in dyslipidemic men (Kovanen et al. 1998). It is possible that smokers with one or more copies of the variant allele Val75 have a lower risk of MI relative to smokers with the wildtype (Ile-Ile) genotype due to their decreased serum IgE levels. Since nonsmokers do not have elevated IgE levels, having a "protective" genotype may not alter their risk of MI if their IgE levels are already low.

In contrast to the *SELP* and *IL-4R* polymorphisms, the *IL-6* −174G > C polymorphism has been widely studied in relation to MI. Five studies have examined the association between the *IL-6* −174G > C polymorphism

and risk of MI (Basso et al. 2002; Georges et al. 2001; Humphries et al. 2001; Jenny et al. 2002; Nauck et al. 2002). Two of these studies examined the interaction of this polymorphism with smoking status on the risk of MI (Georges et al. 2001; Humphries et al. 2001). No interaction was observed in the ECTIM study, an analysis of 640 male MI cases and 719 controls (Georges et al. 2001). In the Northwick Park Heart Study II (NPHSII), a prospective cohort of 3,052 healthy men, the GC genotype was associated with a significantly increased risk of MI compared to the GG genotype, an effect that was confined to the smokers (GC smokers vs. GG nonsmokers: RR = 2.66; 95%CI: 1.64–4.32) (Humphries et al. 2001). The CC genotype did not confer increased risk compared to the GG genotype. However, the interaction of current smoking with *IL-6* genotype under the additive model was not statistically significant. Since the NPHSII investigators only considered an additive model, it is not known whether they would have found a significant interaction term for smoking using a recessive model as was found in our analysis.

The *IL-6* −174G > C polymorphism has been shown to be of functional significance. One study found that individuals carrying the C allele had higher plasma levels of interleukin-6 (Humphries et al. 2001). If the C allele is associated with increased plasma levels of interleukin-6, it would be a possible explanation for the increased risk of MI in individuals with one or more C alleles.

There are several limitations in our analysis. The subjects consisted of Caucasian middle-aged men and thus the results may not be generalizable to females or to other racial/ethnic groups. Additionally, there could have been misclassification of smoking status since smoking status was assessed only at baseline and some subjects may have quit during the follow-up period. However, this misclassification is likely to be nondifferential with respect to genotype and therefore, would have biased the results toward the null. Another limitation of our analysis was that the cases and controls were matched on smoking status and so it was not possible to examine the main effect of smoking in these data. However, the matching can also be viewed as a strength since it ensured that there were a sufficient number of smokers in the control group. Another strength of our analysis is that it is a prospective nested case-control study with a large sample size. Of note, in a previous investigation using the PHS cohort, we found no evidence of population stratification (Zee et al. 2004b).

In conclusion, it appears that there is a possibility of an interaction between smoking status and certain inflammation-related gene polymorphisms (*IL-4R* ile75val, *IL-6* −174G > C, *SELP* ser330asn) on the risk of MI in men. However, after adjustment for multiple comparisons, these interactions were not significant. A haplotype-based analysis revealed a significant association between IL-4R haplotypes and the risk of MI. These data require confirmation in other large prospective studies in different populations.

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