Genetic Influence on Inflammation Variables in the Elderly

Moniek P.M. de Maat, Else Marie Bladbjerg, Jacob von Bornemann Hjelmborg, Lise Bathum, Jørgen Jespersen, Kaare Christensen

Background—Inflammation variables (C-reactive protein [CRP], fibrinogen, and soluble intercellular adhesion molecule-1 [sICAM-1]) have been identified as risk factors for cardiovascular disease. It is still not known how much the regulation of inflammatory risk factors is determined by genetic factors, and the aim of this study was to determine the heritability of these inflammation variables and of the acute phase regulating cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) at older ages.

Methods and Results—The heritability of CRP, fibrinogen, sICAM-1, IL-6, and TNF-α was determined in a twin study consisting of 129 monozygotic twin pairs and 153 dizygotic same-sex twins aged 73 to 94 years who participated in the Longitudinal Study of Aging of Danish Twins. Furthermore, we determined the influence of selected genetic polymorphisms on the plasma level variations. Genetic factors accounted for 20% to 55% of the variation in plasma levels of the inflammation variables. The highest heritability was found for sICAM-1. The genetic polymorphisms we studied explained only a small, insignificant part of the heritability.

Conclusions—This study in elderly twins provides evidence for a substantial genetic component of inflammatory cardiovascular risk factors among the elderly. (***Arterioscler Thromb Vasc Biol*. 2004;24:2168-2173.)**

Key Words: inflammation ■ twins ■ heritability ■ cardiovascular disease

A positive family history of cardiovascular disease is a strong determinant of the risk of cardiovascular events in epidemiological studies,[1,2] and this observation was confirmed by a Swedish twin study in which death from coronary heart disease showed a substantial genetic component.[3] Within families, the genetic background and environmental factors are shared, and twin studies are among the best studies for disentangling the effects of genetic and environmental components.

Inflammation is an important mechanism in cardiovascular disease. The acute phase proteins C-reactive protein (CRP),[4,5] fibrinogen,[6] and soluble intercellular adhesion molecule-1 (sICAM-1)[7] are consistently associated with the risk of cardiovascular events in prospective population studies. The levels of these inflammatory markers reflect the severity of the underlying atherosclerosis in the vessel wall, but they may also causally contribute to the development of cardiovascular disease.[8] As an example, CRP activates the classical pathway of the complement system, functions as a scavenger factor, and determines the uptake of low-density lipoprotein by macrophages.[9] Fibrinogen levels determine the rigidity and fibrinolysis rate of clots; fibrinogen functions as a bridging molecule for many types of cell–cell adhesion events and provides a critical provisional matrix.[10] ICAM-1 participates in the adhesion of neutrophils and monocytes to the endothelium, an essential step in extravasation of neutrophils and monocytes at the site of inflammation.

The plasma levels of these variables show a large interindividual and intraindividual variation.[11] Inflammatory triggers are the main determinants of the levels of these factors, but also, genetic variations have been reported to explain part of the variation in inflammatory variables. Studies of CRP[12–15] and fibrinogen[13,16–18] in healthy young and middle-aged twins and in family studies suggest that a considerable part of the variation in these factors can be explained by genetic factors.

Because cardiovascular disease is primarily a disease of the elderly, it is important to assess how important genetic variation in risk factors is in the elderly because they are subject to many more triggers or risks than young individuals. It may be expected that genetic factors make a different contribution to the variation in the elderly compared with the younger age groups. On one hand, it is possible that functional genetic variations in the promoter region of the genes, which determine response to triggers, contribute more in the elderly because there are more triggers. On the other hand, the surplus and variety of triggers present in later age may also decrease the importance of genetic variation. Thus, it is not clear whether a larger or smaller contribution of genetic variation is expected, and evolution predicts the first, and traditional gerontology predicts the latter.

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From the Department for Thrombosis Research (M.P.M.dM., E.M.B., J.J.), University of Southern Denmark, and the Department of Clinical Biochemistry (M.P.M.d.M., E.M.B., J.J., Ribe County Hospital, Esbjerg, Denmark; the Department of Hematology (M.P.M.dM.), Erasmus Medical Center, Rotterdam, the Netherlands; and Institute of Public Health (J.v.B.H., K.C.), Research Unit of Epidemiology, University of Southern Denmark.

Correspondence to Dr M.P.M. de Maat, Department of Hematology, Erasmus University Medical Center, Room Ee 13.93, Dr Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. E-mail m.demaat@erasmusmc.nl

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In addition to functional variations in genes of inflammation variables, functional genetic variations in factors that regulate these variables may contribute to the variation in plasma levels of inflammatory variables. Interleukin-6 (IL-6)[19] and tumor necrosis factor-α (TNF-α)[20] are 2 important regulatory cytokines for acute phase proteins[21,22] that both have a genetic contribution to their levels[23] and that both have functional genetic polymorphisms in their promoter region that have been associated with their plasma levels.[24,25] These functional polymorphisms, for which an association with levels has been shown repeatedly and is biologically plausible, are the most likely genetic variants to explain part of the hereditary component that determines the plasma levels of the inflammatory variables.

The aim of this study was to assess the overall genetic role in variations of inflammation variables among the elderly. Therefore, we determined the genetic contribution to the variations in CRP, fibrinogen, sICAM-1 and the acute phase-regulating cytokines IL-6 and TNF-α in elderly twins (73 to 94 years old).

Subjects and Methods

Participants

A total of 584 individuals (190 males and 394 females) aged 73 to 94 years participated in this study. These individuals made up 129 intact monozygotic (MZ) twin pairs and 153 intact dizygotic (DZ) same-sex twin pairs. The twin pairs were identified using the Danish Twin Registry and participated in the 1997 Longitudinal Study of Aging of Danish Twins.[26] The aim was to include a random sample of twins so all twins who were able to finalize the interview and give informed consent were eligible for participation in the study.

The Danish Twin Registry, a nationwide and population-based registry established in 1954, comprises twins born between 1870 and 1930, surviving to the age of 6 years.[26] Only same-sex twin pairs were included in the present study. Zygosity was established through a questionnaire on the degree of similarity between twins in a pair. Zygosity classification was evaluated by comparison with blood group determinants, and the misclassification rate was $<5\%$.[27]

Methods

Blood samples (20 mL of EDTA blood) were collected within a 6-month period. The samples were centrifuged within 12 hours after sampling at 1000*g* for 10 minutes, and plasma was frozen in aliquots at $-80°C$ within 2 days (usually within the same day). Plasma samples were rapidly thawed in a water bath at 37°C. The protein concentration of CRP was measured using a high-sensitivity in-house enzyme immunoassay using rabbit anti-human CRP IgG as capture and tagging antibody (DAKO). Human CRP Standard (Dade Behring) was used as a calibrator. Protein concentrations of IL-6, TNF-α, and sICAM-1 were determined using high-sensitivity human ELISAs (R&D Systems). The protein concentration of fibrinogen was determined using a nephelometric method (Dade Behring). Interassay coefficients of variation for the assays we used were: for fibrinogen, $<5.0\%$; IL-6, $<10.1\%$; ICAM, $<5.6\%$; TNF, $<14.5\%$; CRP, $<6.5\%$ based on 17 determinations from a single normal plasma sample on different days.

Genomic DNA samples were analyzed using polymerase chain reaction (PCR)-based methods. The fibrinogen β-455G/A promoter polymorphism was analyzed as described by Thomas et al.[28] The CRP-1059G/C polymorphism was analyzed as described by Zee et al.[29] The ICAM-1 K/E469 polymorphism was analyzed as described by Nishimura et al.[30] The IL-6–174C/T promoter polymorphism was analyzed as described by Fishman et al.[24] The TNF-α–238G/A promoter polymorphism was analyzed as described by Wennberg et al.[31] The genotype was determined in all individuals and monozygosity was confirmed. In each PCR run, samples with known genotypes were included. We also reanalyzed 10% of the samples (at random), and the results were confirmed.

Analyses of Twin Similarity

The similarity in MZ and DZ twins was assessed using intraclass correlations for inflammatory variables. The classic twin study methodology is based on the fact that MZ twins have identical genotypes, whereas DZ twins share, on average, half of their genes and are no more genetically related than ordinary siblings. A greater phenotypic similarity in MZ twins is to be expected if there is a significant genetic component in the etiology of the component. Analyses were adjusted (by linear regression) for age, sex, and body mass index (BMI). To approximate a bivariate normal distribution, data were transformed to the logarithmic scale, and probit plots showed that the marginal distributions could be assumed to be normal. Plasma levels of CRP >10 mg/L and levels of IL-6 >10 pg/mL (6% of the individuals) are considered to reflect "clinical inflammation"; these data and the data from the cotwin were removed from the data set.

Estimation of Heritability

Heritability of the 5 traits, effects of covariates on traits and the degree of dependence between the traits, were determined using multivariate variance components models.[32] Phenotypic (co)variances are decomposed into genetic and environmental components by the Cholesky decomposition method. According to standard biometric practice, assuming no epistasis (genetic inter locus interaction), no gene–environment interaction or correlation, and no assortative mating, the phenotypic variance can be separated into 4 variance components: (A) variance attributable to additive genetic effects; (D) genetic dominance; (C) shared (family) environment; and (E) nonshared (individual-specific) environment.[32] Only nonshared environments contribute to dissimilarity within MZ twin pairs because of their genetic identity, whereas the effects of additive genetic factors and genetic dominance may also contribute to dissimilarity within DZ pairs, who share, on average, half of the additive and one-quarter of the dominant genetic factors. For each trait, the heritability is the ratio of genetic variance to the total variance. Effects of the covariates of each trait are given by the regression coefficients coming from the covariate that is linearly added to the model (which corresponds to linear regression of the covariates on traits and subsequent heritability analysis of residuals thereof.

The multivariate model incorporates the 5 traits and associated covariates and allows estimating the degree of dependence between the traits (ie, correlations between the traits). Furthermore, the extent to which the same genetic factors contribute to the observed phenotypic correlation (ie, the degree of pleiotropy of possible genes influencing the traits) is obtained from the model by estimating the genetic correlations. The method for selecting the best model followed standard procedures (structural-equation analyses) using the Mx statistical program.[32,33]

Association Between Genotype and Phenotype

Association between the 5 polymorphisms and 5 trait levels in the whole population was initially assessed by 1-way ANOVA (with robust estimation of variance of phenotypes for individual twins clustered into twin pairs because of possible within-pair dependence). Sib-pair–based association analysis was performed using the variance components models of association implemented in the Quantitative Transmission Disequilibrium Test (QTDT) program,[34] and in all analyses, gender, age, and BMI were included as covariates. We tested for association in QTDT using the model by Fulker et al,[35] allowing for the effects of polygenes, shared family environment, nonshared environment, and effect of linkage at the locus under study. This model allows for the partitioning of observed association into between-pair and within-pair components, thus providing a check on whether results could have arisen because of unsuspected population stratification among the pairs studied. Subsequent permutation tests[36] of association, which do not rely on the bivariate normal distribution assumption, were performed using the

TABLE 1. General Characteristics and Concentrations of Inflammation Variables in MZ and DZ Elderly Twins

	MZ Twins	DZ Twins
Age (years)	78.1 (0.26)	77.9 (0.24)
Gender (intact pairs men/women)	44/88	51/109
BMI (kg/m^2)	24.4 (0.23)	24.2 (0.23)
Current smokers	65 (25%)	92 (30%)
CRP (mg/L)§	1.88 (1.64, 2.15)	2.05 (1.80, 2.32)
Fibrinogen (μmol/L)§	12.8 (12.4, 13.2)	12.9 (12.5, 13.2)
sICAM-1 (pg/mL)§	286 (278, 294)	276 (270, 283)
IL-6 (pg/mL)§	2.64 (2.46, 2.85)	2.68 (2.51, 2.86)
TNF-α (pg/mL)§	2.44 (2.35, 2.54)	2.54 (2.44, 2.65)

Values are mean (SE) or §geometric mean (95% CI). The SE is estimated, correcting for the correlation within twin pairs.

QTDT program. Secondly, the amount of variation in the plasma levels of the inflammatory variables attributable to the inflammatory polymorphisms was studied using parametric linkage analysis implemented in the Merlin program.[36] There is no adjustment of the mean levels for zygosity. It is an assumption that MZ and DZ pairs come from the same population. There is no sign of violation of this assumption (Table 1).

Allele frequencies were determined by gene counting, and 95% CIs of the allele frequencies were calculated from sample allele frequencies. Deviations of the genotype distributions from that expected for a population in Hardy–Weinberg equilibrium were analyzed using the χ^2 test.

Results

Table 1 shows the general characteristics of our elderly twin population and the concentrations of inflammation variables in the MZ and DZ twins. All characteristics are comparable in the MZ and DZ twins. Concentrations of the inflammatory variables are comparable in MZ and DZ twins.

The model chosen to explain the factors contributing to the variance in the inflammatory cardiovascular risk factors is the model including additive genetic effects, common environmental effects, and nonshared environmental effects (ACE). In all the traits, the common environmental effects were small and nonsignificant (data not shown). The ACE model was then used to estimate the heritability coefficients, effects of covariates, and dependencies among the traits (ie, mutual correlation of the traits and genetic correlation of the traits).

As covariates in our multivariate variance components model, we included gender, age, and BMI. Plasma concentrations of IL-6 and TNF-α increase with age, and the plasma concentration of IL-6 decreases with increasing BMI, whereas levels of CRP and fibrinogen increase with increasing BMI (Table 2). We observed no significant gender effect for any of the traits (Table 2).

The intraclass correlations in MZ twins were consistently higher than those in DZ twins for all variables (Table 2). The difference was statistically significant for ICAM-1 ($P<0.001$). Heritability estimates, when adjusting for effects of gender, age, and BMI, and when allowing for possible dependencies between the traits, varied between 0.17 for IL-6 and 0.55 for sICAM-1 (Table 2). Mutual correlations between the traits are given in Table 3. Between the plasma levels we observed, as expected, significantly positive correlations ranged from 0.17 to 0.55, indicating substantial relationship among the traits. Significant genetic correlations, ranging between 0.27 and 0.41, were seen between the cytokines and CRP, fibrinogen, and sICAM-1, indicating a substantial degree of pleiotropy (ie, a gene affecting 2 or more traits [Table 3]). Small (and not significant) genetic correlations were observed among CRP, fibrinogen, and sICAM-1, which may indicate that the common genetic contribution to their regulation is insignificant.

Polymorphisms in the inflammatory genes (-455G/A in fibrinogen-β, -1059G/C in CRP, K/E469 in ICAM-1, -174C/T in IL-6, and -238G/A in TNF-α) were all in Hardy–Weinberg equilibrium, and the frequencies of the alleles (Table 4) were comparable to those reported in other white populations.[25,37–40]

When we studied the effect of polymorphisms in factors upstream in the regulatory pathway of the cytokines and acute phase proteins, evidence for an association between genotype at the TNF–238G/A polymorphism and IL-6 was found by ANOVA ($P=0.02$; Table 5). This association was confirmed using the sib-pair–based analysis (QTDT, $\chi^2=5.7$ [1 *df*]; $P=0.02$) performed using the genetic model by Fulker et al,[35] which takes possible population stratification into account. Associations were also seen for TNF–238G/A polymorphism with CRP levels ($P=0.03$) and for the fibrinogen–455G/A polymorphism with fibrinogen levels ($P=0.03$) by ANOVA,

TABLE 2. Heritability Estimates for Inflammatory Cardiovascular Risk Factors

	CRP	Fibrinogen	sICAM1	IL-6	TNF-α
n pairs (MZ/DZ)	(112/129)	(129/153)	(129/153)	(118/130)	(126/151)
Age	−0.0147	0.0026	0.0027	0.0204*	0.0160*
Sex	1.0911	0.1727	−0.0192	0.5337	0.0547
BMI	0.0432*	0.0069*	−0.0092	−0.0957*	0.0618
ICC MZ	0.30 (0.25, 0.30)	0.30 (0.19, 0.41)	0.59 (0.48, 0.67)	0.27 (0.15, 0.38)	0.35 (0.21, 0.48)
ICC DZ	0.20 (0.19, 0.21)	0.19 (0.16, 0.28)	0.31 (0.24, 0.40)	0.18 (0.10, 0.23)	0.21 (0.14, 0.32)
Heritability	0.20 (0.16, 0.34)	0.21 (0.07, 0.25)	0.55 (0.34, 0.65)	0.17 (0.12, 0.28)	0.26 (0.01, 0.34)

Heritability estimates (95% CI) when assuming the multivariate model including additive genetic effects, common environmental effects, and nonshared environmental effects (ACE).

The model allows adjustment (by linear regression) of covariate gender, age, and BMI.

Effects of covariates (*$P<0.05$) and estimates of the intraclass correlations (ICC, 95% CI) for MZ and DZ pairs are shown.

Inferences are estimated under bivariate normality assumption.

TABLE 3. Correlations Between Cytokines and Acute Phase Proteins

	CRP	Fibrinogen	ICAM-1	IL-6	TNF-α
CRP (mg/L)		0.52*	0.22*	0.55*	0.22*
Fibrinogen (g/L)	0.13		0.19*	0.42*	0.17*
ICAM-1 (ng/mL)	0.23	0.09		0.24*	0.31*
IL-6 (ng/mL)	0.71#	0.60#	0.27*		0.23*
TNF-α (ng/mL)	0.36*	0.41*	0.33*	0.21	

The multivariate model allows for estimation of correlations between cytokines and acute phase proteins. These correlations are given above the diagonal. Furthermore, genetic correlations between cytokines and acute phase proteins are given below the diagonal.

*$P<0.05$; #P value could not be calculated.

but these associations were not confirmed in the subsequent sib-pair–based analysis.

No evidence for linkage between the polymorphisms and the plasma levels of the inflammation variables was observed, not even between the TNF-α polymorphism and IL-6 levels. However, power is limited for linkage studies in our cohort.

Discussion

This study describes that there is a clear genetic component in levels of inflammatory cardiovascular risk factors in the elderly. Selected functional polymorphisms in the genes of fibrinogen-β, CRP, ICAM-1, IL-6, and TNF-α only explained a very small part of this variation.

The contribution of genetic variation to plasma levels of fibrinogen in the elderly twins in our study was comparable or somewhat smaller than the genetic contributions published for younger, mostly middle-aged twins. For fibrinogen, the additive genetic variance in our elderly twins (0.21) was lower than the estimates determined in other twin studies, which varied from 0.28 to 0.44.[13,16,18] Family studies reported heritabilities for fibrinogen between 0.20 and 0.51,[16,41] and in the Kibbutzim Family Study, it was observed that the heritability decreased from 0.48 in 20-year-old individuals to 0.20 in 80-year-old individuals.[42] It was expected that the effect of genetic factors could be smaller in the elderly twins because of the presence of many inflammatory triggers, such as cardiovascular disease and other age-associated diseases. The results of our study support this hypothesis, but it remains unclear how much of the difference in heritability can be attributed to age versus other population characteristics.

Only a small, insignificant part of this variance could be explained by the -455G/A polymorphism in the promoter region of the fibrinogen-β gene, in accordance with other reports.[43] Polymorphisms in ≈2000 bp of the promoter region of the fibrinogen-β gene were identified, and in whites, these are in very strong linkage disequilibrium.[44] Furthermore, we recently found using luciferase expression studies that the -148C/T polymorphism (which is in perfect linkage disequilibrium with the -455G/A polymorphism in white populations) is the functional polymorphism in the promoter region, explaining all promoter activity.[45] Therefore, it is not expected that any new information will become available when other polymorphisms are studied in this region. Also polymorphisms in the promoter region of IL-6 and TNF-α, 2 major determinants of fibrinogen synthesis, explained only an insignificant part of the variance in fibrinogen levels.

The genetic contribution to variation in the CRP concentration appears somewhat lower in our study than in middle-aged twins, for whom a clear genetic contribution has been reported in a UK study (intraclass correlation coefficient of 0.57 in MZ twins and 0.31 in DZ twins)[12] and in healthy family studies.[14,15] Polymorphisms in the promoter region of CRP, IL-6, or TNF-α did not explain a major part of this genetic variance in our elderly twins, in contrast to the observation of Vickers et al that the IL-6–174C/T polymorphism explains 14% of the CRP concentration.[14] For sICAM-1, no information was published on the heritability of the levels, but the high estimate in our study makes this an interesting candidate gene for further studies. For IL-6, the heritability estimate in our study (0.17) is similar to an estimate of heritability that is available from a family study (0.24).[23] For TNF-α, the heritability estimate in our study (0.26) was much lower than the estimates derived from family studies (0.68 and >0.80).[23,46] The heritability of the in vitro production of TNF-α in a whole-blood stimulation experiment was 0.60,[47] which is also higher than the heritability we saw in vivo in elderly twins. This suggests that the responsiveness may have a stronger genetic component than the baseline levels.

The hypothesis that responsiveness may be genetically determined suggests that functional polymorphisms in the regulatory elements of inflammatory genes may be determinants of the plasma levels of inflammatory variables. However, the regulatory polymorphisms we studied in the genes for fibrinogen, IL-6, CRP, and TNF-α did not explain much of the variance in their gene products, nor did polymorphisms

TABLE 4. Frequencies of Polymorphisms in Inflammation Genes

Polymorphism	Genotype	n
−1059G/C in CRP	GG	418
	GC	48
	CC	0
	f(C)	0.05 (0.04, 0.07)
−455G/A in fibrinogen-β	GG	299
	GA	154
	AA	16
	f(A)	0.20 (0.17, 0.23)
K/E469 in ICAM-1	KK	162
	KE	217
	EE	90
	f(E)	0.42 (0.39, 0.46)
−174C/T in IL-6	CC	142
	CT	227
	TT	100
	f(T)	0.46 (0.42, 0.49)
−238G/A in TNF-α	GG	321
	GA	124
	AA	21
	f(A)	0.18 (0.15, 0.21)

Given are frequencies of the rare alleles with the 95% CI.

TABLE 5. Relationship Between Polymorphisms and Plasma Protein Concentrations

Polymorphism	Genotype	CRP (mg/L)	Fibrinogen (μmol/L)	ICAM (ng/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
−1059G/C in CRP	GG	1.66 (0.28, 9.99)				
	GC	1.83 (0.30, 11.10)				
	CC	–				
	P value	NS				
−455G/A in fibrinogen-β	GG		12.55 (7.69, 20.49)			
	GA		13.46 (8.41, 21.55)			
	AA		13.07 (7.70, 22.18)			
	P value		0.02			
K/E469 in ICAM-1	KK			278 (181, 428)		
	KE			278 (184, 420)		
	EE			290 (184, 455)		
	P value			NS		
−174C/T in IL-6	CC	1.53 (0.29, 8.09)	12.55 (7.84, 20.09)	276 (184, 414)	2.56 (0.76, 8.67)	2.48 (1.11, 5.55)
	CT	1.65 (0.27, 10.00)	12.81 (7.85, 20.91)	284 (177, 457)	2.64 (0.88, 7.96)	2.48 (1.28, 4.84)
	TT	1.91 (0.29, 12.83)	13.20 (7.93, 21.97)	279 (197, 395)	2.81 (0.95, 8.27)	2.51 (1.37, 4.61)
	P value	NS	NS	NS	NS	NS
−238G/A in TNF-α	GG	1.79 (0.29, 11.07)	12.94 (7.92, 21.12)	281 (183, 433)	2.69 (0.85, 8.50)	2.50 (1.28, 4.90)
	GA	1.51 (0.29, 7.72)	12.81 (7.85, 20.91)	281 (179, 442)	2.69 (0.92, 7.87)	2.55 (1.22, 5.32)
	AA	1.09 (0.16, 7.61)	11.82 (7.99, 17.50)	265 (169, 416)	1.91 (0.65, 5.63)	2.13 (1.09, 4.16)
	P value	0.03	NS	NS	0.02	0.05

Presented are the correlations between polymorphisms and plasma levels of the gene product and of proteins for which they are in the regulatory pathway. Geometric mean and 95% CIs are given for the plasma levels.

in the IL-6 and TNF-α genes explain the variance in the levels of the acute phase proteins. We studied polymorphisms for which an association with levels has been shown repeatedly and is biologically plausible. In addition to the fibrinogen promoter, also for the other factors, the gene and a reasonable part of the promoter region (on average up to 2000 bp) were studied for functional polymorphisms, and we selected polymorphisms for which it was most likely that they would explain a considerable part of the heritability.[24,45,48] However, we cannot exclude that other genetic variants have a major impact on the plasma levels of these factors, and studies using full haplotype analysis will be able to give a better estimation of the contribution of genetic variation in a gene to its plasma levels.

We only saw 1 possible association, but no linkage, for any combination of polymorphism and plasma level, indicating that twin pairs that share their alleles are not necessarily close in trait values. This suggests that we did not study the functional polymorphism or that a combination of polymorphisms affects the plasma level. The intriguing question is what the genetic components are if they are not genetic variants in the genes, variations in the promoter regions, or in the regulating cytokine genes. The explanation for the high heritability may therefore be found in enhancer elements further away from the gene or in other genes that determine the regulation of inflammatory factors, such as nuclear proteins. The high genetic correlation between inflammatory variables in our study may indicate a common genetic regulatory mechanism.

BMI has a large genetic component and is also a major determinant of inflammation variables, for example, by increasing the cytokine production by adipose tissue.[49] Furthermore, it is possible that the sensitivity of inflammatory variables to BMI is genetically determined. Adjustment for BMI in the statistical analysis may remove such a relationship. Therefore, we analyzed the data unadjusted and adjusted for BMI and noticed that the adjustment had no significant effect on the heritability estimated in our population. This suggests that different gene pools determine the inflammatory variables and the BMI.

When studying the elderly, it cannot be excluded that there has been a selection of our study population because only individuals that survived to be 73 years old were included in the study. However, that is a problem inherent in all aging research, and the present study benefited from a high response rate (79%).

The calculated heritability may be an underestimate of the underlying heritability because the procedure for processing blood was not optimal, which may have given a spuriously greater variation in the levels of the inflammatory markers. Furthermore, the phenotypes show a great day-to-day variation because the inflammatory variables are very sensitive to environmental factors.

In conclusion, levels of inflammatory cardiovascular risk predictors are, to a large extent, genetically determined, even in elderly individuals, and therefore, it is important to elucidate the genetic factors involved in the regulation of these proteins, especially in cardiovascular disease.

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