



## Original Contribution

# Single Nucleotide Polymorphisms in Inflammation-related Genes and Mortality in a Community-based Cohort in Washington County, Maryland

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Received for publication August 24, 2007; accepted for publication November 30, 2007.

The purpose of this study was to examine the associations between single nucleotide polymorphisms (SNPs) in genes controlling inflammatory processes and mortality. Data were analyzed from 9,933 individuals who participated in two large community-based cohort studies conducted in Washington County, Maryland, in 1974 and 1989, designated "CLUE I" and "CLUE II," respectively. DNA from blood collected in 1989 was genotyped for 47 SNPs in 23 inflammation-related genes, including interferon- $\gamma$  (*IFN* $\gamma$ ), lymphotoxin- $\alpha$  (*LT* $\alpha$ ), tumor necrosis factor- $\alpha$  (*TNF* $\alpha$ ), C-reactive protein (*CRP*), peroxisome proliferator-activated receptor (*PPAR*), and the human endothelial nitric oxide synthase (*eNOS*). All participants were followed from 1989 to the date of death or to June 20, 2005. The results showed no observable patterns of association for the SNPs and the all-cause and cause-specific mortality outcomes, although statistically significant associations were observed between at least one mortality outcome and SNPs in *eNOS* (reference SNP (rs) 1799983), *PPARG* (rs4684847), *CRP* (rs2794521), *IFN* $\gamma$  (rs2069705), *TNF* $\alpha$  (rs1799964), and *LT* $\alpha$  (rs2229094). Additionally, three of the four examined *CRP* SNPs were strongly associated with *CRP* serum concentration among those with *CRP* measurements. The authors' findings from this community-based prospective cohort study suggest that the selected SNPs are not associated with overall or cause-specific death, although *CRP* genotypes may be associated with systemic inflammation.

cohort studies; C-reactive protein; inflammation; mortality; neoplasms; polymorphism, genetic

Abbreviations: *CRP*, C-reactive protein; *ICD*, *International Classification of Diseases*; *IL*, interleukin; *rs*, reference single nucleotide polymorphism; *SNP*, single nucleotide polymorphism.

As an individual ages, systemic levels of inflammatory proteins and cytokines increase, a phenomenon termed "inflamm-aging" (1, 2). This chronic inflammation appears to be detrimental for longevity and has consistently been found to be associated with mortality and the development of age-related adverse health conditions. For example, in several large published studies, the commonly measured inflammatory biomarkers C-reactive protein (*CRP*), interleukin-6 (*IL*6), and tumor necrosis factor- $\alpha$  have been

shown to be associated with cardiovascular disease (3–7), cancer (8, 9), and arthritis (10). The proposed mechanism for the associations between chronic inflammation and disease is thought to be through an imbalance in the equilibrium between the levels of pro- and antiinflammatory cytokines, such that there is a shift toward a proinflammatory profile at the systemic level (1). The etiology for such a shift is unknown, although it is likely that the altered inflammatory profile is a result of cumulative exposure to

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stressful stimuli, such as antigens or chemical agents, over a lifetime (1).

While a substantial amount of literature exists on the associations between circulating inflammatory protein and cytokine levels and adverse health outcomes, less is known about the role of genetics in human longevity. Single nucleotide polymorphisms (SNPs) have been identified in genes encoding inflammatory proteins and cytokines and, if functional, such as those that have been identified in the *CRP* gene (11–24), these changes may influence the strength and trajectory of the inflammatory response associated with morbidity and mortality. Recent studies have shown that certain genotypes in genes encoding inflammatory factors, such as interleukin-10 (IL10), interleukin-6 (IL6), and transforming growth factor  $\beta$ 1, are more frequent among centenarians compared with the general population (25–28). For example, in analysis of *IL10* -1082G>A genotypes, Lio et al. (26) reported an increased frequency of the GG genotype among Italian male centenarians compared with male controls aged less than 60 years from the same geographic area. These studies support the role of genetic variation on overall health and longevity and indicate the need for additional studies to further the understanding of genetic variation in the aging process.

In 1974 and 1989, two community-based cohorts designated “CLUE I” and “CLUE II” were established in Washington County, Maryland, with associated specimen banks. Buffy coat samples were preserved from the 1989 cohort. Genotyping was performed on individuals who contributed to both the 1974 cohort and the 1989 cohort, as well as on an age-stratified random sample of CLUE II. DNA was subsequently genotyped for 47 SNPs in the following 23 inflammation-related genes: tumor necrosis factor- $\alpha$  (*TNF $\alpha$* ), interleukin-1 $\alpha$  (*IL1 $\alpha$* ), interleukin-1 $\beta$  (*IL1 $\beta$* ), interleukin-2 (*IL2*), interleukin-4 (*IL4*), interleukin-6 (*IL6*), interleukin-8 (*IL8*), interleukin-10 (*IL10*), interleukin-13 (*IL13*), interleukin-18 (*IL18*), cyclooxygenase-1 (*COX1*), cyclooxygenase-2 (*COX2*), myeloperoxidase (*MPO*), endothelial nitric oxide synthase (*eNOS*), interferon- $\gamma$  (*IFN $\gamma$* ), colony-stimulating factor 1 (*CSF1*), colony-stimulating factor 2 (*CSF2*), lymphotoxin- $\alpha$  (*LT $\alpha$* ), *CRP*, inducible nitric oxide synthase (*iNOS*), peroxisome proliferator-activated receptor  $\gamma$  (*PPARG*), peroxisome proliferator-activated receptor  $\gamma$ , coactivator C1 (*PPARGC1*), and peroxisome proliferator-activated receptor  $\delta$  (*PPARD*). Collection of genotype data on this cohort, for which 17 years of follow-up data exist, allowed for the investigation of the associations between SNPs in genes controlling the inflammatory processes and overall and cause-specific mortality.

## MATERIALS AND METHODS

### Study population

In 1974 and 1989, the two cohorts CLUE I and CLUE II, based on the campaign slogan “Give us a Clue to Cancer and Heart Disease,” were established in Washington County, Maryland. At baseline for both CLUE I and CLUE II, informed consent was obtained, a blood sample was collected, and a brief questionnaire was administered. This

questionnaire ascertained data on age, gender, marital status, education, height and weight (CLUE II only), cigarette smoking, and medication and vitamin supplement use within the 48 hours prior to blood donation. In addition, blood pressure was measured and, in 1989, total cholesterol was assayed. Individuals who donated blood to both CLUE I and CLUE II constitute the Odyssey Cohort ( $n = 8,394$ ) (29).

In addition to the Odyssey Cohort, a CLUE II subcohort was developed for case-cohort studies that would be conducted using the CLUE II cohort data. The subcohort was identified by taking a 10 percent age-stratified, random sample of CLUE II participants who donated a blood specimen and were adult residents of Washington County, Maryland. Of the 2,460 participants identified for the subcohort, 807 were also in the Odyssey Cohort.

Of the participants in the Odyssey Cohort and the CLUE II subcohort, DNA was successfully extracted from the buffy coat samples of 9,960 individuals (99 percent). DNA from these participants was genotyped for polymorphisms in genes controlling biologic processes such as inflammation that have been associated with multiple diseases. For the study presented in this paper, Odyssey and subcohort participants who were missing data on all of the chosen SNPs were excluded from the analysis ( $n = 27$ ). This study was approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health.

### Genotyping

SNPs in inflammation-related genes analyzed in the present study were chosen because the minor allele frequency was estimated to be at least 5 percent among Caucasians, and the polymorphisms were either known to be functional or were likely to alter function because they encoded for a nonsynonymous amino acid change or were located within the 5'- or the 3'-untranslated region of the gene and, thus, could potentially alter messenger RNA stability. Descriptions and SNP database (dbSNP) identifiers of the polymorphisms in the inflammation pathways selected are shown in table 1.

DNA extracted from the preserved buffy coat samples collected in 1989 was used for genotyping. Blood samples were centrifuged at  $1,500 \times g$  for 30 minutes at room temperature; subsequently separated into plasma, buffy coat, and red blood cells; and frozen at  $-70^{\circ}\text{C}$  within 24 hours of collection. The buffy coat remained frozen until DNA extraction was performed. The DNA extraction procedures used the alkaline lysis method (30). Following DNA isolation, DNA samples were resuspended in 10 mM Tris-HCl/1 mM ethylenediaminetetraacetic acid, and the DNA concentration was adjusted to 100  $\mu\text{g}/\text{ml}$ . Genotyping was performed by Celera Genomics Co. (Rockville, Maryland) for the SNPs with reference SNP (rs) numbers 1800629, 1800587, 1143634, 16944, 2243250, 1800795, 4073, 1800871, 1800872, 2143416, 2745557, 2206593, 4684847, 709158, and 1175543. Genotyping was performed by Applied Biosystems, Inc. (Foster City, California) for the SNPs with rs numbers 3842787, 5275, 1205, 1800947, 1130864, 2794521, 105885, 1469149, 25882, 2069705, 17561, 2069762, 1800797, 1800890, 1800896, 20541,

**TABLE 1. Description of single nucleotide polymorphisms in inflammation genes investigated in the Odyssey Cohort, Washington County, Maryland, 1989–2005**

Gene	Gene symbol	dbSNP* rs number*	Single nucleotide polymorphism	Amino acid substitution	Genotyping success rate (%)
Tumor necrosis factor- $\alpha$	<i>TNF<math>\alpha</math></i>	1800629	-487A>G		95
Tumor necrosis factor- $\alpha$	<i>TNF<math>\alpha</math></i>	1799724	-1036T>C		97
Tumor necrosis factor- $\alpha$	<i>TNF<math>\alpha</math></i>	1799964	-1210T>C		96
Interleukin-1 $\alpha$	<i>IL1<math>\alpha</math></i>	1800587	Ex1+12C>T		95
Interleukin-1 $\alpha$	<i>IL1<math>\alpha</math></i>	17561	Ex5+21G>T	A114S	95
Interleukin-1 $\beta$	<i>IL1<math>\beta</math></i>	1143634	Ex5+14C>T	F105F	87
Interleukin-1 $\beta$	<i>IL1<math>\beta</math></i>	16944	-1060T>C		88
Interleukin-2	<i>IL2</i>	2069762	-384G>T		94
Interleukin-4	<i>IL4</i>	Unknown			94
Interleukin-6	<i>IL6</i>	1800795	-236C>G		93
Interleukin-6	<i>IL6</i>	1800797	-660A>G		97
Interleukin-8	<i>IL8</i>	4073	-351A>T		94
Interleukin-10	<i>IL10</i>	1800871	-853C>T		93
Interleukin-10	<i>IL10</i>	1800872	-626A>C		93
Interleukin-10	<i>IL10</i>	1800896	-1116A>G		96
Interleukin-10	<i>IL10</i>	1800890	-3584A>T		96
Interleukin-13	<i>IL13</i>	20541	Ex4+98A>G	Q144R	96
Interleukin-13	<i>IL13</i>	1800925	-1069C>T		96
Interleukin-18	<i>IL18</i>	187238	-9211C>G		96
Interleukin-18	<i>IL18</i>	1946518	-9681C>A		96
Cyclooxygenase 1	<i>COX1</i>	3842787	Ex2+43C>T	P17L	95
Cyclooxygenase 2	<i>COX2</i>	2143416	-4342T>G		95
Cyclooxygenase 2	<i>COX2</i>	2745557	IVS1+150T>C		96
Cyclooxygenase 2	<i>COX2</i>	2206593	Ex10-1461T>C		96
Cyclooxygenase 2	<i>COX2</i>	5275	Ex10+837T>C		93
Myeloperoxidase	<i>MPO</i>	2243828	-764T>C		96
Myeloperoxidase	<i>MPO</i>	2333227	-642G>A		92
Endothelial nitric oxide synthase	<i>eNOS</i>	1799983	Ex8-63T>G		93
Interferon- $\gamma$	<i>IFN<math>\gamma</math></i>	2069705	-1615C>T		93
Colony-stimulating factor 1	<i>CSF1</i>	1058885	Ex6-347C>T	L408P	93
Colony-stimulating factor 2	<i>CSF2</i>	1469149	-674C>A		97
Colony-stimulating factor 2	<i>CSF2</i>	25882	Ex4+23T>C	I117T	93
Lymphotoxin- $\alpha$	<i>LT<math>\alpha</math></i>	2229094	Ex2+46T>C	C13R	90
Lymphotoxin- $\alpha$	<i>LT<math>\alpha</math></i>	2229092	Ex3+53A>C	H51P	97
Lymphotoxin- $\alpha$	<i>LT<math>\alpha</math></i>	1041981	Ex3-27C>A	T60N	96
Lymphotoxin- $\alpha$	<i>LT<math>\alpha</math></i>	909253	IVS1+90A>G		96
C-reactive protein	<i>CRP</i>	1205	Ex2-155G>A		95
C-reactive protein	<i>CRP</i>	1800947	Ex2+491G>C	L184L	86
C-reactive protein	<i>CRP</i>	1130864	Ex2+838T<C		94
C-reactive protein	<i>CRP</i>	2794521	-820G>A		94
Inducible nitric oxide synthase	<i>iNOS</i>	2297518	Ex16+14C>T	S608L	96
Peroxisome proliferative activated receptor- $\gamma$	<i>PPARG</i>	4684847	IVS2-6622C>T		95
Peroxisome proliferative activated receptor- $\gamma$	<i>PPARG</i>	709158	IVS9+4523A>G		95
Peroxisome proliferative activated receptor- $\gamma$	<i>PPARG</i>	1175543	IVS9+7780A>G		95
Peroxisome proliferative activated receptor- $\gamma$	<i>PPARG</i>	1801282	Ex4-49C>G	P12A	96
Peroxisome proliferative activated receptor- $\delta$	<i>PPARD</i>	2016520	Ex4+15C>T		96
Peroxisome proliferative activated receptor, $\gamma$ , coactivator C1	<i>PPARGC1</i>	8192678	Ex8-350G>A	G482S	96

\* dbSNP, single nucleotide polymorphism (SNP) database (db) of the National Center for Biotechnology Information; rs number, reference SNP (RefSNP) accession identifier.

1800925, 187238, 1946518, 2229094, 2229092, 1041981, 909253, 2243828, 2333227, 2297518, 1799983, 2016520, 1801282, 8192678, 1799724, and 1799964. Primer and probe sequences are available upon request.

All polymorphisms were genotyped using TaqMan technology (Applied Biosystems, Inc.). Laboratory technicians were masked to disease status. The genotyping success rates of the selected polymorphisms ranged from 86 percent to 97 percent.

### Outcome assessment

All participants were followed from the date of blood draw to the date of death or the end of follow-up (June 20, 2005), whichever came first. In the CLUE cohorts, deaths are identified through daily searches of obituaries, cross-linking with death certificates for Washington County, and searches of the Social Security Administration for individuals aged 65 years or older and the National Death Index. Cause of death is ascertained from the underlying cause on Maryland State death certificates as coded by state nosologists. Of specific interest in this study were cancer deaths, for which the underlying cause was given as *International Classification of Diseases* (ICD), Revision 9 or Revision 10, codes 140–239 or C00–C97, respectively, and cardiovascular disease deaths, for which the underlying cause was given as ICD-9 codes 390–459 or ICD-10 codes I00–I99. During the follow-up period, 2,159 deaths were documented in the Odyssey Cohort and the CLUE II subcohort. Of these, 791 (36.6 percent) were cardiovascular deaths, 574 (26.6 percent) were cancer deaths, and 775 (35.9 percent) were deaths due to other causes. Approximately 4 percent ( $n = 334$ ) of the Odyssey Cohort and the CLUE II subcohort participants were lost to follow-up. Because these individuals were not documented to have died during the follow-up period, they were considered alive at the end of follow-up and censored at June 20, 2005.

### Statistical analysis

The Hardy-Weinberg equilibrium for each SNP was tested by a goodness-of-fit approach. As reported in another publication, most of the SNPs were in Hardy-Weinberg equilibrium, with the exceptions of rs3842787 (*COX1*), rs1800947 (*CRP*), rs1469149 (*CSF2*), rs16944 (*IL1 $\beta$* ), rs2243250 (*IL4*), rs187238 (*IL18*), rs2229094 (*LT $\alpha$* ; reported as rs2857713; rs2857713 was merged into rs2229094), rs2229092 (*LT $\alpha$* ; reported as rs3093543; rs3093543 was merged into rs2229092), and rs2297518 (*iNOS*) (31).

Multiple imputation (32) was used to account for missing data. Five independent, complete data sets were constructed by predicting missing genotypes with decision trees that used all the variables relevant for the model building (other SNPs in linkage disequilibrium, demographic variables/covariates associated with the SNP, and the outcome). If no variables were associated with the SNP, imputation was based on the marginal distribution of the SNP with the missing data. Relative risk estimates

were then obtained by combining estimates and standard errors from analyzing the five complete data sets. Cox proportional hazards regression was used to obtain the relative risk estimates and 95 percent confidence intervals for each SNP and mortality outcomes. For cause-specific death outcomes, all competing deaths were treated as censored observations at the time of death. Genetic variations were coded by genotype, with the reference category being the homozygote of the major allele. Further, the significance of the association between each genotype with respect to each outcome was examined by putting the genotype variable in the model as a single variable and generating an overall  $p$  value. All analyses were age adjusted and stratified by 10-year age groups. Other variables considered for adjustment included gender, body mass index, education (<12, 12, >12 years), cigarette smoking status (never, former, current), systolic blood pressure, and diastolic blood pressure. Because the relative risk estimates did not change after multivariable adjustment or by using the imputation methods, only age-adjusted and stratified estimates using the nonimputed data are presented.

SNP-SNP interactions for all single-locus markers were examined by use of the imputed data by logic regression, an adaptive regression approach based on Boolean combinations of binary variables (33, 34). Each SNP was recorded in two binary variables with a dominant and recessive coding, except for SNPs with low minor allele frequencies, where the heterozygote and the less common homozygote were combined. Using a logistic link with death being the outcome, we examined Boolean combinations of those binary predictors as new possible covariates, adjusting for age simultaneously. Model selection was carried out using permutation tests.

Further, to explore the potential for each SNP to be associated with premature death (all cause or cause specific), we examined Kaplan-Meier curves for the associations between each SNP and the mortality outcomes. There were no indications that any of the three genotype curves for each SNP diverged early in the follow-up time period rather than later; therefore, it was decided that no further analyses for premature death would be considered or displayed in the results.

Additional analyses were conducted to examine the associations between the SNPs in the *IL6* and *CRP* genes and serum concentrations of IL6 and CRP among participants whose levels were measured as a part of other studies using the Odyssey Cohort. Concentrations of these proteins were measured by use of blood collected in 1989. For these analyses, the IL6 and CRP serum concentration data were log transformed because of nonnormal distributions. Age-adjusted associations between the SNPs in these genes and their respective protein levels were assessed with generalized linear models (SAS, version 9.0, software; SAS Institute, Inc., Cary, North Carolina).

All analyses were performed with R, version 2.3.1 (The R Project for Statistical Computing; <http://www.r-project.org/>), unless otherwise specified. The two-sided  $p < 0.05$  was considered statistically significant.

**TABLE 2. Characteristics of study sample at the 1989 blood draw, Odyssey Cohort, Washington County, Maryland, 1989–2005**

Characteristic	
No. (%)	9,933 (99.8)
Mean age in years (standard deviation)	53.1 (15.5)
Female (%)	61.5
Married (%)	72.9
Cigarette smoking status (%)	
Never	53.9
Former	29.6
Current	16.5
Mean education (years)	12
Treatment for hypertension	
Yes (%)	24.2
Mean systolic blood pressure (mmHg)	138.8
Mean diastolic blood pressure (mmHg)	82.7
No (%)	75.8
Mean systolic blood pressure (mmHg)	124.4
Mean diastolic blood pressure (mmHg)	78
Treatment for elevated cholesterol	
Yes (%)	5.3
No (%)	94.7
Mean cholesterol (mg/dl)	208.8
Body mass index (%)	
<25.0 kg/m <sup>2</sup>	42.9
25.0–29.9 kg/m <sup>2</sup>	37.8
≥30.0 kg/m <sup>2</sup>	19.3

## RESULTS

Reflecting the demographics of Washington County, Maryland, 99 percent of the participants were of Caucasian race. In 1989, participants ranged in age from 5 to 95 years; the mean age was 53.1 (standard deviation: 15.5) years (table 2). Approximately 62 percent were female, 16.5 percent were current smokers, and 57.1 percent had a body mass index of greater than 25 kg/m<sup>2</sup>. About a quarter of the participants were taking hypertension medications; 5.3 percent were taking medication for elevated cholesterol.

In general, there were no observable patterns of association for the selected SNPs and the all-cause and cause-specific mortality outcomes, although several of the SNPs were significantly associated with the mortality outcomes (Web table 1). (This information is described in the first of three supplementary tables; each is referred to as “Web table” in the text and is posted on the *Journal's* website (<http://aje.oxfordjournals.org/>.) Specifically, *eNOS* rs1799983 genotype, *IFN $\gamma$*  rs2069705 genotype, *CRP* rs2794521 genotype, and *PPARG* rs4684847 genotype were significantly associated with all-cause mortality. Further, *TNF $\alpha$*  rs1799964, *LT $\alpha$*  rs2229094, and *PPARG* rs4684847 were significantly associated with cancer-related mortality. There were no signifi-

**TABLE 3. Associations between polymorphisms in the *IL6* and *CRP* genes and concentrations of serum protein, Odyssey Cohort, Washington County, Maryland, 1989–2005**

Genotype	rs number*	No.	Age-adjusted geometric mean (pg/ml)	95% CI*	p value
<i>IL6</i>	1800795				0.7
GG		31	2.20	1.72, 2.80	
GC		72	2.42	2.07, 2.84	
CC		23	2.60	1.96, 3.43	
<i>IL6</i>	1800797				0.9
GG		35	2.39	1.89, 3.01	
AG		65	2.37	2.00, 2.81	
AA		27	2.54	1.94, 3.31	
<i>CRP</i>	1205				<0.01
CC		338	2.18	1.95, 2.45	
CT		349	1.54	1.37, 1.72	
TT		103	1.13	0.92, 1.40	
<i>CRP</i>	1800947				<0.01
CC		589	1.84	1.68, 2.01	
CG		89	1.04	0.83, 1.31	
GG		21	2.40	1.51, 3.83	
<i>CRP</i>	1130864				<0.01
CC		398	1.63	1.47, 1.82	
CT		303	1.71	1.51, 1.93	
TT		70	2.63	2.04, 3.40	
<i>CRP</i>	2794521				0.08
TT		408	1.61	1.45, 1.80	
CT		318	1.78	1.57, 2.00	
CC		66	2.21	1.69, 2.89	

\* rs number, reference SNP (RefSNP) accession identifier; CI, confidence interval.

cant associations between the genotypes and cardiovascular-related mortality. When the joint effects of the SNPs were considered, no statistically significant interactions were observed with permutation tests. (The associations between the SNPs and cardiovascular and cancer mortality by specific type of cardiovascular and cancer mortality are displayed in Web tables 2 and 3.)

Three of the four SNPs in the *CRP* gene (rs1205, rs1800947, rs1130864) that were examined were strongly associated with the CRP serum concentration among the subset of participants who had had CRP measured in other studies ( $p < 0.05$ ) (table 3). The association between the remaining *CRP* SNP that was examined (rs2794521) and the CRP serum concentration was marginally significant ( $p = 0.08$ ). Neither *IL6* SNP was associated with *IL6* serum concentration (table 3) or overall and cause-specific mortality.

## DISCUSSION

Using data from a large, community-based cohort in Washington County, Maryland, we found no strong evidence

that the 47 investigated SNPs in the 23 inflammation-related genes were associated with overall or cause-specific mortality. We did, however, find statistically significant or marginally statistically significant associations between the four chosen *CRP* SNPs and CRP serum concentrations; these results are consistent with those of previous investigations (14, 15, 17, 20–22). Despite the apparent functionality of the *CRP* SNPs, the *CRP* SNPs were not, in general, associated with overall or cause-specific mortality in this study, as only weak associations were observed for two of the specific SNPs and cardiovascular death. Some previous studies, but not all, have reported null results for the associations between the investigated *CRP* SNPs and cancer and adverse cardiovascular events that are consistent with ours (14, 15, 22). Most of the existing literature has centered on disease events, such as cancer incidence or myocardial infarction, and not on mortality, as was the focus of this study.

For the remainder of the genes investigated, although some of these genes encode inflammatory markers that have been reported to be associated with the development of disease, no statistically significant associations (or statistically significant weak associations in some cases) were observed between the SNPs and overall or cause-specific mortality. It may be that some of the SNPs examined in this study are not functional, although they were selected on the basis of the high probability that they influence the amount of circulating protein produced during the inflammatory process. For example, the *IL6* SNPs in this study were found not to be associated with overall or cause-specific mortality and, further, were also found not to be associated with the IL6 plasma concentration. In contrast, it may also be that associations with single SNPs were not observed because it takes multiple functional polymorphisms in a single gene or multiple genes, not individually functional polymorphisms, to modify inflammation levels to the point of increasing mortality or disease risk. Joint SNP-SNP associations among the genotypes were examined in this study, but genes in other pathways not investigated could potentially modify these associations. Finally, it is also a possibility that environmental influences and/or other factors affecting post-translational function of the encoded proteins may be the relevant factors influencing health outcomes; these were not examined in this study.

Despite the overall null results between the inflammation-related SNPs and mortality reported in this study, the overall body of published research suggests that the process of inflammation is an important factor in aging, the development of disease, and eventual death. Numerous epidemiologic studies have demonstrated the associations between inflammatory factors and both morbidity and mortality in the general population (35). For example, high concentrations of circulating inflammatory markers have been shown to be predictive of cardiovascular disease (5, 6), Alzheimer's disease (36, 37), certain types of cancer (8, 9), and other diseases that are related to shorter survival (35). At the present time, serologic measures, which result from both genetic and environmental influences, are the best way to assess risk, and they are the best target for interventions aimed at decreasing the risk of disease. However, future research should also explore whole-genome scanning approaches to

find clues for multiple pathway interactions, particularly in terms of the inflammatory process. The apparent importance of inflammation in the disease process points toward the need to identify individuals who are at risk for higher levels of non-age-related inflammation; this would allow for preventive and/or therapeutic measures to be used to ensure successful aging and survival (35).

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## ACKNOWLEDGMENTS

The study was supported by research grant 1U01AG18033 from the National Institute on Aging.

Conflict of interest: none declared.

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