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Inflammation and stress-related candidate genes, plasma interleukin-6 levels, and longevity in older adults

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ABSTRACT

Interleukin-6 (IL-6) is an inflammatory cytokine that influences the development of inflammatory and aging-related disorders and ultimately longevity. In order to study the influence of variants in genes that regulate inflammatory response on IL-6 levels and longevity, we screened a panel of 477 tag SNPs across 87 candidate genes in >5000 older participants from the population-based Cardiovascular Health Study (CHS). Baseline plasma IL-6 concentration was first confirmed as a strong predictor of all-cause mortality. Functional alleles of the *IL6R* and *PARP1* genes were significantly associated with 15%–20% higher baseline IL-6 concentration per copy among CHS European–American (EA) participants (all $p < 10^{-4}$). In a case/control analysis nested within this EA cohort, the minor allele of *PARP1* rs1805415 was nominally associated with decreased longevity ($p = 0.001$), but there was no evidence of association between *IL6R* genotype and longevity. The *PARP1* rs1805415 – longevity association was subsequently replicated in one of two independent case/control studies. In a pooled analysis of all three studies, the "risk" of longevity associated with the minor allele of *PARP1* rs1805415 was 0.79 (95%CI 0.62–1.02; $p = 0.07$). These findings warrant further study of the potential role of *PARP1* genotype in inflammatory and aging-related phenotypes.

1. Introduction

Aging is associated with low-grade elevation of circulating inflammatory markers (Franceschi et al., 2007; Vasto et al., 2007). Interleukin-6 (IL-6) is a multi-functional inflammatory cytokine that plays an important role in the response to environmental stress and has been implicated in the pathogenesis of many chronic diseases associated with aging (Ershler and Keller, 2000). Increased circulating levels of IL-6 have been associated with the subsequent occurrence of coronary heart disease (Danesh et al., 2008) as well as with physical disability, frailty, increased all-cause and disease-specific mortality and decreased longevity in older adults (Harris et al., 1999; Ferrucci et al., 1999; Volpato et al., 2001; Reuben et al., 2002; Roubenoff et al., 2003; Cohen et al 2003; Penninx et al., 2004; Störk et al., 2006; Heikkilä et al., 2007). While increased IL-6 levels may signal the occurrence of underlying pathological mechanisms, IL-6 may also contribute directly to a wide variety of chronic degenerative processes, including atherosclerosis (Huber et al., 1999; Ershler and Keller, 2000).

Circulating IL-6 levels (de Maat et al., 2004; de Craen et al., 2005; Dupuis et al., 2005; Su et al., 2008; Wörns et al., 2006) and life span (vB Hjelmborg et al., 2006) are likely heritable traits in older adults, although specific genetic factors responsible for inter-individual differences in IL-6 levels and human longevity are largely unknown. Determination of specific contributing variants to IL-6 and longevity is difficult in part because of the complexity of inflammatory signaling, and because populations studied to date were not recruited for the study of longevity or inflammatory phenotypes. A common promoter variant of the *IL6* gene, −174 G/C (rs1800795) was found to be weakly associated with plasma IL-6 levels and CRP levels in older adults, but was not associated with

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mortality (Walston et al., 2007). In a recent meta-analysis of eight European case-control studies, there was no significant overall difference in IL-6 genotype frequencies between long-lived and controls, though the −174 GG genotype was less common in a subgroup of Italian male centenarians (Di Bona et al., 2009). Given the evidence for genetic influence on IL-6 levels, we hypothesized that polymorphisms in additional inflammation or stress-response genes may account for inter-individual variation in plasma IL-6 concentration and may also contribute to human longevity. In order to test this hypothesis, we designed a candidate gene study that utilized a large cohort study of older adults and two additional nested case-control samples of older adults for validation as described below.

2. Materials and methods

2.1. Human subjects, data collection by population

2.1.1. The Cardiovascular Health Study (CHS)

In order to test our hypothesis in the larger candidate gene panel, we utilized stored DNA and previously measured and collected data from CHS. CHS is a prospective, population-based cohort study of 5888 adult men and women 65 y and older recruited from four field centers: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania (Fried et al., 1991). Baseline examination for the original cohort, of whom 4925 or 95% self-identified their ethnicity as white, was performed over 1 y beginning in May 1989. A second CHS cohort of African–American (AA) participants ($n = 687$) was recruited between 1992 and 1993. All procedures were conducted under institutionally approved protocols for use of human subjects.

Medication and lifestyle histories, physical examinations and phlebotomy samples were obtained at the baseline exam. Phlebotomy and blood processing methods were described previously (Cushman et al., 1995). Baseline IL-6 was measured from stored plasma samples in 4517 self-identified EA and 829 self-identified AA using a highly sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; RD Systems, Minneapolis, MN). The analytical coefficient of variation was 6.3% (Jenny et al., 2002). Of the participants who had baseline IL-6 levels measured, 4190 EA and 766 AA consented to genetic testing and had DNA aliquots available for genotyping.

Complete follow-up data for the CHS cohort were available through June 2005. Fatal events due to all-causes were defined and adjudicated by physician review, according to medical records and death certificates. The National Death Index provided complete mortality follow-up. Cause of death was adjudicated by using published criteria: cardiovascular disease (CVD) death was defined as death due to atherosclerotic coronary heart disease, cerebrovascular disease, atherosclerotic disease, or other CVD (Ives et al., 1995). For our candidate gene – longevity phenotype association analysis, we defined longed-lived cases as subjects who lived to at least 92 y of age ($n = 479$; 61% female) and shorter-lived "controls" as subjects who died before the age 79 y ($n = 900$; 46% female).

2.2. Centenarian case-control study of Ashkenazi Jews

In order to validate significant results identified in the CHS population, we utilized previously collected DNA and phenotypic data from a study of three hundred and eighty eight Ashkenazi Jewish cases with exceptional longevity [289 females and 99 males, average age 97.7 (0.2) years (mean SE), age range 95–108 y; 20% over the age of 100] and Ashkenazi Jewish control subjects [$n = 364$, mean age 79.5 (0.4) years (mean SE), 58% female] without a family history of unusual longevity as previously described (Barzilai et al 2003; Atzmon et al., 2006; Suh et al. 2008; Atzmon et al., 2008). Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

2.3. Study of Osteoporotic Fractures (SOF)

The SOF cohort served as an additional validation population for this study. The original SOF cohort comprised $n = 9704$ women of EA descent age 65 and older recruited between September 1986 through October 1988 from 4 U.S. sites: Baltimore, Maryland; Monongahela Valley, Pennsylvania; Minneapolis, Minnesota; and Portland, Oregon (Cummings et al., 1995). The study protocol was approved by the institutional review committee and written informed consent was obtained from all participants. The cohort has been followed for over 20 y with complete mortality follow-up and included 896 SOF participants selected for inclusion in a longevity case-control study (293 long-lived cases $\geqslant$92 y, mean = 95.3 ± 2.1 y and 603 shorter-lived controls age at death $\leqslant$79 y, mean = 75.7 ± 2.6 y).

2.4. Gene and SNP selection

Our primary hypotheses were that one or more common SNPs in genes from pathways related to inflammation and other stress-responses related to aging were associated with (a) circulating IL-6 levels; (b) longevity case-control status. Candidate genes were chosen based on their known influence on acute and chronic inflammation and involvement in components of IL-6 regulated innate immunity, including pathogen recognition, cytokine signaling, NFκB activation, and the generation of acute phase reactants. The complete list of genes is shown in Supplementary Table 1. Within each candidate gene, "tag" SNPs were selected to capture common linkage disequilibrium patterns using the SeattleSNPs candidate gene SNP discovery resource (http://pga.mbt.washington.edu/) and/or the HapMap data base (http://www.hapmap.org). A comprehensive assessment of *IL6* and *CRP* gene polymorphisms, IL-6 levels, and mortality risk in CHS has been reported previously (Walston et al., 2007; Hindorff et al., 2008); therefore we did not include *IL6* or *CRP* tagSNPs in the current candidate gene screening analysis. TagSNPs were selected separately in EA and AA using the pair-wise LD binning procedure implemented in the LDSelect algorithm of Carlson et al (2004), such that all known common variants [defined as minor allele frequency (MAF) $\geqslant$5%] are correlated with at least one tagSNP with $r^2 > 0.8$. For some LD bins, multiple tagSNPs were typed in case of assay failure. These redundant tags ($r^2 > 0.8$) were removed prior to analysis.

TagSNP genotyping was performed for the majority of our candidate genes by the Center for Inherited Disease Research (CIDR) using the Illumina GoldenGate 1536 SNP platform. Overall genotype missing rate was <0.1%; genotyping failure rate did not exceed 4.2% for any of the SNPs under study. Concordance rates for blind duplicate samples was >99.5% for all SNPs assays across both genotyping platforms. We excluded SNPs from analysis with minor allele frequency less than 5%. Using this procedure, we identified 477 and 727 tagSNPs bins among EAs and AAs, respectively, across 87 candidate genes. Of the total number of tagSNPs analyzed ($n = 791$), 413 or 52% were common to both EA and AA, while 64 and 314 were unique to EA and AA, respectively.

For the Ashkenazi Jewish validation study, genotyping was performed at the Albert Einstein College of Medicine using Sequenom MASSarray® System (Sequenom Inc., San Diego, CA) according to the manufacturer's instructions. SOF validation genotyping was performed using the SNPstream 48plex genotyping platform

(Beckman Coulter, Fullerton CA) according to manufacturer's instructions.

2.5. Statistical analysis

To assess the association of baseline plasma IL-6 concentration with mortality during follow-up, we used Cox proportional hazards to model quartiles of IL-6 level. Results were reported as hazard ratios with 95% confidence intervals (95%CI). Hazard ratios were adjusted for baseline age, sex, race, body mass index, total cholesterol, diabetes, systolic blood pressure, smoking, race, clinic site, and clinical CVD at baseline.

We performed our primary analysis of association between each SNP and logarithmically transformed IL-6 concentration levels using multiple linear regression that included covariate terms for age, sex, clinic, body mass index, and smoking. Analyses were performed separately in CHS EA and AA participants. SNP genotype was coded 0/1/2 for the number of copies of the minor allele assuming an additive genetic model. Logistic regression was used to assess associations between each SNP genotype and longevity case/control status. Cox regression was used to model associations between SNP genotype and total mortality or cause-specific mortality (cardiovascular or non-cardiovascular). Covariates for the initial case-control longevity analysis included sex, and recruitment site. The Cox models were also adjusted for baseline age. We also performed more fully adjusted multivariable models that included baseline hypertension, diabetes, smoking status, clinical CVD, and diagnosis of cancer. Clinical CVD, used for adjusting multivariable models, was defined as self-reported myocardial infarction, stroke, angina, transient ischemic attack, claudication, or re-vascularization procedure.

In the CHS AA, genotype data were also available for a genome-wide set of 24 unlinked ancestry-informative SNP markers, which were used to adjust for European admixture and population stratification in AAs (Reiner et al., 2005). To examine the potential effects of within-Europe population stratification in CHS, we repeated our analysis for any candidate gene SNP associated with IL-6 or longevity following correction for population stratification. Specifically, we computed a Bayesian model-averaged population structure estimate from 1038 autosomal SNPs typed in CHS (excluding SNPs from pairs with $r^2 > 0.8$), and then assigned individuals to six genetically similar clusters (Corander and Tang, 2007). Genetic background cluster was then used as an adjustment variable in regression models assessing the association between candidate gene SNP and phenotype. A subsequent generalized analysis of molecular variance showed that these clusters explained a significant proportion (6.6%, $p < 0.001$) of the genetic background among CHS European–Americans (Nievergelt et al., 2007).

To assess the overall statistical significance and correct for multiple SNP comparisons, we used the false discovery rate (FDR) to control the type I error (Storey and Tibshirani, 2003). The FDR procedure provides an estimate of significance in terms of the q-value, the proportion of significant features expected to be false among the total # of tested SNPs. Results were considered statistically significant if $q < 0.05$. SNPs found to be nominally associated with longevity in CHS ($p < 0.02$) were genotyped in SOF and the Ashkenazi Jewish centenarian case-control studies.

Summary effect estimates for longevity status were calculated under an additive genetic model using a meta-analysis approach with inverse-variance weighting. Meta-analysis was performed across all three studies using either a fixed-effects or random-effects model (DerSimonian and Laird, 1986) with the STATA software package v.8.2 (Stata Corporation, College Station, Texas). The random-effects model allows for heterogeneity of results between studies.

3. Results

3.1. Baseline IL-6 levels predict all-cause mortality in CHS

CHS participant baseline characteristics by race are summarized in Table 1. In univariate and multivariable-adjusted regression analyses, older age, male sex, current smoking, higher BMI, alcohol consumption, and prevalent CVD were significantly associated with higher IL-6 (all $p < 0.001$). When analyzed by quartiles, and adjusted for other risk factors, baseline IL-6 levels were strongly and independently associated with a linear increase in all-cause mortality, as well as mortality due to cardiovascular and non-cardiovascular causes (Table 2).

3.2. Candidate gene SNP associations with IL-6 phenotype in CHS

Using a false discovery rate threshold of 5%, three tagSNPs within the *IL6R* gene on chromosome 1q21.3 [rs4537545 (q-value $< 10^{-15}$), rs4553185 (q-value $< 10^{-8}$), and rs4072391 (q-value $< 10^{-3}$)] and one tag SNP within the *PARP1* gene on 1q42.12 (rs1805415; q-value < 0.01) were significantly associated with baseline IL-6 concentration in EA (Table 3). *IL6R* encodes a subunit of the IL-6 receptor complex, while *PARP1* encodes the DNA repair enzyme poly-ADP ribose polymerase. While both genes are located on the short arm of chromosome 1, there was no evidence of linkage disequilibrium between *IL6R* and *PARP1*.

The three *IL6R* tagSNPs most strongly associated with IL-6 phenotype were moderately correlated with one another in CHS EA (pair-wise r^2 between rs4537545 and rs4553185 = 0.44; rs4537545 and rs4072391 = 0.15; rs4553185 and rs4072391 = 0.26). When IL-6 phenotype was regressed simultaneously on these three *IL6R* tagSNPs, only rs4537545 remained statistically significant; each additional copy of the rs4537545 minor allele was associated with a 20% increase in mean log IL-6 levels

Table 1
Baseline characteristics of European–American and African–American CHS participants.

Characteristic	European–Americans	African–Americans
Number	4190	766
Mean age, years (range)	73 (65–98)	73 (65–92)
Female sex	1064 (57)	202 (62)
Current smokers	206 (11)	50 (15)
Body mass index (kg/m^2)	26.3 ± 4.5	28.4 ± 5.3
Total cholesterol (mg/dL)	212 ± 40	210 ± 37
HDL cholesterol (mg/dL)	54 ± 16	59 ± 17
Triglycerides (mg/dL)	141 ± 75	116 ± 69
Systolic blood pressure (mm Hg)	136 ± 22	142 ± 23
Diagnosis of hypertension	1020 (55)	239 (73)
Diabetes	277 (15)	74 (23)
Prevalent CVD[a]	315 (17)	52 (16)
IL-6 (pg/mL)	240 ± 541	374 ± 830

Data are presented as number (%) or mean ± standard deviation, unless otherwise indicated.
[a] Self-reported myocardial infarction, stroke, angina, transient ischemic attack, claudication, or re-vascularization procedure.

Table 2
Baseline IL-6 concentration and subsequent risk of mortality in CHS.

IL-6 level	All-cause Hazard ratio (95% CI)	CVD Hazard ratio (95% CI)	Non-CVD Hazard ratio (95% CI)
<1.2 pg/mL	1	1	1
1.2–1.7 pg/mL	1.34 (1.17–1.52)	1.61 (1.29–2.01)	1.19 (1.01–1.41)
1.8–2.6 pg/mL	1.71 (1.49–1.94)	2.07 (1.67–2.58)	1.52 (1.29–1.75)
⩾2.6 pg/mL	2.34 (2.05–2.65)	2.65 (2.14–3.30)	2.17 (1.85–2.56)
P for trend	5×10^{-43}	1×10^{-20}	6×10^{-24}

Adjusted for age, race, sex, CVD, cancer, smoking, hypertension, diabetes, and BMI.

Table 3
SNPs associated with plasma IL-6 concentration in CHS European–Americans.

SNP rs#	Gene	Chr	Position (bp)	Location	Minor allele frequency	Correlated SNP	r^2 with reference SNP	Regression coefficient ± standard error[a]	*p*-Value
rs4537545	IL6R	1	152685503	Intron	0.41	Tag SNP	–	0.114 ± 0.013	3×10^{-18}
rs7518199	IL6R	1	152674043	Intron	0.41	Typed SNP	0.88	0.101 ± 0.013	3×10^{-18}
rs8192284	IL6R	1	152693594	Ala358Asp	0.35	HapMap SNP	0.96	–	–
rs4553185	IL6R	1	152677579	Intron	0.43	Tag SNP	–	−0.082 ± 0.013	3×10^{-10}
rs4845618	IL6R	1	152666639	Intron	0.43	Typed SNP	0.96	−0.078 ± 0.013	3×10^{-10}
rs4072391	IL6R	1	152705504	3′ UTR	0.19	Tag SNP		−0.076 ± 0.017	5×10^{-6}
rs2229238	IL6R	1	152704520	3′ UTR	0.19	Typed SNP	0.98	−0.079 ± 0.017	5×10^{-6}
rs1805415	PARP1	1	224637463	Lys352Lys	0.15	Tag SNP	–	0.095 ± 0.025	1×10^{-4}
rs752307	PARP1	1	224618152	Intron	0.16	Typed SNP	0.997	0.067 ± 0.018	2×10^{-4}
rs1136410	PARP1	1	224621925	Val762Ala	0.17	HapMap SNP	1.00	–	–

Adjusted for age, sex, clinic, BMI, and smoking.
[a] Regression coefficient equals the estimated change in log(IL-6) level per each additional copy of the minor allele.

(p-value < 10^{-15}). Notably, *IL6R* rs4537545 tags another IL6R SNP rs8192284, which encodes a functional Ala358Asp polymorphism (Table 3). The minor allele of *PARP1* rs1805415 (which encodes a synonymous coding SNP) was associated with ~18% increase in mean log IL-6 levels. *PARP1* rs1805415 is in strong linkage disequilibrium with rs1136410, which encodes a non-synonymous Val762Ala amino acid substitution. Overall, *IL6R* genotype explained 2% of the inter-individual variation in IL-6 phenotype among EA, while PARP1 genotype explained 0.2%. By comparison, age, sex, clinic, BMI, and smoking explained 12% of the IL-6 phenotypic variance.

In EA, tagSNPs within several genes encoding components of the nuclear factor kappa B (NFκB) transcription factor complex (*IKBKE*, *IKBKB*, and *RELA*) or the IL-6 signal transducer and activator of transcription-3 gene (*STAT3*) showed weaker evidence of association with IL-6 phenotype (Supplemental Table 2). Among the smaller CHS African–American cohort, an intronic SNP within the Toll-like receptor 4 gene (*TLR4*) had a borderline association with higher IL-6 levels (q-value = 0.07). Several additional tagSNPs of *IL6R*, *STAT3*, or *IL6ST* (which encodes the IL-6 signal transducer component of the IL-6 receptor complex) showed weaker evidence of association with IL-6 phenotype among AAs (Supplemental Table 3).

3.3. Candidate gene SNP associations with longevity in CHS

At an experiment-wide significance threshold (FDR < 0.05), none of the candidate gene SNPs were significantly associated with longevity in either CHS EA or AA (Supplemental Tables 2 and 3). Nonetheless, it is notable that the tagSNP with the strongest evidence of association with longevity in EA was rs1805415, the *PARP1* variant associated with higher IL-6 concentration. The odds ratio for each additional copy of the rs1805415 minor allele was 0.66 (95%CI 0.55–0.81; $p = 0.001$). In a time-to-event analysis among the entire CHS EA cohort, each additional copy of the *PARP1* rs1805415 variant allele was associated with a 1.11-fold increased risk in all-cause mortality (95%CI 1.02–1.18; $p = 0.01$). Adjustment for major risk factors at the baseline examination (age, sex, hypertension, diabetes, cigarette smoking, diagnosis of cancer or clinical CVD) did not alter these results (hazard ratio = 1.12; 95%CI 1.03–1.22; $p = 0.006$). On the other hand, adjustment for baseline plasma IL-6 levels reduced the relative risk of death associated with the rs1805415 variant allele to 1.05 (95%CI 0.98–1.14; $p = 0.18$). There was no evidence of association between longevity or all-cause mortality and any of the *IL6R* tag SNPs associated with IL-6 levels.

3.4. Replication of PARP-1 longevity result

PARP1 rs1805415 showed an association with decreased risk of longevity in the Ashkenazi Jewish longevity cases and controls (odds ratio = 0.75; 95%CI 0.56–0.99; $p = 0.04$). In contrast, there was no association between *PARP1* rs1805415 among SOF longevity cases and controls (odds ratio = 1.02; 95%CI 0.77–1.36; $p = 0.88$). There was no evidence that the association between *PARP1* rs1805415 and longevity differed by gender in either CHS or the Ashkenazi Jews (data not shown). In the pooled analysis of all three studies, the risk of longevity associated with the minor allele of *PARP1* rs1805415 was 0.79 (95%CI 0.67–0.92; $p = 0.003$) under a fixed-effect model. Under a random-effect model, the pooled odds ratio was 0.79 (95%CI 0.62–1.02; $p = 0.07$), with an estimate of between-study variance of 0.03. Seven other SNPs nominally associated with longevity in CHS ($p < 0.02$) showed little evidence of association with longevity in either SOF or the Ashkenazi Jewish centenarian study (Supplemental Table 4).

4. Discusssion

Our results confirm that higher circulating IL-6 levels strongly predict future mortality in a large community-based sample of older EA and AA adults from CHS. By screening a panel of inflammation and stress-response candidate genes, we demonstrate that SNPs tagging common functional polymorphisms of *IL6R* and *PARP1* are associated with circulating IL-6 levels among European–American CHS participants. Though no SNPs contained within our candidate gene panel reached the experiment-wide significance threshold of association with longevity in our screening analysis, it is noteworthy that the minor allele of *PARP1* rs1805415, which was significantly associated with higher baseline IL-6 levels (an important longevity endo-phenotype), was nominally associated with decreased longevity in CHS EA. In contrast, there was no evidence of association between *IL6R* genotype and longevity in CHS EA. The *PARP1* rs1805415 variant showed a similar pattern of association with longevity in one of two independent replication longevity case-control samples.

PARP-1 is a nuclear enzyme that catalyzes the post-translational poly(ADP-ribosyl)ation of target proteins in response to DNA damage and appears to have diverse roles in various aging-related process such as DNA repair, transcription, chromatin remodeling, mitosis, telomere maintenance, apoptosis, and inflammation (Beneke and Bürkle, 2007). Based on HapMap data, *PARP1* rs1805415 is in strong linkage disequilibrium ($r^2 > 0.9$) with an extended haplotype of least 13 other common *PARP1* SNPs among Europeans; these include rs1136410, which encodes a non-synonymous Val762Ala amino acid substitution. The Val762Ala polymorphism is located within the catalytic domain of PARP-1 and has been associated with reduced enzymatic activity and increased cancer susceptibility among Europeans and Asians (Lockett et al., 2004; Wang et al., 2007).

PARP activity in peripheral blood mononuclear cells has been correlated with maximal life span in mammalian species (Grube

and Bürkle, 1992), and also reported to be higher in immortalized lymphocytes from centenarians than from shorter-lived controls (Muiras et al., 1998). PARP-1 also activates various pro-inflammatory pathways by interacting with NFκB, a key regulator of innate immunity (Csiszar et al., 2008). *PARP1* genotype was recently reported to be associated with plasma C-reactive protein, another well-characterized inflammation and stress-responsive marker associated with mortality risk in older adults (Reiner et al., 2008). The same *PARP1* variant associated with higher IL-6 levels was associated with higher CRP levels in CHS. Therefore, *PARP1* genotype may have pleiotropic effects on various inflammatory and other aging-related traits, thereby representing an important link between inflammatory responses to environmental or cellular stress, aging, and mortality in older adults.

The observed *IL6R* genotype – IL6 phenotype association in CHS confirms recent findings from several other population-based studies of middle-aged and older adults, including the Women's Health Study (WHS) (Qi et al., 2007), the InCHIANTI Italian cohort (Rafiq et al., 2007), and the Health ABC cohort (Reich et al., 2007). Based on the HapMap data base, the *IL6R* tagSNP rs4537545 showing the strongest association with plasma IL-6 levels in CHS is in linkage disequilibrium ($r^2 > 0.8$) with at least seven other common *IL6R* SNPs among Europeans; these include rs8192284, which encodes a functional Ala358Asp non-synonymous amino acid substitution located at the site where the IL-6 receptor is cleaved to form soluble IL-6 receptor (sIL-6R) (Galicia et al., 2004; Reich et al., 2007). The *IL6R* rs8192284 Ala358Asp polymorphism has been associated with higher sIL-6R levels in Health ABC and InCHIANTI, explaining a substantial part of sIL-6R phenotypic variance (Reich et al., 2007; Rafiq et al., 2007).

Despite the strong evidence for association with IL-6 and sIL-6R phenotypes, there was no evidence for association between *IL6R* genotype and longevity in CHS, nor with other systemic markers of inflammation in InCHIANTI (Rafiq et al., 2007). In fact, the *IL6R* rs8192284 variant allele was recently reported in a genome-wide association study from WHS (Ridker et al., 2008) to be associated with *lower* CRP levels, which is in the opposite direction to its reported effect on IL-6 and sIL-6R levels. Unlike other soluble receptors, sIL-6R is generally thought to act as an agonist of IL-6, thus allowing gp130-mediated IL-6 signaling to occur on cells without a membrane-bound IL-6R (so-called 'trans-signaling') (Jones et al., 2001; Knüpfer and Preiss, 2008). However, the biology of IL-6 signaling is complex, and can have both pro- and anti-inflammatory effects (Gabay, 2006; Knüpfer and Preiss, 2008). These observations suggest a different mechanism of action of PARP-1 versus soluble IL-6 receptor gene variants on the expression of distinct inflammatory- and aging-related phenotypes. Therefore, the association of *PARP1* genotype with *higher* plasma levels of both CRP and IL-6 (two important inflammation/stress-response proteins, each of which predict mortality), as well as the probable influence of PARP-1 as a more global regulator of transcription and other genes related to aging, may account for the apparent association between *PARP1* genotype and the more complex clinical outcome of longevity.

Strengths of the current study include use of a relatively large cohort of older adults with extensive baseline and follow-up data, the examination of an important quantitative intermediate phenotype (IL-6) as well as the more complex outcome of longevity, the use of tagSNPs to cover common linkage disequilibrium patterns of across a large set of inflammation and stress-related genes, and the use of independent samples for validation of candidate genotype – longevity association findings. Several potential limitations should also be noted. The smaller size of the CHS AA cohort as well as the lack of any suitable replication sample limited our statistical power and ability to detect candidate gene associations among AA. As with any indirect association study using common tagSNPs, we cannot distinguish which SNP is the true functional variant(s), nor exclude the possible contribution of rare variants within our candidate genes. Due to multiple testing issues and the observed heterogeneity in *PARP1* – longevity association results between studies, additional validation of in other samples of older adults and analysis of other aging-related phenotypes in older adults is warranted. Given that PARP-1 inhibitors are currently under investigation for the treatment of various chronic inflammatory conditions (de la Lastra et al., 2007), *PARP1* genotype may ultimately prove to have potential clinical or pharmacogenomic utility for a wide range of inflammatory and aging-related disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.exger.2009.02.004.

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