**Plasma Levels of Soluble Interleukin-2 Receptor α**

**Associations With Clinical Cardiovascular Events and Genome-Wide Association Scan**

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**Objective**—Interleukin (IL)-2 receptor subunit α regulates lymphocyte activation, which plays an important role in atherosclerosis. Associations between soluble IL-2Rα (sIL-2Rα) and cardiovascular disease (CVD) have not been widely studied and little is known about the genetic determinants of sIL-2Rα levels.

**Approach and Results**—We measured baseline levels of sIL-2Rα in 4408 European American (EA) and 766 African American (AA) adults from the Cardiovascular Health Study (CHS) and examined associations with baseline CVD risk factors, subclinical CVD, and incident CVD events. We also performed a genome-wide association study for sIL-2Rα in CHS (2964 EAs and 683 AAs) and further combined CHS EA results with those from two other EA cohorts in a meta-analysis (n=4464 EAs). In age, sex- and race- adjusted models, sIL-2Rα was positively associated with current smoking, type 2 diabetes mellitus, hypertension, insulin, waist circumference, C-reactive protein, IL-6, fibrinogen, internal carotid wall thickness, all-cause mortality, CVD mortality, and incident CVD, stroke, and heart failure. When adjusted for baseline CVD risk factors and subclinical CVD, associations with all-cause mortality, CVD mortality, and heart failure remained significant in both EAs and AAs. In the EA genome-wide association study analysis, we observed 52 single-nucleotide polymorphisms in the chromosome 10p15-14 region, which contains IL2RA, IL15RA, and RMB17, that reached genome-wide significance ($P=5\times10^{-8}$). The most significant single-nucleotide polymorphism was rs7911500 ($P=1.31\times10^{-75}$). The EA meta-analysis results were highly consistent with CHS-only results. No single-nucleotide polymorphisms reached statistical significance in the AAs.

**Conclusions**—These results support a role for sIL-2Rα in atherosclerosis and provide evidence for multiple-associated single-nucleotide polymorphisms at chromosome 10p15-14. (*Arterioscler Thromb Vasc Biol. 2015;35:2246-2253. DOI: 10.1161/ATVBHA.115.305289.*)

**Key Words:** atherosclerosis | genome-wide association study | inflammation | interleukin-2 receptor subunit α | heart failure

Interleukin (IL)-2 and IL-2 receptor (IL-2R) signaling play an important role in regulating both tolerance and immunity. IL-2 is a T-cell growth factor, inducing the proliferation and differentiation of antigen-activated T cells, and is particularly important in the development of regulatory T cells in the thymus. The IL-2R is a trimeric receptor composed of the IL-2Rα subunit (CD25), the IL-2Rβ subunit (CD122), and the IL-2γc subunit (CD132). IL-2Rα is specific for IL-2R, whereas IL-2Rβ and IL-2γc are shared components of other cytokine receptors (eg, IL-15). Soluble IL-2 receptor α (sIL-2Rα) results from the proteolytic cleavage of IL-2Rα at the cell surface by a membrane metalloprotease (ectodomain shedding); which is encoded by IL2RA on human chromosome 10. The function of sIL-2Rα has not been fully elucidated. Although the sIL-2Rα has IL-2-binding kinetics similar to the membrane form, sIL-2Rα may serve to mitigate the immune responses by binding and sequestering IL-2.

High plasma levels of sIL-2Rα have been associated with autoimmune diseases, including Crohn disease, rheumatoid arthritis, and type 2 diabetes mellitus.
arthritis,\textsuperscript{9} and multiple sclerosis\textsuperscript{10} and higher levels have been observed in patients with coronary artery disease.\textsuperscript{11} Murine models have shown that IL-2 increases regulatory T-cell numbers in atherosclerotic plaques and also reduces the size of those plaques.\textsuperscript{12} When the IL-2R is blocked in the same model, the plaque reduction is negated.

Despite its potential importance in the immune system and cardiovascular disease (CVD), sIL-2R\textalpha has not been widely investigated in large prospective population-based studies of CVD. A 2003 study in the Health, Aging, and Body Composition (Health ABC) study did not result in evidence for a significant association between sIL-2R\textalpha and CVD; however, sIL-2R\textalpha measurements were only available in a subset of n=499 participants. In addition, little is known about the genetic determinants for sIL-2R\textalpha levels. Although genome-wide association studies (GWASs) have identified single-nucleotide polymorphisms (SNPs) in the IL-2RA gene for several autoimmune diseases,\textsuperscript{10} there have been no published reports for GWAS of serum levels of sIL-2R\textalpha.

In this study, we examined sIL-2R\textalpha levels in the Cardiovascular Health Study (CHS), a cohort of older adults with follow-up for incident clinical CVD and mortality for ≤20 years. We examined the relationships between sIL-2R\textalpha at baseline and incident events as well as cross-sectionally with other CVD and inflammatory markers (fibrinogen, C-reactive protein [CRP], and IL-6). We then conducted a GWAS and region-specific conditional analyses to identify

### Table 1. Associations Between sIL-2R\textalpha and Other Cardiovascular Risk Factors and Atherosclerosis at the CHS Baseline Examination

<table>
<thead>
<tr>
<th>Baseline Characteristics (Mean±SD or %)</th>
<th>Model A, β±SE</th>
<th>Model B, β±SE</th>
<th>Model C, β±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (5.6)</td>
<td>0.074±0.005***</td>
<td>0.078±0.006***</td>
<td>0.067±0.006***</td>
</tr>
<tr>
<td>Female sex (57.2%)</td>
<td>−0.029±0.011*</td>
<td>−0.031±0.011*</td>
<td>0.017±0.012</td>
</tr>
<tr>
<td>Black race (14.8%)</td>
<td>−0.120±0.007***</td>
<td>−0.133±0.008***</td>
<td>−0.134±0.008***</td>
</tr>
<tr>
<td>Current smoking (54.0%)</td>
<td>0.100±0.164***</td>
<td>0.108±0.017***</td>
<td>0.079±0.017***</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus (16.2%)</td>
<td>0.056±0.014**</td>
<td>0.042±0.015**</td>
<td>−0.002±0.015</td>
</tr>
<tr>
<td>Hypertension (44.5%)</td>
<td>0.042±0.006***</td>
<td>0.046±0.007***</td>
<td>0.030±0.007***</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg (136.6±21.8)</td>
<td>0.013±0.005</td>
<td>−0.011±0.026</td>
<td>0.003±0.007</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL (129.8±35.6)</td>
<td>−0.030±0.005***</td>
<td>−0.030±0.005***</td>
<td>−0.032±0.007***</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL (54.2±15.7)</td>
<td>−0.055±0.006***</td>
<td>−0.050±0.006***</td>
<td>−0.046±0.006***</td>
</tr>
<tr>
<td>Triglycerides, mg/dL (139.8±76.7)</td>
<td>0.007±0.005</td>
<td>−0.002±0.006</td>
<td>−0.025±0.008*</td>
</tr>
<tr>
<td>Glucose, mg/dL (111.1±35.9)</td>
<td>0.013±0.005</td>
<td>−0.009±0.008</td>
<td>−0.013±0.007</td>
</tr>
<tr>
<td>Insulin, IU/mL (17.4±27.4)</td>
<td>0.023±0.005***</td>
<td>0.017±0.005*</td>
<td>0.011±0.005</td>
</tr>
<tr>
<td>BMI, kg/m² (26.6±4.7)</td>
<td>0.009±0.006</td>
<td>0.005±0.006</td>
<td>−0.016±0.006*</td>
</tr>
<tr>
<td>Waist circumference, cm (94.4±13.1)</td>
<td>0.014±0.005*</td>
<td>0.016±0.010</td>
<td>0.003±0.010</td>
</tr>
<tr>
<td>C-reactive protein, mg/L (4.8±8.0)</td>
<td>0.083±0.005***</td>
<td>0.080±0.005***</td>
<td>0.043±0.008***</td>
</tr>
<tr>
<td>IL-6, pg/mL (2.2±1.8)</td>
<td>0.059±0.005***</td>
<td>0.051±0.005***</td>
<td>0.024±0.006***</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL (323.8±67.3)</td>
<td>0.067±0.005***</td>
<td>0.063±0.054***</td>
<td>0.032±0.006***</td>
</tr>
<tr>
<td>Internal carotid wall thickness, mm (1.5±0.7)</td>
<td>0.027±0.006***</td>
<td>0.014±0.006</td>
<td>0.009±0.006</td>
</tr>
</tbody>
</table>

Each variable was examined for association with sIL-2R\textalpha in a separate model, adjusting for the variables listed in each model; the exception is that a variable is not adjusted for itself when it is being tested. β for all measures except sex, race, diabetes mellitus, and hypertension are for a 1-SD change in the predictor. Model A: adjusted for age, race, and sex. Model B: adjusted for age, race, sex, smoking, diabetes mellitus, hypertension, systolic blood pressure, and BMI. Model C: adjusted for age, race, sex, smoking, diabetes mellitus, hypertension, systolic blood pressure, BMI, LDL cholesterol, HDL cholesterol, C-reactive protein, IL-6, and fibrinogen. BMI indicates body mass index; CHS, Cardiovascular Health Study; IL, interleukin; LDL, low-density lipoprotein; and HDL, high-density lipoprotein. sIL-2R\textalpha ln-transformed P values: *P<0.01, **P<0.001, ***P<0.0001.
genetic variants associated with sIL-2Rα levels. Finally, we performed a GWAS meta-analysis, including results from two additional studies: the Health ABC study and the Multiethnic Study of Atherosclerosis (MESA), to increase our power to detect associated variants not detected in CHS alone.

Materials and Methods
Materials and Methods are available in the online-only Data Supplement.

Results
Associations Between sIL-2Rα and Baseline CVD Risk Factors and Other Inflammation Biomarkers
The characteristics of the 5174 CHS participants with sIL-2Rα measurements at the baseline examination are summarized in Table 1, and Spearman correlation coefficients for sIL-2Rα with each continuous CVD risk factor and intima media thickness are given in Table I in the online-only Data Supplement. sIL-2Rα levels were on average higher in older individuals, higher in men, and higher in European Americans (EAs). At baseline, mean sIL-2Rα levels were 1146.4 pg/mL (SD=507.5 pg/mL) and 1101.6 pg/mL (SD=556.4 pg/mL) in EA men and women, respectively; and 873.1 pg/mL (SD=505.5 pg/mL) and 910.9 pg/mL (SD=581.2 pg/mL) in African American (AA) men and women, respectively; and 873.1 pg/mL (SD=505.5 pg/mL) and 910.9 pg/mL (SD=581.2 pg/mL) in African American (AA) men and women, respectively. In age-, race- and sex-adjusted models, sIL-2Rα was additionally associated with current smoking, type 2 diabetes mellitus, hypertension, fasting insulin, waist circumference, CRP, IL-6, fibrinogen, and internal carotid wall intima media thickness and negatively associated with low-density lipoprotein and high-density lipoprotein cholesterol. After further adjustment, sIL-2Rα levels remained associated with age, race, smoking, hypertension, lipids, and inflammation.

Table 2. Hazard Ratios Between sIL-2Rα and Incident Events in CHS

<table>
<thead>
<tr>
<th>Model</th>
<th>European Americans</th>
<th>African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All-Cause Mortality (n=2985 events)</td>
<td>Cardiovascular Mortality (n=1202 events)</td>
</tr>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Minimal† (1)</td>
<td>1.17 (1.14–1.19)***</td>
<td>1.16 (1.11–1.20)***</td>
</tr>
<tr>
<td>Multivariable† (2)</td>
<td>1.16 (1.13–1.19)***</td>
<td>1.15 (1.10–1.20)***</td>
</tr>
<tr>
<td>Subclinical† (3)</td>
<td>1.14 (1.11–1.18)***</td>
<td>1.13 (1.07–1.19)***</td>
</tr>
<tr>
<td>2nd Q vs 1st Q (3)</td>
<td>1.17 (1.04–1.32)*</td>
<td>1.19 (0.98–1.45)</td>
</tr>
<tr>
<td>3rd Q vs 1st Q (3)</td>
<td>1.25 (1.11–1.41)**</td>
<td>1.38 (1.10–1.62)**</td>
</tr>
<tr>
<td>4th Q vs 1st Q (3)</td>
<td>1.63 (1.45–1.83)***</td>
<td>1.64 (1.36–1.99)***</td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age, sex, and study site; Model 2: model 1+smoking, diabetes mellitus, hypertension, systolic blood pressure, low-density lipoprotein; Model 3: model 2+CRP, IL-6, fibrinogen, and carotid intima-media thickness. CI indicates confidence interval; CHS, Cardiovascular Health Study; HR, hazard ratio; and IL, interleukin. *P<0.05, **P<0.005, ***P<0.0001.†HRs for a 1-SD unit increase in sIL-2R. †HRs comparing quartiles to first quartile of sIL-2R.
seems to be driven by the highest quartile of sIL-2Rα when compared with a more graded effect in EAs. We estimated a 63% (EA)/67% (AA) increased risk for all-cause mortality, and a 57%/71% increased risk of heart failure for individuals in the fourth quartile versus those in the first quartile, after adjustment for both established CVD risk factors, inflammation biomarkers, and subclinical measures of CVD. For cardiovascular mortality, this estimated increased risk was 64% in EAs and not significant in AAs; and 28% in EAs and 130% in AAs for stroke.

GWAS of sIL-2Rα in CHS EA and AA

We conducted a race-stratified GWAS in 2964 EAs and 683 AAs from CHS that had both sIL-2Rα measurement and GWAS data available. A total of 52 SNPs in the chromosome 10p15-p14 region (containing IL2RA, IL15RA, and RBM17) reached genome-wide significance ($P<5\times10^{-8}$) in the EA analysis. The most significant SNP was rs7911500 ($P=1.31\times10^{-75}$), which is located between IL2RA and IL15RA. No other regions reached genome-wide significance in the EA analysis. No SNPs reached genome-wide significance in the AA analysis. The top findings in AAs were for an intergenic SNP between BRE and FOSL2 on chromosome 2 (rs7602568, $P=5.8\times10^{-8}$) and an intronic SNP in ADK (rs12220238, $P=8.3\times10^{-8}$), nearly 70 Mb from IL2RA on chromosome 10. IL2RA SNP rs7911500 ($P=0.52$) demonstrated no evidence for association in AAs, although the minor allele frequency for this variant in AAs was only 2.5% (compared with 13.4% in EAs). Several chromosome 10p15-p14 SNPs between IL15RA and

![Figure 1](http://ahajournals.org)
**IL2RA (lead SNP rs8177607, P=3.2×10^{-4}) provided nominal evidence for an association in AAs. rs8177607 showed no evidence for association in EAs (P=0.65).**

**Conditional and Multiple Variant Analysis of IL2RA Region in CHS EA**
In the CHS EAs, as described in Methods, we performed an iterative, forward-selection conditional analysis of the chromosome 10p14-15 region (≈200 Kb span), beginning with conditioning on the rs7911500 SNP (Figure 1). The order of additional SNP conditioning was rs7911590 (pcond=7.0×10^{-35}, an intronic SNP in IL2RA), rs8177757 (pcond=2.3×10^{-10}, located between IL15RA and IL2RA), rs10905716 (pcond=3.3×10^{-9}, located between IL2RA and RBM17), and finally rs7924005 (pcond=4.4×10^{-10}, located in LOC101928080 downstream from RBM17). There was still nominal evidence for further association of SNPs in the region after adjusting for these five, although none reached genome-wide significance. The multiple variant penalized regression method LLARRMA identified six SNPs (resample model inclusion probability (RMIP) >0.8; namely, rs2104286 [RMIP =1.00], rs7924005 [RMIP=0.995], rs10905716 [RMIP=0.995], rs4749955 [RMIP=0.911], rs11256497 [RMIP=0.899], and rs7898980 [RMIP=0.871]) that were consistently associated with sIL-2Rα levels across alternative resamplings of the data. Our top SNP in our initial GWAS, rs7911500 (RMIP=0.002) was not predicted to be important in the multi-SNP LLARRMA model. However, LLARRMA did include the top variant, rs7911590 (RMIP=0.592), from the conditional analysis after conditioning on rs7911500, more often than not in the final multi-SNP model across different resamplings of the data. The five index SNPs identified in the conditional analysis, in total explain ≈14% of the variation in sIL-2Rα levels after adjusting for age, sex, and principle components to account for population admixture. When we further examined these five SNPs individually for association with incident cardiovascular events in CHS, none of them was significant. We also observed no evidence for an association between a genetic risk score (equal to the sum of the alleles individually associated with an inflammatory phenotype based on the CRP-IL-6 pattern, were statistically significant with the sIL-2Rα levels in this candidate variant analysis after Bonferroni correction for 1093 test (P<4.6×10^{-3}). In addition, we searched the CARDIoGRAM+C4D database containing data from multiple GWAS (63746 case and 130681 controls) combined to determine variants associated with coronary heart disease and myocardial infarction (http://www.cardiogramplusc4d.org14–16). No significant associations (all P>0.05) between our SNPs and CVD were identified.

**Discussion**
We report the first large-scale assessment of sIL-2Rα for association with CVD-related traits and events in a prospective cohort and the first GWAS for SNPs associated with sIL-2Rα levels. The major findings from this study are (1) sIL-2Rα levels are associated with many established CVD risk factors and carotid intima media thickness, a measure of subclinical CVD, (2) plasma sIL-2Rα predicted all-cause mortality and cardiovascular mortality independently of CVD risk factors and baseline subclinical CVD, (3) in CHS alone (n=2961), we identified 52 SNPs in the chromosome 10p15-p14 region with genomewide significance for association with plasma sIL-2Rα levels; most significant was rs7911500, intergenic to IL15RA and IL2RA; (4) conditional analysis indicated that there are multiple SNPs independently associated in this region; the five most significant loci, in total explain ≈14% of the variation in plasma sIL-2Rα levels in CHS EAs, (5) combining results from EAs in CHS and two additional cohort studies, MESA and Health ABC (n=4464), did not result in any additional significantly associated loci, (6) we did not identify any significant associations in the CHS AAs, although we did observe nominal evidence for association in the IL15RA/IL2RA region, and (7) there was no evidence that sIL-2Rα-associated SNPs were associated with incident clinical events in CHS; we also observed no evidence of association with coronary heart disease and myocardial infarction in a search of the CARDIoGRAM+C4D database results for these SNPs.14–16

Activated T lymphocytes play an important role in atherosclerosis promoting chemokine secretion, inflammation, and eventually, the formation of atherosclerotic plaques. IL-2, produced by T-helper 1 cells, has been found in plaques and contributes to the development of atherosclerosis by its interaction with the IL-2 receptor increasing lymphocyte activation.17 IL-2 stimulates the synthesis of interferon-γ, thereby promoting an increased immune response and atherosclerotic progression. However, IL-2 also promotes regulatory T cells, and may have an atheroprotective role as well.12

Although sIL-2Rα is a strong biological candidate for use as a biomarker for CVD morbidity and mortality, epidemiological studies have been limited. Analysis in the Health ABC study did not identify evidence for an association between sIL-2Rα and either subclinical (P=0.27) or clinical CVD (P=0.27), but measured sIL-2Rα levels were only available on a subset of 499 of the 3045 participants with incident event data. Although it was not statistically significant, median sIL-2Rα level was slightly higher in those with incident clinical CVD when compared with those with no CVD (1.4 µg/mL
versus 1.2 µg/mL. Investigators from another study of 286 Japanese patients that underwent angiography (167 coronary artery disease cases and 119 controls) reported a significant positive association of sIL-2Rα and cross-sectional coronary artery disease case status based on extreme quartiles of sIL-2Rα (P=0.005 for minimally adjusted model and P=0.035 for model with additional adjustment for CVD risk factors). This study represents the first well-powered effort examining sIL-2Rα level prospectively with clinical CVD events and all-cause mortality. We observed statistically significant evidence for all incident events examined (all-cause mortality, CVD mortality, incident coronary heart disease, stroke, and heart failure) in minimally adjusted models, and for all-cause mortality, CVD mortality, and incident heart failure in fully adjusted models. We found sIL-2Rα levels to be significantly associated with carotid intima media thickness in the minimally adjusted model; although this did not remain significant when other cardiovascular risk factors were added to the model.

Fifty-two chromosome 10p15-p14 SNPs were significantly associated (P<5x10−6) with plasma sIL-2Rα levels in CHS EAs; no other regions reached genome-wide significance. The most significant SNP, rs7911500, was located between IL15RA and IL2RA. Iterative conditional analyses identified a total of five significant independent SNPs across the region. LLARRMA identified six SNPs that were consistently associated with sIL-2Rα levels across alternative resamplings of the data. Both iterative conditional analyses and LLARRMA provide compelling evidence for the existence of multiple important causal variants in the region, although they did not agree with respect to the importance of our most significant SNP, rs7911500. Higher density genotype data, including both common haplotype-tagging variants and less-common putative functional variants, will be necessary to fine map the association signals in this region. Two of our significant SNPs in the region, rs2104286 (P=4.9×10−59; the top SNP identified by LLARRMA) and rs11594656 (P=1.5×10−44), have been shown to function in transcription factor binding.

These SNPs have also been reported to be associated with sIL-2Rα levels and type 1 diabetes mellitus and multiple sclerosis. No regions reached genome-wide significance in the smaller cohort of CHS AAs. Nominal evidence for association in AAs was detected between IL15RA and IL2RA (best result: rs8177607, P=3.2×10−6). The lead SNP in EAs, rs7911500, was less polymorphic in AAs and demonstrated no evidence for association. Similarly, no evidence for association was found for rs791590 (P=0.31) or rs10905716 (P=0.43), two significant variants in EAs in the conditional analyses. The two other significant SNPs in the conditional analyses, rs8177757 and rs7924005, were not successfully imputed in the AAs. The difference in findings between EAs and AAs could suggest different risk variants in the two populations, be reflective of different linkage disequilibrium structures in the region that mask common underlying causal variants, or be the result of lower power in AAs. There are strong allele frequency differences between the two populations for many of the EA SNPs in the region (see Table II in the online-only Data Supplement for frequencies in HapMap CEU and YRI populations) and the AA sample size is considerably smaller than for EAs.

Interestingly, the top SNP from LLARRMA, rs2104286, in EAs was nominally significant in AAs (P=0.011), despite the lower estimated frequency of the minor allele in AAs (minor allele frequency=0.065) compared with EAs (minor allele frequency=0.27). The effect estimates for the SNP were similar in AAs (β=−0.17) and EAs (β=−0.15), where carriers of the minor allele were predicted to have lower sIL-2Rα levels. Elevated sIL-2Rα levels have been shown to be associated with several autoimmune diseases and may predict a relapse of those diseases. We found many IL2RA SNPs previously associated with autoimmune-related diseases to be significantly associated with sIL-2Rα levels. Many significant SNPs have also been observed to be associated in GWAS, fine mapping studies and SNP-specific genotyping studies for autoimmune diseases including Graves’ disease (rs11594656, odds ratio (OR), 1.54; P=0.0053), vitiligo (rs706779, OR, 1.27; P=3×10−6), Crohn disease (rs12722489, OR, 1.11; P=3×10−6), type 1 diabetes mellitus (rs7090530, OR, 1.23; P=0.003), and multiple sclerosis (rs2104286, OR, 0.81; P=0.017).

Our two most significant SNPs, rs7911500 and rs12722605, were found to be significantly associated with an inflammatory
phenotype derived from the high-sensitivity CRP-IL-6 pattern in a GWAS of the Genetics of Lipid Lowering Drugs and Diet Network ($P=5 \times 10^{-8}$ and $P=5 \times 10^{-10}$). The nature of this association is uncertain; it is possible that these variants or others in linkage disequilibrium with them are directly increasing the sIL-2Rα levels, which, in turn, results in downstream production.

There are several limitations in this study, which should be noted. We only analyzed common variants; rare polymorphisms may account for much of the variability in the sIL-2Rα levels. Also, we had weak statistical power to detect associations in AAs. Finally, our study was focused on older adults and the results may not be generalizable to other populations.

Our findings suggest that serum sIL-2Rα, a surrogate marker of T-lymphocyte activation, may be a valuable novel biomarker for all-cause mortality, cardiovascular morality, stroke, and heart failure in older adults. Additional studies are needed to assess whether sIL-2Rα levels predict mortality in younger populations. Also, further studies are needed (1) to identify the causal variants in the chromosomal region harboring IL15RA and IL2RA influencing sIL-2Rα, (2) to provide large and multiethnic samples to identify additional genetic loci for this trait, and (3) to determine the complex biology of the genetic control of IL-2/IL-2R interactions with respect to regulatory T-cell promotion and proinflammatory cytokine production.

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Disclosures

None.

References


**Significance**

This study found that soluble IL-2 receptor α, a regulator of white blood cells, is associated with many cardiovascular disease risk factors, as well as with all-cause mortality, cardiovascular disease mortality, and heart failure in the Cardiovascular Health Study. Analysis of genetic variants in European Americans found several variants in the chromosome 10 region containing the genes IL2RA, IL15RA, and RMB17 to be significantly associated with soluble IL-2 receptor α. These results provide support for a role of soluble IL-2 receptor α in atherosclerosis and cardiovascular disease.
Plasma Levels of Soluble Interleukin-2 Receptor α: Associations With Clinical Cardiovascular Events and Genome-Wide Association Scan

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METHODS

Study samples
The Cardiovascular Health Study (CHS) is a prospective population-based cohort study of men and women recruited at age 65 or older at baseline. The original cohort of 5201 participants was recruited between 1988 and 1989 at four field centers: Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA. Between 1992 and 1993, an additional 687 mostly African-American (AA) participants were recruited for a total cohort of 5888. The baseline examination for CHS participants included a medical history, demographic and lifestyle history, physical exam, fasting blood collection and an assessment of vascular disease by carotid ultrasound and ankle-brachial index.

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study comprised of 6814 European American (EA), African-American, Hispanic, and Asian participants between the ages of 45 and 84 recruited at six sites from 2000 to 2002. The six study sites were: Wake Forest University, University of Minnesota, Northwestern University, University of California at Los Angeles, Columbia University, and Johns Hopkins University. At baseline, participants had no clinical CVD or atrial fibrillation. Baseline examinations included medical, demographic and lifestyle history, measurement of coronary calcium, ventricular mass, carotid intimal-medial wall thickness, ankle and brachial blood pressures, and standard CVD risk factors. Fasting blood was also collected. Our study used data from 699 EA participants and 647 AA participants.

The Health, Aging and Body Composition Study (Health ABC) is a cohort study of 3075 participants age 70 – 79 residing in Memphis, TN or Pittsburgh, PA who were enrolled between 1997 and 1998. Participants were interviewed for medical and social history. The baseline clinical exam included a general physical, tests of physical performance and body composition as well as a blood collection. This analysis used data from 786 EA participants and 561 AA participants.

Biomarker and Genotype Measurement
sIL-2Rα was measured in plasma by ELISA (R&D Systems) with a detectable range of 312 – 20,000 pg/mL. The coefficients of variation in the current study ranged from 5.11% to 7.59%.

A total of 3388 EA and 607 AA CHS samples were genotyped using the Illumina 370CNV platform. In ancestry specific quality control (QC) analyses, SNPs were excluded from consideration if any of the following applied: 1) minor allele frequency < 0.005, 2) missing rate across subjects > 5%, or 3) Hardy-Weinberg equilibrium p-value < 1.0x10^-5. Genotype imputation was performed to expand the coverage of common variants in our GWAS to SNPs that were not included on the genotype panel or that were included but were lost during QC. Ancestry-specific imputation was performed using the software package MaCH [1,2]. Genotype data for 314,364 SNPs in EAs and 311,324 SNPs in AAs, after QC SNP removal, were used to impute 2.2 million SNPs from HapMap Phase 2 and HapMap Phase 3 reference samples. For EAs, HapMap CEU (Phases 2 and 3) and TSI (Phase 3) reference samples were included. For AAs, CEU (Phases 2 and 3), YRI (Phases 2 and 3), TSI (Phase 3), LWK (Phase 3), ASW (Phase 3) reference samples were used. Finally, sets of unrelated subjects for analyses (n=3232 EA and n=594 AA) were identified by iteratively removing one subject at a time from subject-pairings with a global identity-by-descent (IBD) estimate > 0.10 until no subject pairs had a global IBD estimated greater than that threshold. IBD estimation was performed using a linkage-disequilibrium-pruned set of SNPs that had similar frequencies in EAs and AAs (to minimize confounding of IBD with background ancestry similarity). QC analyses and IBD estimation were performed using the software PLINK [3].

MESA participants were genotyped using the Affymetrix Human SNP array 6.0 (Affymetrix Inc. Santa Clara, CA). Ancestry-specific imputation was performed using IMPUTE v2 [4] using HapMap Phase 2 CEU reference samples for the European American (EA) participants.

In the Health ABC study, genotyping was performed using the Illumina Human1M-DuoBeadChip system. Imputation in the EAs was performed using Mach version 1.0.16 using HapMap Phase 2 CEU reference samples.

Statistical Analysis
To satisfy model assumptions, sIL-2Rα was natural log-transformed for association analyses with CVD risk factors and genetic variants. Associations between sIL-2Rα and quantitative traits (systolic blood pressure [SBP], LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose, fasting insulin, BMI, waist circumference, CRP, IL-6, fibrinogen, and carotid intima media thickness [IMT]) and binary traits (diabetes mellitus and hypertension) were analyzed using multiple linear regression and logistic regression, respectively. Hypertension was defined as current use of antihypertensive medication or SBP>140 and DBP>90.
Cox proportional hazards models were used to test for association between sIL-2Rα and the risk of incident coronary heart disease (CHD), incident stroke, congestive heart failure (CHF), CVD mortality and all-cause mortality, separately for EAs and AAs. All events were adjudicated by an expert review panel. Incident CHD included non-procedure-related fatal or nonfatal MI. CVD mortality included fatal events where death was adjudicated as due to atherosclerotic CHD or cerebrovascular disease, including definite fatal MI, definite fatal stroke and definite or probable fatal CHD. Participants with adjudicated baseline prevalent disease for the corresponding incident disease were excluded from analysis (e.g. individuals with a history of myocardial infarction at first visit were excluded from incident CHD analysis). Three progressive levels of covariate adjustments were used to assess risk of incident events associated with sIL-2Rα levels. The first model was minimally adjusted for the potential confounders baseline age, sex and study site. The second model was additionally adjusted for CVD risk factors (baseline measures of current smoking status, type 2 diabetes, hypertension, systolic blood pressure (SBP), and low density lipoprotein (LDL) cholesterol) and baseline CVD (for the mortality outcomes). The third model added adjustments for baseline measures of inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen), and carotid IMT.

For the genetic analyses of CHS data, the associations between sIL-2Rα and individual genotyped and imputed SNPs, scored as dosage values (expected number of copies of the minor alleles), were tested in linear regression models implemented in Mach2qtl. Covariates in the regression models included age, sex, study site, and the first two principal components (PCs), used to control for potential population substructure. PCs were calculated using the program EIGENSOFT. The statistical significance threshold used for defining significance was set to 5x10^{-8}.

Targeted conditional analysis was performed in regions where multiple SNPs achieved statistical significance to ascertain how many sIL-2Rα-associated SNPs provided independent evidence for association in the region of interest. The conditional analysis was performed by iteratively adding the most significant genotyped or imputed SNP in with other model covariates and re-assessing the region for any SNP meeting genome-wide significance using forward-stepwise linear regression. A series of regional association plots showing results after each successive model iteration were constructed using the software LocusZoom. Given the rigidity of the forward step wise conditional analysis approach with respect to order of SNP inclusion, we additionally applied the LASSO local automatic regularization resample model averaging (LLARRMA) method to assess the number of important SNPs across the region. Both the conditional analyses and the LLARRMA analyses were restricted to CHS EA HapMap Phase 2 imputed data.

Estimation of sIL-2Rα phenotypic variance explained by individual SNPs was performed using the REG procedure with PCORR2 option in SAS. Cox proportional hazards models were used to assess whether SNPs associated with sIL-2Rα were also associated with incident events, both before and after adjusting for sIL-2Rα level. The significance threshold for these analyses was set at p=0.05 and analyses were performed using STATA statistical software.

Tests of association between imputed SNP dosage and sIL-2Rα were performed using SNPTEST version 2.4.1 in MESA and Mach2qtl in Health ABC. We used fixed effects inverse-variance weighted meta-analysis implemented in Meta to combine results from CHS, MESA and Health ABC EAs.
REFERENCES

3. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. Plink: A tool set for whole-genome association and population-based linkage analyses. American journal of human genetics. 2007;81:559-575.
**Supplemental Table I**: Spearman correlation coefficients for sIL2sR and continuous CVD risk factors and subclinical CVD.

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Spearman correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.19***</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.037*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.084***</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.16***</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.042*</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.038*</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.080***</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.038**</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.018</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.17***</td>
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<tr>
<td>Interleukin-6</td>
<td>0.24***</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.14***</td>
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<tr>
<td>Internal carotid wall thickness</td>
<td>0.13***</td>
</tr>
</tbody>
</table>

*P<0.01; **P<0.001; ***P<0.0001
**Supplemental Table II.** Minor allele frequencies for 5 CHS independently associated SNPs, using HapMap data.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>CEU</th>
<th>YRI</th>
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<tr>
<td>rs7911500</td>
<td>T</td>
<td>0.14</td>
<td>0</td>
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<tr>
<td>rs791590</td>
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<td>0.10</td>
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<tr>
<td>rs8177757</td>
<td>T</td>
<td>0.04</td>
<td>0</td>
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<tr>
<td>rs10905716</td>
<td>T</td>
<td>0.24</td>
<td>0.31</td>
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<tr>
<td>rs7924005</td>
<td>C</td>
<td>0.19</td>
<td>0.18*</td>
</tr>
</tbody>
</table>

*Not available in Hapmap, based on 1000 Genomes YRI data.*