Interleukin-18 (IL-18) is a proinflammatory cytokine involved in the processes of innate and acquired immunities and associated with cardiovascular disease and type 2 diabetes. We sought to identify the common genetic variants associated with IL-18 levels.

Methods and Results—We performed a 2-stage genome-wide association study among women of European ancestry from the Nurses’ Health Study (NHS) and Women’s Genome Health Study (WGHS). IL-18 levels were measured by ELISA. In the discovery stage (NHS, n=1523), 7 single-nucleotide polymorphisms (SNPs) at the IL18-BCO2 locus were associated with IL-18 concentrations at the \(1 \times 10^{-7}\) significance level. The strongest association was found for SNP rs2115763 in the BCO2 gene (\(P=6.31 \times 10^{-8}\)). In silico replication in WGHS (435 women) confirmed these findings. The combined analysis of the 2 studies indicated that SNPs rs2115763, rs1834481, and rs7106524 reached a genome-wide significance level (\(P<5 \times 10^{-8}\)). Forward selection analysis indicated that SNPs rs2115763 and rs1834481 were independently associated with IL-18 levels (\(P=0.0002\) and 0.0006, respectively). The 2 SNPs together explained 2.9% of variation of plasma IL-18 levels.

Conclusion—This study identified several novel variants at the IL18-BCO2 locus associated with IL-18 levels. (Arterioscler Thromb Vasc Biol. 2010;30:885-890.)

Key Words: GWA study ■ IL-18 ■ IL18 gene

Interleukin-18 (IL-18) is a proinflammatory cytokine involved in the processes of innate and acquired immunities.\(^1\)\(^-\)\(^4\) Originally identified as an interferon-\(\gamma\)-inducing factor in Kupffer cells and macrophages,\(^4\) IL-18 induces production of interferon-\(\gamma\) in T lymphocytes and natural killer cells.\(^5\) In addition, IL-18 can combine with IL-12 to promote the T helper 1 responses.\(^1\)\(^-\)\(^3\)\(^-\)\(^6\) Epidemiology studies have indicated that plasma concentrations of IL-18 are associated with diverse conditions, including type 2 diabetes,\(^7\)\(^-\)\(^9\) cardiovascular disease,\(^10\)\(^-\)\(^11\) and the severity of coronary atherosclerosis.\(^12\) Animal studies have also found that IL-18 can promote atherosclerosis.\(^13\)\(^-\)\(^14\) In ApoE knockout mice, IL18 gene knockout reduces atherosclerosis.\(^15\) Despite the important role of IL-18, the loci affecting IL-18 concentrations have not been well established.

To comprehensively identify the common genetic variants associated with IL-18 levels, we carried out a genome-wide association (GWA) study in 1523 women from the Nurses’ Health Study (NHS). A total of 704,409 single-nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) \(\geq 0.02\), genotype call rate \(\geq 0.98\), and Hardy-Weinberg equilibrium \(P \geq 1 \times 10^{-8}\) were analyzed. Furthermore, we conducted an in silico replication in the Women’s Genome Health Study (WGHS).

Methods

Study Population

The NHS began in 1976 with the recruitment of 121,700 female registered nurses (aged 30 to 55 years). Between 1989 and 1990, a total of 32,826 women provided blood samples. Medical history, lifestyle information, and disease diagnoses were updated every 2 years using validated questionnaires.\(^16\) Participants in the current study were a subset of those who had both IL-18 measurements and genome-wide scan data. Those who were unrelated, genetically identified women of European ancestry with high-quality genotyping data were included. After further excluding participants with missing phenotype and covariate data, 1523 participants were included in the final analysis (679 type 2 diabetes cases and 844 diabetes-free
controls). The NHS study has been approved by the institutional review board of the Brigham and Women’s Hospital in Boston.

**Replication Sample**

The participants included in our replication study were currently enrolled in the WGHS. A total of 435 women with genotype data and plasma IL-18 measurement were included. The participants in WGHS are from the Women’s Health Study with no prior history of major chronic illness including cardiovascular disease, diabetes, and cancer. The WGHS study has been approved by the institutional review board of the Brigham and Women’s Hospital.

**Measurements of IL-18 and Other Inflammatory Biomarkers**

In NHS, blood sample collection (between 1989 and 1990) and processing were previously reported. To decrease systematic bias and interassay variation, study samples were measured in randomly ordered case-control pairs. Plasma IL-18 levels were measured using an enzyme-linked immunosorbent assay (Diaclone, Besançon, France). The sensitivity of this assay is 0.9 pg/mL. The average intraassay coefficient of variation for IL-18 was 7.3%. The methods for measuring other biomarkers are described in detail elsewhere. Briefly, the coefficients of variation were 3.8% for plasma C-reactive protein, 3.3% to 4.8% for soluble intercellular cell adhesion molecule-1, 5.7% to 8.8% for soluble E-selectin, 5.9% for IL-6, 2.6% to 4.8% for tumor necrosis factor-α receptor 2, and 8.5% to 9.8% for soluble vascular cell adhesion molecule-1. IL-18 measurement in WGHS is described in detail elsewhere.

**Genotyping and Quality Control**

In NHS, DNA was extracted from white blood cells using the QIAamp blood kit (Qiagen, Chatsworth, CA) and prepared following the manufacturer’s instructions. Genome-wide genotyping was performed using the Affymetrix Genome-Wide Human 6.0 array. Genotype calls were made using the Birdseed calling algorithm (version 2). All samples used in the present study achieved a call rate of ≥98%. Individual SNPs were excluded if their MAF was <0.02 (n=141 930), missing call rate was ≥2% (n=17 889), or Hardy-Weinberg equilibrium test probability value was <1×10⁻⁸ (n=3312). Finally, 704 409 SNPs were available for analysis. In WGHS, genotyping and quality control have been described in detail elsewhere. Briefly, samples were genotyped using Infinium II technology from Illumina (HumanHap300 Duo-Plus chip). Genotype calls were made using Beadstudio v3.3 (Illumina). Those samples with >2% missing genotypes were removed first. SNPs with a call rate of <90%, Hardy-Weinberg equilibrium test probability value <1×10⁻⁶, or MAF ≤0.01 were also excluded. The quality control on genotyping left a total of 336 108 SNPs for final analysis.

**Population Stratification**

In the NHS, principal component analysis was used to investigate population structure. We used a set of 12 021 SNPs in whites with very low levels of linkage disequilibrium (LD) and MAF greater than 0.05 and 3 369 subjects passing quality control and having 209 HapMap II founders (59 CEU, 60 Yoruba [YRI], 45 Japanese [JPT], and 45 Chinese [CHB]) for the analysis (Supplemental Figure I, available online at http://atvb.ahajournals.org). Subjects with first and second principal components defined by the means of the first and second principal components among self-described whites ±3 SD were classified as having primarily European ancestry. Population stratification in WGHS has been described in detail elsewhere.

**Statistical Analysis**

The associations between each SNP and log-transformed plasma IL-18 levels were examined using linear regression models, adjusting for age, body mass index (BMI), and diabetes status. An additive model was assumed for each SNP. To control for potential confounding by population stratification, we further adjusted for the top 3 principal components of genetic variation, and the results were similar. A probability value cut-off of 5×10⁻⁸ was used as a genome-wide significance level in this study to correct for the roughly 1 000 000 independent statistical tests thought to correspond to all the common genetic variation of the human genome. We used the hidden Markov model implemented in Mach 1.0 software to impute SNPs that are not covered in Affymetrix 6.0 chip. The associations of IL-18 levels with genotyped data were tested by linear regression in PLINK. Imputed data (expressed as allele dosage) were analyzed by using linear regression models with ProbABEL software. We used predicted (undetyped) SNPs on the basis of genotyped SNPs and haplotype structure in the reference samples (HapMap CEU). The imputed genotypes are not actually observed genotypes, and the ambiguity of their prediction has to be included in the interpretation of associations with them. In this regard, we used the posterior genotype probability (allele dosage) in the analyses to reflect the ambiguity of the genotypes. The LD structure was inferred on the basis of HapMap CEU Release 22 data.

To further evaluate the independence of genetic associations, we conducted a forward selection linear multiple regression analysis. We forced age, BMI, and diabetes status into the linear regression model. The selection criterion was P<0.05 for a SNP to enter the final model. The SNPs selected were further analyzed for haplotype associations. We used the standard E-M algorithm to impute haplotypes and analyze the association with haplotypes by using the multimarker haplotype tests implemented in the PLINK software. We used fixed effect metaanalysis to combine evidence for association from the discovery (NHS) and replication (WGHS) samples.

**Results**

At the discovery stage, 1523 women from NHS were included in the final analysis. All subjects have European ancestry as defined by the principal component analysis of genetic variations. The basic characteristics of the participants are presented in Supplemental Table I. The mean age was 56 years, and the mean IL-18 concentration was 320.1 pg/mL (SD=158.8).

The quantile-quantile plot indicates that only a minor number of SNPs deviated from the expected distribution (beyond the 95% confidence interval), and there was no evidence of systematic bias (A=0.991; Supplemental Figure II). The distribution of probability values for the association of SNPs with IL-18 levels, according to chromosome, is presented in Figure 1. In the initial GWA scan, 7 SNPs were associated with IL-18 concentrations at the 1×10⁻⁵ significance level (Table 1). All top SNPs clustered within a ~100-kb region on chromosome 11q22–11q23, which harbors 3 genes, IL18, TEX12, and BCO2 (GeneIDs 3606, 56158, and 83875, respectively; Figure 2). The strongest association was found for SNP rs2115763 in intron 2 of the BCO2 gene (P=6.31×10⁻⁸). This SNP was highly prevalent in our sample, with an MAF of 33%, slightly lower than the 41.5% in the HapMap CEU samples. Another SNP, rs1834481, located in intron 3 of the IL18 gene, was associated with IL-18 levels, with a P value of 8.38×10⁻⁷. SNPs rs2115763 and rs1834481 were weakly correlated (r²=0.19).

Among the remaining top SNPs, 2 SNPs, rs12420140 and rs4935984, are located in introns of the BCO2 gene; another 3 SNPs, rs7106524, rs10891329, and rs5744280, are located in the IL18 gene (Table 1). The LD of the top SNPs is presented in Supplemental Table II.

To assess whether any other, stronger genetic markers of IL-18 levels exist in the IL18 or another region, we expanded our genome scan by imputing 130 020 SNPs spanning chromosome 11. Three additional SNPs in IL18 or BCO2 genes...
were found with probability values of less than $5 \times 10^{-7}$; another 14 top SNPs were also mapped to the \textit{IL18} or \textit{BCO2} gene ($P = 1.22 \sim 6.44 \times 10^{-6}$; Supplemental Table III).

We also replicated the association of the 6 top SNPs with IL-18 levels in the WGHS, except for rs10891329, which was not available in WGHS. Among these 6 SNPs, 2 SNPs of rs7344280 and rs1834481 were experimentally genotyped with call rates 99.99% and 99.97% respectively. The remaining 4 SNPs were imputed with 100% completion and high quality scores. Each SNP was in Hardy-Weinberg equilibrium ($P > 0.05$) and was significantly associated with IL-18 levels after adjustment for age, BMI, and diabetes status ($P < 0.05$). In the combined analysis of NHS and WGHS, the strongest association of SNP rs2115763 with IL-18 concentration had a probability value of 3.88$	imes 10^{-9}$. Two other SNPs, rs1834481 and rs7106524, also reached a genome-wide significance level ($P < 5 \times 10^{-8}$), with probability values of 1.02$	imes 10^{-8}$ and 3.22$	imes 10^{-8}$, respectively (Table 1).

To assess the independence of the associations, we applied the model selection algorithm for the top SNPs (Table 1) in NHS samples. We forced age, BMI, and diabetes status into the model. SNPs rs2115763 and rs1834481 entered the final model ($P = 0.0002$ and 0.0006, respectively; Table 2). These 2 SNPs together explained 2.9% of the residual variation of plasma IL-18 levels after adjustment for age, BMI, and diabetes status. We also performed haplotype analysis for SNPs rs2115763 and rs1834481 with IL-18 levels. The frequencies were 33% for haplotype TC, 24% for AG, and 43% for AC. Haplotypes of TC and AG showed directionally consistent associations with IL-18 levels, as reported in single-SNP analysis, with probability values of 1.12$	imes 10^{-7}$ and 4.05$	imes 10^{-7}$, respectively (global $P = 3.85 \times 10^{-9}$; Table 3).

Finally, we performed exploratory analyses for the associations of SNPs rs2115763 and rs11834481 with other inflammatory biomarkers and diabetes in NHS. No significant associations were observed between these SNPs and inflammatory biomarkers, including C-reactive protein, tumor necrosis factor-α receptor 2, IL-6, soluble E-selectin, soluble intracellular cell adhesion molecule-1, and soluble vascular cell adhesion molecule-1 ($P > 0.05$; Supplemental Table IV). These SNPs were not associated with diabetes risk, either when analyzed individually or in haplotypes.

### Discussion

By analyzing a GWA scan in 1523 women and performing an in silico replication in an independent sample of 435 women from the WGHS, we identified 2 SNPs at the \textit{IL18}-\textit{BCO2} locus associated with plasma IL-18 levels at the genome-wide significance level.

### Table 1. Top SNPs for Plasma IL-18 Levels (pg/mL)*

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Chromosome</th>
<th>Position (kb)†</th>
<th>Alleles</th>
<th>Gene</th>
<th>Function</th>
<th>MAF</th>
<th>HWE‡</th>
<th>$\beta$</th>
<th>$P$</th>
<th>$\bar{\beta}$</th>
<th>$P$</th>
<th>Combined $P$</th>
<th>A1/A1</th>
<th>A1/A2</th>
<th>A2/A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2115763</td>
<td>11</td>
<td>111556.4</td>
<td>A/T</td>
<td>\textit{BCO2}</td>
<td>Intron</td>
<td>0.33</td>
<td>0.85</td>
<td>0.09</td>
<td>6.31$	imes 10^{-6}$</td>
<td>0.09</td>
<td>0.02</td>
<td>3.88$	imes 10^{-9}$</td>
<td>299.8 (139.6)</td>
<td>330.0 (175.8)</td>
<td>354.1 (145.7)</td>
</tr>
<tr>
<td>rs1834481</td>
<td>11</td>
<td>111529.0</td>
<td>C/G</td>
<td>\textit{IL18}</td>
<td>Intron</td>
<td>0.24</td>
<td>0.60</td>
<td>−0.09</td>
<td>8.38$	imes 10^{-7}$</td>
<td>−0.13</td>
<td>0.003</td>
<td>1.02$	imes 10^{-6}$</td>
<td>334.7 (89.3)</td>
<td>303.7 (143.8)</td>
<td>279.6 (121.3)</td>
</tr>
<tr>
<td>rs7106524</td>
<td>11</td>
<td>111538.8</td>
<td>G/A</td>
<td>\textit{IL18}</td>
<td>Intron</td>
<td>0.35</td>
<td>0.32</td>
<td>0.08</td>
<td>1.15$	imes 10^{-6}$</td>
<td>0.10</td>
<td>0.009</td>
<td>3.22$	imes 10^{-8}$</td>
<td>304.1 (148.6)</td>
<td>325.9 (168.7)</td>
<td>352.3 (150.1)</td>
</tr>
<tr>
<td>rs12400140</td>
<td>11</td>
<td>111575.5</td>
<td>G/A</td>
<td>\textit{BCO2}</td>
<td>Intron</td>
<td>0.27</td>
<td>0.39</td>
<td>−0.08</td>
<td>5.83$	imes 10^{-6}$</td>
<td>−0.09</td>
<td>0.02</td>
<td>3.98$	imes 10^{-7}$</td>
<td>336.3 (170.6)</td>
<td>302.5 (142.9)</td>
<td>297.9 (138.9)</td>
</tr>
<tr>
<td>rs49350484</td>
<td>11</td>
<td>111578.1</td>
<td>G/A</td>
<td>\textit{BCO2}</td>
<td>Intron</td>
<td>0.33</td>
<td>0.95</td>
<td>0.07</td>
<td>6.16$	imes 10^{-6}$</td>
<td>0.09</td>
<td>0.002</td>
<td>4.47$	imes 10^{-7}$</td>
<td>304.2 (146.6)</td>
<td>329.7 (172.7)</td>
<td>346.7 (141.6)</td>
</tr>
<tr>
<td>rs10891328</td>
<td>11</td>
<td>111551.1</td>
<td>C/T</td>
<td>\textit{IL18}</td>
<td>Intron</td>
<td>0.32</td>
<td>0.66</td>
<td>0.07</td>
<td>9.52$	imes 10^{-6}$</td>
<td>0.13</td>
<td>0.003</td>
<td>3.90$	imes 10^{-7}$</td>
<td>306.8 (149.4)</td>
<td>325.2 (170.0)</td>
<td>354.2 (151.7)</td>
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<tr>
<td>rs5744280</td>
<td>11</td>
<td>111521.7</td>
<td>G/A</td>
<td>\textit{IL18}</td>
<td>Intron</td>
<td>0.32</td>
<td>0.30</td>
<td>0.07</td>
<td>9.83$	imes 10^{-6}$</td>
<td>0.10</td>
<td>0.01</td>
<td>3.90$	imes 10^{-7}$</td>
<td>307.2 (148.4)</td>
<td>325.9 (170.1)</td>
<td>351.9 (151.6)</td>
</tr>
</tbody>
</table>

*IL-18 levels were log transformed for linear regression model adjusted for age, diabetes status, and BMI.
†Position based on National Center for Biotechnology Information build 36.3.
‡HWE, deviation from Hardy-Weinberg equilibrium $P$ value in NHS.
§A1, major allele; A2, minor allele. Mean±SD of IL-18 levels are values in NHS.
The strongest association was found for SNP rs2115763, which is located in intron 2 of the BCO2 gene (which encodes β-carotene oxygenase 2) (PubMed Entrez Database: http://www.ncbi.nlm.nih.gov/sites/entrez) and also in the same block with the IL18 gene. β-Carotene oxygenase 2 (BCO2) catalyzes the asymmetrical oxidative cleavage of β-carotene metabolism.30 β-Carotene oxygenase 2 may also be involved in biological processes other than vitamin A synthesis, because its gene is expressed in some tissues that are not related to vitamin A deficiency.31 Although vitamin A may affect the immune system,32 there is no evidence connecting vitamin A and plasma IL-18 levels. Thus, the mechanism underlying the association between SNP rs2115763 in the BCO2 gene and IL-18 levels is unclear. Because the BCO2 gene is located upstream of the IL18 gene, SNP rs2115763 might directly regulate IL18 gene transcription. It is also possible that the SNP rs2115763 is a marker of functional mutation in the IL18 gene affecting plasma IL-18 levels. SNP rs1834481 is an intronic SNP of IL18 gene.33 Several candidate gene studies found that SNPs in IL-18 gene were associated with plasma IL-18 levels in healthy and diseased individuals.34–36 Thompson et al indicated that SNP rs2043055 in the IL-18 gene was associated with plasma IL-18 levels,34 which was suggestively associated with IL-18 levels in our imputed SNP analysis (P = 1.47 × 10−6) and tagged by top SNP rs2115763 (r2 = 0.87). Similar results were obtained for SNP rs5744256, which was reported in another candidate gene study36 and tagged by SNP rs1834481 (r2 = 0.95). A recent GWA study found that 5 SNPs in or close to the IL-18 gene were associated with IL-18 levels at the genome-wide significance level.37 One SNP, rs2250417, was not, but the other SNPs (rs2043055, rs5744222, rs7123686, and 12420140) were also tagged by the 2 SNPs of rs2115763 and rs1834481 (r2 for rs2043055, rs7123686, and 12420140: 0.87 and 0.88; r2 for rs5744222, rs12420140, and rs1834481: 1.0 and 0.87), confirming the findings in the present study that the 2 SNPs were associated with plasma IL-18 levels. SNPs rs2115763 and rs1834481 are weakly correlated. In the forward selection model, SNPs rs2115763 and rs1834481 were independently associated with IL-18 levels. In addition, haplotypes with the effect allele of either SNP were associated with IL-18 concentration. These data suggest that these SNPs may confer independent genetic effects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1*</th>
<th></th>
<th></th>
<th></th>
<th>Model 2†</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>P Value</td>
<td>Variance Explained (%)‡</td>
<td>β (SE)</td>
<td>P Value</td>
<td>Variance Explained (%)‡</td>
<td></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>0.004 (0.001)</td>
<td>4.9 × 10⁻³</td>
<td>0.50</td>
<td>0.004 (0.001)</td>
<td>4.8 × 10⁻³</td>
<td>0.54</td>
<td></td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.01 (0.002)</td>
<td>6.0 × 10⁻⁷</td>
<td>1.67</td>
<td>0.01 (0.002)</td>
<td>3.2 × 10⁻⁷</td>
<td>1.80</td>
<td></td>
<td></td>
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<tr>
<td>Diabetes status</td>
<td>0.098 (0.023)</td>
<td>3.2 × 10⁻⁵</td>
<td>1.16</td>
<td>0.094 (0.023)</td>
<td>5.4 × 10⁻⁵</td>
<td>1.10</td>
<td></td>
<td></td>
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<tr>
<td>rs2115763</td>
<td>0.087 (0.016)</td>
<td>6.4 × 10⁻⁸</td>
<td>2.0</td>
<td>0.065 (0.017)</td>
<td>0.0002</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1834481</td>
<td>−0.066 (0.019)</td>
<td>0.0006</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Independent variables included age, BMI, diabetes status (yes/no), and rs2115763.
†Based on model 1, rs1834481 was also included in the model as an independent variable.
‡Measured by R², which was the difference of model sum of squares between models with and without the SNP of interest divided by the corrected total sum of squares of the full model.
We recently found that higher plasma IL-18 levels were associated with increased diabetes risk. However, no associations were observed between the IL-18-associated SNPs and diabetes in the present study. The null association is not surprising because the genetic variants account for only a small proportion of the variance of IL-18 levels. IL-18-associated SNPs were not associated with other inflammatory biomarkers, suggesting that the genetic variants specifically affect IL-18 levels.

Our findings are less likely to be false positives because of strong replications in independent samples. Also, all top SNPs were cis effects in that they were present in or close to the IL18 gene. Nevertheless, this study has several limitations. First, measurement errors in IL-18 measurements are inevitable. However, these errors are likely to be random, which would only bias the association toward the null. Second, most SNPs identified in our study are in introns. These SNPs may be only markers for functional variants. Third, using metaanalysis to combine associations from the discovery and replication samples might introduce bias. However, the high consistency in associations between the 2 stages indicated that our findings are robust. In addition, this GWA study had 80% power to identify SNPs associated with ≥2.5% of the variation in IL-18 levels at P<5×10^{-8}. Our study may be underpowered to identify other quantitative trait loci with more modest effects. Finally, the participants included in this study are women of European ancestry. It remains to be determined whether our findings apply to men or other ethnicities.

In summary, we identified novel SNPs at the IL18-BOC2 locus associated with plasma IL-18 levels in 2 independent GWA study samples. Detailed fine mapping or sequencing at this locus and subsequent functional characterization are warranted to identify causal variants.

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**Disclosures**

None.

**References**


Genome-Wide Association Study Identifies Variants at the IL18-BCO2 Locus Associated With Interleukin-18 Levels
Meian He, Marilyn C. Cornelis, Peter Kraft, Rob M. van Dam, Qi Sun, Cathy C. Laurie, Daniel B. Mirel, Daniel I. Chasman, Paul M. Ridker, David J. Hunter, Frank B. Hu and Lu Qi

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Supplemental Material

Supplementary Figure I. Eigenvectors 1 and 2 from the principal components analysis of all unduplicated NHS samples (N=3,369) and all unduplicated and unrelated HapMap controls (N=209, including 59 CEU, 60 YRI, 45 JPT, 45 CHB). Color- and symbol-coding is by self-identified ethnic group.

Supplementary Figure II. Quantile-Quantile (QQ) $P$ value plot for genome-wide scan for log-transformed plasma IL-18 levels in the NHS discovery sample of 1,523 women.
Supplementary table I Distribution of plasma IL-18 levels and other characteristics among the study population in Nurses’ Health Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>1,523</td>
<td>56.0 (8.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1,520</td>
<td>28.0 (5.6)</td>
</tr>
<tr>
<td>Physical activity (MET-hr/week)</td>
<td>1,490</td>
<td>14.1 (22.2)</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>1,454</td>
<td>4.41 (8.87)</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td>1,518</td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td></td>
<td>725 (47.8)</td>
</tr>
<tr>
<td>Past smoker</td>
<td></td>
<td>631 (41.6)</td>
</tr>
<tr>
<td>Current smoker</td>
<td></td>
<td>162 (10.7)</td>
</tr>
<tr>
<td>Menopausal status (%)</td>
<td>1,523</td>
<td></td>
</tr>
<tr>
<td>Pre-menopause</td>
<td></td>
<td>317 (20.8)</td>
</tr>
<tr>
<td>Post-menopause, hormone never user</td>
<td></td>
<td>505 (33.2)</td>
</tr>
<tr>
<td>Post-menopause, hormone past user</td>
<td></td>
<td>239 (15.7)</td>
</tr>
<tr>
<td>Post-menopause, hormone current user</td>
<td></td>
<td>462 (30.3)</td>
</tr>
<tr>
<td>Fasting status (%)</td>
<td>1,513</td>
<td>1,025 (67.8)</td>
</tr>
<tr>
<td>Type 2 diabetes (%)</td>
<td>1,523</td>
<td>679 (44.6)</td>
</tr>
<tr>
<td>Total energy (kcal/day)</td>
<td>1,454</td>
<td>1,794 (512)</td>
</tr>
<tr>
<td>Glycemic load</td>
<td>1,454</td>
<td>105.1 (19.6)</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>1,523</td>
<td>320.1 (158.8)</td>
</tr>
</tbody>
</table>

*Mean (SD) for continuous variables; n (%) for categorical variables.
Supplementary Table II Pair-wise linkage disequilibrium ($r^2$) among top SNPs associated with IL-18 concentrations

<table>
<thead>
<tr>
<th>SNPs</th>
<th>rs2115763</th>
<th>rs1834481</th>
<th>rs7106524</th>
<th>rs12420140</th>
<th>rs4935984</th>
<th>rs10891329</th>
<th>rs5744280</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2115763</td>
<td>1.0</td>
<td>0.19</td>
<td>0.87</td>
<td>0.22</td>
<td>0.90</td>
<td>0.73</td>
<td>0.69</td>
</tr>
<tr>
<td>rs1834481</td>
<td>1.0</td>
<td>0.22</td>
<td>0.87</td>
<td>0.20</td>
<td>0.18</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>rs7106524</td>
<td></td>
<td>1.0</td>
<td>0.25</td>
<td>0.90</td>
<td>0.86</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>rs12420140</td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.23</td>
<td>0.23</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>rs4935984</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.77</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>rs10891329</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>rs5744280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
Supplementary Table III Top imputed SNPs for plasma IL-18 levels∗

| SNPs           | Position (kb)† | Alleles (+/-)‡ | Gene  | Function             | MAF§   | r2|| | Beta¶ | se   | P value¶ |
|----------------|----------------|----------------|-------|----------------------|--------|------|-------|------|----------|
| rs11214093     | 111497802      | T/C            | IL18  | 3’ near gene         | 0.43   | 0.565| 0.108 | 0.020| 1.22E-07 |
| rs10891343     | 111585594      | T/C            | BCO2  | intron               | 0.48   | 0.971| 0.079 | 0.015| 3.63E-07 |
| rs2250417      | 111590526      | T/C            | BCO2  | intron               | 0.48   | 0.950| 0.079 | 0.016| 4.75E-07 |
| rs5744222      | 111542224      | G/T            | IL18  | 5’ near gene         | 0.25   | 0.987| 0.087 | 0.018| 1.22E-06 |
| rs7121554      | 111553261      | C/T            | BCO2  | intron               | 0.26   | 0.874| 0.091 | 0.019| 1.26E-06 |
| rs2043055      | 111536834      | G/A            | IL18  | intron               | 0.36   | 0.997| 0.075 | 0.016| 1.47E-06 |
| rs12797880     | 111554401      | T/C            | BCO2  | intron               | 0.25   | 0.980| 0.086 | 0.018| 1.49E-06 |
| rs12796114     | 111554350      | A/C            | BCO2  | intron               | 0.25   | 0.981| 0.086 | 0.018| 1.50E-06 |
| rs5744256      | 111528058      | A/G            | IL18  | intron               | 0.25   | 0.999| 0.084 | 0.018| 2.02E-06 |
| rs5744258      | 111526977      | C/G            | IL18  | intron               | 0.25   | 0.998| 0.084 | 0.018| 2.05E-06 |
| rs7131094      | 111550127      | C/T            | BCO2  | 5’ near gene         | 0.25   | 0.962| 0.086 | 0.018| 2.11E-06 |
| rs10891334     | 111562310      | A/G            | BCO2  | intron               | 0.34   | 0.988| 0.076 | 0.016| 2.38E-06 |
| rs5744276      | 111522081      | C/G            | IL18  | intron               | 0.25   | 0.998| 0.084 | 0.018| 2.58E-06 |
| rs4937176      | 111574183      | A/G            | BCO2  | intron               | 0.34   | 0.992| 0.074 | 0.016| 4.39E-06 |
| rs7123686      | 111570693      | C/A            | BCO2  | intron               | 0.34   | 0.991| 0.074 | 0.016| 4.43E-06 |
| rs10789863     | 111582588      | T/A            | BCO2  | intron               | 0.34   | 0.994| 0.073 | 0.016| 5.68E-06 |
| rs1972421      | 111583330      | T/C            | BCO2  | intron               | 0.34   | 0.990| 0.073 | 0.016| 6.44E-06 |

∗N= 1,523; IL-18 levels were log transformed.
†Position based on NCBI build 36.3.
‡”+”, effect allele; “-“, reference allele.
§Minor allele frequency among study participants.
||r2 refers to the measurement of SNPs imputation quality.
¶Regression coefficients and P values for every one copy of effect allele were estimated from linear regression models adjusted for age, diabetes status, and BMI.
Supplementary Table IV Associations between top SNPs and other inflammatory factors as well as type 2 diabetes risk in the Nurses’ Health Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Beta ± s.e.</th>
<th>P</th>
<th>Beta ± s.e.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rs2115763</td>
<td></td>
<td>rs1834481</td>
<td></td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>989</td>
<td>0.056 ± 0.049</td>
<td>0.25</td>
<td>-0.033 ± 0.054</td>
<td>0.54</td>
</tr>
<tr>
<td>TNF-α R2 (pg/mL)</td>
<td>1003</td>
<td>0.014 ± 0.018</td>
<td>0.45</td>
<td>-0.008 ± 0.022</td>
<td>0.71</td>
</tr>
<tr>
<td>IL-6 (ng/mL)</td>
<td>973</td>
<td>0.04 ± 0.03</td>
<td>0.24</td>
<td>0.013 ± 0.036</td>
<td>0.72</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>1005</td>
<td>0.04 ± 0.02</td>
<td>0.06</td>
<td>0.004 ± 0.024</td>
<td>0.87</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>1005</td>
<td>0.014 ± 0.013</td>
<td>0.28</td>
<td>0.004 ± 0.013</td>
<td>0.76</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>1006</td>
<td>0.011 ± 0.013</td>
<td>0.39</td>
<td>-0.012 ± 0.014</td>
<td>0.40</td>
</tr>
<tr>
<td>Type 2 diabetes*</td>
<td>3217</td>
<td>-0.039 ± 0.058</td>
<td>0.50</td>
<td>-0.086 ± 0.064</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Both incident and prevalent cases were included. To convert beta ± s.e. to odds ratio (95% CI), exponentiate beta ± 1.96×s.e.
Supplementary Figure I.
Supplementary Figure II.

\[ \lambda = 0.991 \]