NIH Public Access

Author Manuscript

Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2011 April 1.

Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2010 April ; 30(4): 885–890. doi:10.1161/ATVBAHA.109.199422.

Genome-wide Association Study Identifies Variants at *IL18-BCO2* Locus Associated with Interleukin-18 Levels

Meian He[1,2,3], **Marilyn C. Cornelis**[1], **Peter Kraft**[4], **Rob M. van Dam**[1,4,5], **Qi Sun**[1], **Cathy C. Laurie**[6], **Daniel B. Mirel**[7], **Daniel I. Chasman**[8,9], **Paul M. Ridker**[8,9], **David J. Hunter**[4,5], **Frank B. Hu**[1,4,5], and **Lu Qi**[1,5]

[1] Department of Nutrition, Harvard School of Public Health, Boston 02115, Massachusetts, USA

[2] Institute of Occupational Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China

[3] Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China

[4] Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, Boston 02115, Massachusetts, USA

[5] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, 02115, Massachusetts, USA

[6] Department of Biostatistics, University of Washington, Seattle 98195, WA, USA

[7] Center for Genotyping and Analysis, Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge 02142, MA,USA

[8] Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Harvard Medical School, Boston 02115, Massachusetts, USA

[9] Donald W. Reynolds Center for Cardiovascular Research, Brigham and Women's Hospital, Harvard Medical School, Boston 02115, Massachusetts, USA

Abstract

Objective—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 two-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 SNPs at *IL18-BCO2* locus were associated with IL-18 concentrations at 1×10^{-5} significance level. The strongest association was found for SNP rs2115763 in the *BCO2* gene (P value = 6.31×10^{-8}). In silico replication in WGHS (435 women) confirmed these findings. The combined analysis of the two studies indicated that SNPs rs2115763, rs1834481, and rs7106524 reached genome-wide significance level ($P < 5 \times 10^{-8}$). Forward selection analysis indicated SNPs rs2115763 and

Address correspondence and requests for reprints to Lu Qi, MD, PhD, Department of Nutrition, Harvard School of Public Health, Boston 02115, MA, 02115, USA. Tel: 1-617 432 4116, Fax: 1-617-432-2435, nhlqi@channing.harvard.edu; Frank B. Hu, MD, PhD, Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA, USA. Tel: 617 432 0113, Fax: 617 432 2435, Frank.hu@channning.harvard.edu.

Disclosure

None.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

rs1834481 were independently associated with *IL-18* levels ($P = 0.0002$ and 0.0006, respectively). The two SNPs together explained 2.9% of variation of plasma IL-18 levels.

Conclusions—This study identified several novel variants at *IL18-BCO2* locus associated with IL-18 levels.

Keywords

GWAS; IL-18; *IL18* gene

IL-18 is a proinflammatory cytokine involved in the processes of innate and acquired immunities [1–4]. Originally identified as an interferon (IFN)-γ –inducing factor in Kupffer cells and macrophages[4], IL-18 induces production of IFN-γ in T lymphocytes and natural killer cells[5]. In addition, IL-18 can combine with IL-12 to promote the T helper 1 (T_H1) responses[1–3, 6]. Epidemiology studies indicated that plasma concentrations of IL-18 have been associated with diverse conditions including type 2 diabetes[7–9], cardiovascular disease[10, 11], and the severity of coronary atherosclerosis[12]. Animal studies also found that IL-18 can promote atherosclerosis[13, 14]. In ApoE knockout mice, IL-18 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 1,523 women from the Nurses' Health Study (NHS). A total of 704,409 single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) ≥ 0.02, genotype call rate ≥ 0.98, and Hardy-Weinberg equilibrium $P \geq 1\times10^{-8}$ 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 30–55 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 for the current study were a subset of those who had both IL-18 measurements and genome-wide scan data. Those unrelated, genetically identified women of European ancestry with high quality genotyping data were included. After further excluding participants with missing phenotype and covariate data, 1,523 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 [17]. 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.

IL-18 and other inflammatory biomarkers measurements

In NHS, blood sample collection (between 1989 and 1990) and processing were previously reported[18]. In order to decrease systematic bias and inter-assay variation, study samples were measured in randomly ordered case-control pairs. Plasma IL-18 levels were measured using

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

enzyme-linked immunosorbent assay (Diaclone Besancon, France). The sensitivity of this assay is 0.9 pg/mL. The average intra-assay coefficient of variation for IL-18 was 7.3%. The methods for measuring other biomarkers are described in detail elsewhere [19, 20]. Briefly, the coefficients of variation were 3.8% for plasma C- reactive protein (CRP), 3.3–4.8% for soluble intracellular cell adhesion molecule-1 (sICAM-1), 5.7–8.8% for soluble E-selectin (sE-selectin), 5.9% for IL-6, 2.6–4.8% for tumor necrosis factor-α receptor 2 (TNF-αR2) and 8.5 to 9.8% for soluble vascular cell adhesion molecule-1 (sVCAM-1). IL-18 measurement in WGHS was described in detail elsewhere [21].

Genotyping and quality control

In NHS, DNA was extracted from white blood cells using the QIAmpTM 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)[22]. All samples used in the present study achieved a call rate ≥ 98%. Individual SNPs were excluded if their MAF < 0.02 (n = 141,930), missing call rate ≥ 2% (n = 17,889) or Hardy Weinberg equilibrium test P value $< 1\times10^{-8}$ (n = 3,312). Finally, 704,409 SNPs were available for analysis. In WGHS, genotyping and quality control have been described in details elsewhere[23]. Briefly, samples were genotyped using Infinium II technology from Illumina (HumanHap300 Duo-Plus chip). Genotype calls were made using Beadstudio v3.3 (Illumina, USA). Those samples with missing genotypes percentage > 2% were removed first. SNPs with call rates < 90%, Hardy Weinberg equilibrium test P value $< 1\times10^{-6}$, 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 NHS, principle component analysis was used to investigate population structure[24]. We used a set of 12,021 SNPs in Caucasians with very low levels of linkage disequilibrium (LD) and MAF greater than 0.05 and 3,369 subjects passing quality control and 209 HapMap II founders (59 CEU, 60 YRI, 45 JPT and 45 CHB) for the analysis (Supplementary Figure 1). Subjects with first and second principal components defined by the means of the first and second principal components among self-described whites ± 3 s.d. were classified as having primarily European ancestry. Population stratification in WGHS has been described in detail elsewhere [23].

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. Additive model was assumed for each SNP. In order to control for potential confounding by population stratification, we further adjusted for the top three principal components of genetic variation and the results were similar. A P-value cut-off of 5×10^{-8} is used as 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[25, 26]. We used the hidden Markov model implemented in MACH 1.0 software[27] 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[28]. Imputed data (expressed as allele dosage) were analyzed by using linear regression models with ProbABEL software[29]. We imputed (predicted) untyped SNPs based on genotyped SNPs and haplotype structure in the reference samples (Hapmap CEU). The imputed genotypes are not actually observed genotypes and that 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

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

to reflect the ambiguity of the genotypes. The LD structure was inferred based on CEU HapMap 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 PLINK software. We used fixed effect meta-analysis to combine evidence for association from the discovery (NHS) and replication (WGHS) samples.

Results

At the discovery stage, 1,523 women from NHS were included in the final analysis. All subjects have European ancestry defined by the principal component analysis of genetic variations. The basic characteristics of the participants were presented in Supplementary Table 1. 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 95% confidence interval) and there was no evidence of systematic bias ($\lambda = 0.991$; Supplementary Figure 2). The distribution of *P* values for the association of SNPs withIL-18 levels, according to chromosome, is presented in Figure 1. In the initial GWA scan, 7 SNPs were associated with IL-18 concentrations at 1×10^{-5} significance level (Table 1). All top SNPs clustered within a ~100 kb region on chromosome 11q22 ~ 11q23, which harbors three genes including *IL18, TEX12,* and *BCO2* (GeneID are 3606, 56158, and 83875 respectively; Figure 2). The strongest association was found for SNP rs2115763 in intron 2 of *BCO2* gene (*P* values = 6.31×10^{-8}). This SNP is highly prevalent in our sample, with a MAF of 33%, slightly lower than 41.5% in 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 ($r^2 = 0.19$). Among the remaining top SNPs, 2 SNPs rs12420140 and rs4935984 are located in introns of *BCO2* gene; other 3 SNPs rs7106524, rs10891329, and rs5744280 are located in the *IL18* gene (Table 1). The LD of the top SNPs was presented in Supplementary Table 2.

To assess whether any other stronger genetic markers of IL-18 levels exist in the *IL18* or other 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 *P* values of less than 5×10^{-7}; other 14 top SNPs were also mapped to *IL18* or *BCO2* genes ($P = 1.22 \sim 6.44 \times 10^{-6}$; Supplementary Table 3).

We further 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, two SNPs of rs5744280 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, 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 *P* value 3.88×10^{-9}. Two other SNPs rs1834481 and rs7106524 also reached genome-wide significance level ($P < 5 \times 10^{-8}$), with *P* values of 1.02×10^{-8} and 3.22×10^{-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.

NIH-PA Author Manuscript

SNPs rs2115763 and rs1834481 entered the final model ($P = 0.0002$ and 0.0006, respectively; Table 2). These two SNPs together explained 2.9% of the residual variation of plasma IL-18 levels after adjusting 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 P values of 1.12 $\times 10^{-7}$ and 4.05 $\times 10^{-7}$respectively (the global P value = 3.85×10^{-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 CRP, TNF-αR2, IL-6, sE-selectin, sICAM-1, and sVCAM-1 ($P > 0.05$; Supplementary Table 4). These SNPs were not associated with diabetes risk, either when analyzed individually or in haplotypes.

Discussion

By analyzing a GWA scan in 1,523 women and performing an in silico replication in an independent sample of 435 women from the WGHS, we identified 2 SNPs at *IL18*- *BCO2* locus associated with plasma IL-18 levels at genome-wide significance level.

The strongest association was found for SNP rs2115763, which is located in intron 2 of *BCO2* (codes beta-carotene oxygenase 2) gene[30] and also in the same block with the *IL18* gene. Beta-carotene oxygenase 2 (BCO2) catalyzes the asymmetric oxidative cleavage of beta-carotene metabolism[31]. BCO2 may also be involved in biological processes other than vitamin A synthesis, because the gene is expressed in some tissues that are not related to vitamin A deficiency [32]. Although vitamin A may affect the immune system[33], there is no evidence connecting vitamin A and plasma IL-18 levels. Thus, the mechanism underlying the association between SNP rs2115763 in *BCO2* gene and IL-18 levels is unclear. Because the *BCO2* gene is located on the upstream of *IL18* gene, SNP rs2115763 might directly regulate the *IL18* gene transcription. It is also possible that the SNP rs2115763 is a marker of functional mutation in *IL18* gene affecting plasma IL-18 levels. SNP rs1834481 is an intronic SNP of *IL18* gene [34]. Several candidate gene studies found that SNPs in *IL-18* gene were associated with plasma IL-18 levels in healthy and diseased individuals [35–37]. Thompson et al. indicated that SNP rs2043055 in *IL-18* gene was associated with plasma IL-18 levels[35], which was suggestively associated with IL-18 levels in our imputed SNP analysis ($P = 1.47 \times 10^{-6}$) and tagged by top SNP rs2115763 ($r^2 = 0.87$). Similar results were obtained for SNP rs5744256, which was reported in another candidate gene study[37] and tagged by SNP rs1834481 ($r^2 = 0.95$). A recent GWA study found that 5 SNPs in or close to *IL-18* gene were associated with IL-18 levels at the genome-wide significance level[38]. Except for one SNP rs2250417, other 4 SNPs (rs2043055, rs5744222, rs7123686, and 12420140) were also tagged by the two SNPs of rs2115763 and rs1834481 (r^2 for rs2043055, rs7123686, and rs2115763 is 0.87 and 0.88; r^2 for rs5744222, rs12420140, and rs1834481 is 1.0 and 0.87), confirming the findings in the present study that the two 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.

We recently found that higher plasma IL-18 levels were associated with increased diabetes risk[9]. 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 positive 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 [38]. 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. Thirdly, using meta-analysis to combine associations from the discovery and replication samples might introduce bias.[39] However, the high consistency in associations between the two stages indicated our findings are robust. In addition, this genome-wide association study had 80% power to identify SNPs associated with ~2.5% of the variation in IL-18 levels at $P < 5 \times 10^{-8}$. 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 *IL18-BOC2* locus associated with plasma IL-18 levels in two independent GWAS samples. Detailed fine mapping or sequencing at this locus and subsequent functional characterization are warranted to identify causal variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The NHS type 2 diabetes GWA study (U01HG004399) is a component of a collaborative project that includes 13 other GWA studies (U01HG004738, U01HG004422, U01HG004402, U01HG004729, U01HG004726, U01HG004735, U01HG004415, U01HG004436, U01HG004423, U01HG004728, RFAHG006033; National Institute of Dental & Craniofacial Research: U01DE018993, U01DE018903) funded as part of the Gene Environment-Association Studies (GENEVA) under the NIH Genes, Environment and Health Initiative (GEI). Assistance with phenotype harmonization and genotype cleaning, as well as with general study coordination, was provided by the GENEVA Coordinating Center (U01HG004446). Assistance with data cleaning was provided by the National Center for Biotechnology Information. Genotyping was performed at the Broad Institute of MIT and Harvard, with funding support from the NIH GEI (U01HG04424), and Johns Hopkins University Center for Inherited Disease Research, with support from the NIH GEI (U01HG004438) and the NIH contract "High throughput genotyping for studying the genetic contributions to human disease" (HHSN268200782096C). Additional funding for the current research was provided by the National Cancer Institute (P01CA087969, P01CA055075), the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK058845), and Merck & Co., Inc.. L.Q is supported by National Institutes of Health grants RO1 HL71981, American Heart Association Scientist Development Award and the Boston Obesity Nutrition Research Center (DK46200).

References

1. Dinarello CA, Novick D, Puren AJ, Fantuzzi G, Shapiro L, Muhl H, Yoon DY, Reznikov LL, Kim SH, Rubinstein M. Overview of interleukin-18: more than an interferon-gamma inducing factor. J Leukoc Biol 1998;63:658–664. [PubMed: 9620656]
2. McInnes IB, Gracie JA, Leung BP, Wei XQ, Liew FY. Interleukin 18: a pleiotropic participant in chronic inflammation. Immunol Today 2000;21:312–315. [PubMed: 10871869]
3. Dinarello CA. Interleukin-18, a proinflammatory cytokine. Eur Cytokine Netw 2000;11:483–486. [PubMed: 11203186]
4. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 1995;378:88–91. [PubMed: 7477296]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

5. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001;19:423–474. [PubMed: 11244043]

6. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, Hartley SB, Menon S, Kastelein R, Bazan F, O'Garra A. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity 1997;7:571–581. [PubMed: 9354477]

7. Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK. Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. Clin Immunol 2005;117:152–160. [PubMed: 16112617]

8. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984–2002. Diabetes 2005;54:2932–2938. [PubMed: 16186395]

9. Hivert MF, Sun Q, Shrader P, Mantzoros CS, Meigs JB, Hu FB. Circulating IL-18 and the risk of type 2 diabetes in women. Diabetologia 2009;52:2101–2108. [PubMed: 19669125]

10. Troseid M, Seljeflot I, Hjerkinn EM, Arnesen H. Interleukin-18 is a strong predictor of cardiovascular events in elderly men with the metabolic syndrome: synergistic effect of inflammation and hyperglycemia. Diabetes Care 2009;32:486–492. [PubMed: 19092166]

11. Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, Evans A, Cambien F, Tiret L. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Circulation 2003;108:2453–2459. [PubMed: 14581397]

12. Hulthe J, McPheat W, Samnegard A, Tornvall P, Hamsten A, Eriksson P. Plasma interleukin (IL)-18 concentrations is elevated in patients with previous myocardial infarction and related to severity of coronary atherosclerosis independently of C-reactive protein and IL-6. Atherosclerosis 2006;188:450–454. [PubMed: 16405895]

13. Tenger C, Sundborger A, Jawien J, Zhou X. IL-18 accelerates atherosclerosis accompanied by elevation of IFN-gamma and CXCL16 expression independently of T cells. Arterioscler Thromb Vasc Biol 2005;25:791–796. [PubMed: 15604417]

14. de Nooijer R, von der Thusen JH, Verkleij CJ, Kuiper J, Jukema JW, van der Wall EE, van Berkel JC, Biessen EA. Overexpression of IL-18 decreases intimal collagen content and promotes a vulnerable plaque phenotype in apolipoprotein-E-deficient mice. Arterioscler Thromb Vasc Biol 2004;24:2313–2319. [PubMed: 15472128]

15. Elhage R, Jawien J, Rudling M, Ljunggren HG, Takeda K, Akira S, Bayard F, Hansson GK. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res 2003;59:234–240. [PubMed: 12829194]

16. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. J Womens Health 1997;6:49–62. [PubMed: 9065374]

17. Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, Buring JE. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. Clin Chem 2008;54:249–255. [PubMed: 18070814]

18. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004;53:693–700. [PubMed: 14988254]

19. Qi L, van Dam RM, Liu S, Franz M, Mantzoros C, Hu FB. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. Diabetes Care 2006;29:207–211. [PubMed: 16443861]

20. Qi L, Rifai N, Hu FB. Interleukin-6 receptor gene, plasma C-reactive protein, and diabetes risk in women. Diabetes 2009;58:275–278. [PubMed: 18852330]

21. Everett BM, Bansal S, Rifai N, Buring JE, Ridker PM. Interleukin-18 and the risk of future cardiovascular disease among initially healthy women. Atherosclerosis 2009;202:282–288. [PubMed: 18514203]

22. Korn JM, Kuruvilla FG, McCarroll SA, Wysoker A, Nemesh J, Cawley S, Hubbell E, Veitch J, Collins PJ, Darvishi K, Lee C, Nizzari MM, Gabriel SB, Purcell S, Daly MJ, Altshuler D. Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. Nat Genet 2008;40:1253–1260. [PubMed: 18776909]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

23. Pare G, Chasman DI, Kellogg M, Zee RY, Rifai N, Badola S, Miletich JP, Ridker PM. Novel association of ABO histo-blood group antigen with soluble ICAM-1: results of a genome-wide association study of 6,578 women. PLoS Genet 2008;4:e1000118. [PubMed: 18604267]
24. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904–909. [PubMed: 16862161]
25. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbol P, Leal SM, Pasternak S, Wheeler DA, Willis TD, Yu F, Yang H, Zeng C, Gao Y, Hu H, Hu W, Li C, Lin W, Liu S, Pan H, Tang X, Wang J, Wang W, Yu J, Zhang B, Zhang Q, Zhao H, Zhao H, Zhou J, Gabriel SB, Barry R, Blumenstiel B, Camargo A, Defelice M, Faggart M, Goyette M, Gupta S, Moore J, Nguyen H, Onofrio RC, Parkin M, Roy J, Stahl E, Winchester E, Ziaugra L, Altshuler D, Shen Y, Yao Z, Huang W, Chu X, He Y, Jin L, Liu Y, Shen Y, Sun W, Wang H, Wang Y, Wang Y, Xiong X, Xu L, Waye MM, Tsui SK, Xue H, Wong JT, Galver LM, Fan JB, Gunderson K, Murray SS, Oliphant AR, Chee MS, Montpetit A, Chagnon F, Ferretti V, Leboeuf M, Olivier JF, Phillips MS, Roumy S, Sallee C, Verner A, Hudson TJ, Kwok PY, Cai D, Koboldt DC, Miller RD, Pawlikowska L, Taillon-Miller P, Xiao M, Tsui LC, Mak W, Song YQ, Tam PK, Nakamura Y, Kawaguchi T, Kitamoto T, Morizono T, Nagashima A, Ohnishi Y, Sekine A, Tanaka T, Tsunoda T, Deloukas P, Bird CP, Delgado M, Dermitzakis ET, Gwilliam R, Hunt S, Morrison J, Powell D, Stranger BE, Whittaker P, Bentley DR, Daly MJ, de Bakker PI, Barrett J, Chretien YR, Maller J, McCarroll S, Patterson N, Pe'er I, Price A, Purcell S, Richter DJ, Sabeti P, Saxena R, Schaffner SF, Sham PC, Varilly P, Altshuler D, Stein LD, Krishnan L, Smith AV, Tello-Ruiz MK, Thorisson GA, Chakravarti A, Chen PE, Cutler DJ, Kashuk CS, Lin S, Abecasis GR, Guan W, Li Y, Munro HM, Qin ZS, Thomas DJ, McVean G, Auton A, Bottolo L, Cardin N, Eyheramendy S, Freeman C, Marchini J, Myers S, Spencer C, Stephens M, Donnelly P, Cardon LR, Clarke G, Evans DM, Morris AP, Weir BS, Tsunoda T, Mullikin JC, Sherry ST, Feolo M, Skol A, Zhang H, Zeng C, Zhao H, Matsuda I, Fukushima Y, Macer DR, Suda E, Rotimi CN, Adebamowo CA, Ajayi I, Aniagwu T, Marshall PA, Nkwodimmah C, Royal CD, Leppert MF, Dixon M, Peiffer A, Qiu R, Kent A, Kato K, Niikawa N, Adewole IF, Knoppers BM, Foster MW, Clayton EW, Watkin J, Gibbs RA, Belmont JW, Muzny D, Nazareth L, Sodergren E, Weinstock GM, Wheeler DA, Yakub I, Gabriel SB, Onofrio RC, Richter DJ, Ziaugra L, Birren BW, Daly MJ, Altshuler D, Wilson RK, Fulton LL, Rogers J, Burton J, Carter NP, Clee CM, Griffiths M, Jones MC, McLay K, Plumb RW, Ross MT, Sims SK, Willey DL, Chen Z, Han H, Kang L, Godbout M, Wallenburg JC, L'Archeveque P, Bellemare G, Saeki K, Wang H, An D, Fu H, Li Q, Wang Z, Wang R, Holden AL, Brooks LD, McEwen JE, Guyer MS, Wang VO, Peterson JL, Shi M, Spiegel J, Sung LM, Zacharia LF, Collins FS, Kennedy K, Jamieson R, Stewart J. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851–861. [PubMed: 17943122]
26. Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. Genet Epidemiol 2008;32:227–234. [PubMed: 18300295]
27. Li Y, Abecasis GR. Mach 1.0: rapid haplotype reconstruction and missing genotype inference. Am J Hum Genet 2006;S79:2290.
28. 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. Am J Hum Genet 2007;81:559–575. [PubMed: 17701901]
29. Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. GenABEL: an R library for genome-wide association analysis. Bioinformatics 2007;23:1294–1296. [PubMed: 17384015]
30. http://www.ncbi.nlm.nih.gov/sites/entrez.
31. Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE, von Lintig J. Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. J Biol Chem 2001;276:14110–14116. [PubMed: 11278918]
32. Lindqvist A, He YG, Andersson S. Cell type-specific expression of beta-carotene 9′,10′-monooxygenase in human tissues. J Histochem Cytochem 2005;53:1403–1412. [PubMed: 15983114]
33. Pino-Lagos K, Benson MJ, Noelle RJ. Retinoic acid in the immune system. Ann N Y Acad Sci 2008;1143:170–187. [PubMed: 19076350]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

34. Nolan KF, Greaves DR, Waldmann H. The human interleukin 18 gene IL18 maps to 11q22.2–q22.3, closely linked to the DRD2 gene locus and distinct from mapped IDDM loci. Genomics 1998;51:161–163. [PubMed: 9693051]
35. Thompson SR, McCaskie PA, Beilby JP, Hung J, Jennens M, Chapman C, Thompson P, Humphries SE. IL18 haplotypes are associated with serum IL-18 concentrations in a population-based study and a cohort of individuals with premature coronary heart disease. Clin Chem 2007;53:2078–2085. [PubMed: 17962365]
36. Tiret L, Godefroy T, Lubos E, Nicaud V, Tregouet DA, Barbaux S, Schnabel R, Bickel C, Espinola-Klein C, Poirier O, Perret C, Munzel T, Rupprecht HJ, Lackner K, Cambien F, Blankenberg S. Genetic analysis of the interleukin-18 system highlights the role of the interleukin-18 gene in cardiovascular disease. Circulation 2005;112:643–650. [PubMed: 16043644]
37. Frayling TM, Rafiq S, Murray A, Hurst AJ, Weedon MN, Henley W, Bandinelli S, Corsi AM, Ferrucci L, Guralnik JM, Wallace RB, Melzer D. An interleukin-18 polymorphism is associated with reduced serum concentrations and better physical functioning in older people. J Gerontol A Biol Sci Med Sci 2007;62:73–78. [PubMed: 17301041]
38. Melzer D, Perry JR, Hernandez D, Corsi AM, Stevens K, Rafferty I, Lauretani F, Murray A, Gibbs JR, Paolisso G, Rafiq S, Simon-Sanchez J, Lango H, Scholz S, Weedon MN, Arepalli S, Rice N, Washecka N, Hurst A, Britton A, Henley W, van de Leemput J, Li R, Newman AB, Tranah G, Harris T, Panicker V, Dayan C, Bennett A, McCarthy MI, Ruokonen A, Jarvelin MR, Guralnik J, Bandinelli S, Frayling TM, Singleton A, Ferrucci L. A genome-wide association study identifies protein quantitative trait loci (pQTLs). PLoS Genet 2008;4:e1000072. [PubMed: 18464913]
39. Bowden J, Dudbridge F. Unbiased estimation of odds ratios: combining genomewide association scans with replication studies. Genet Epidemiol 2009;33:406–418. [PubMed: 19140132]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

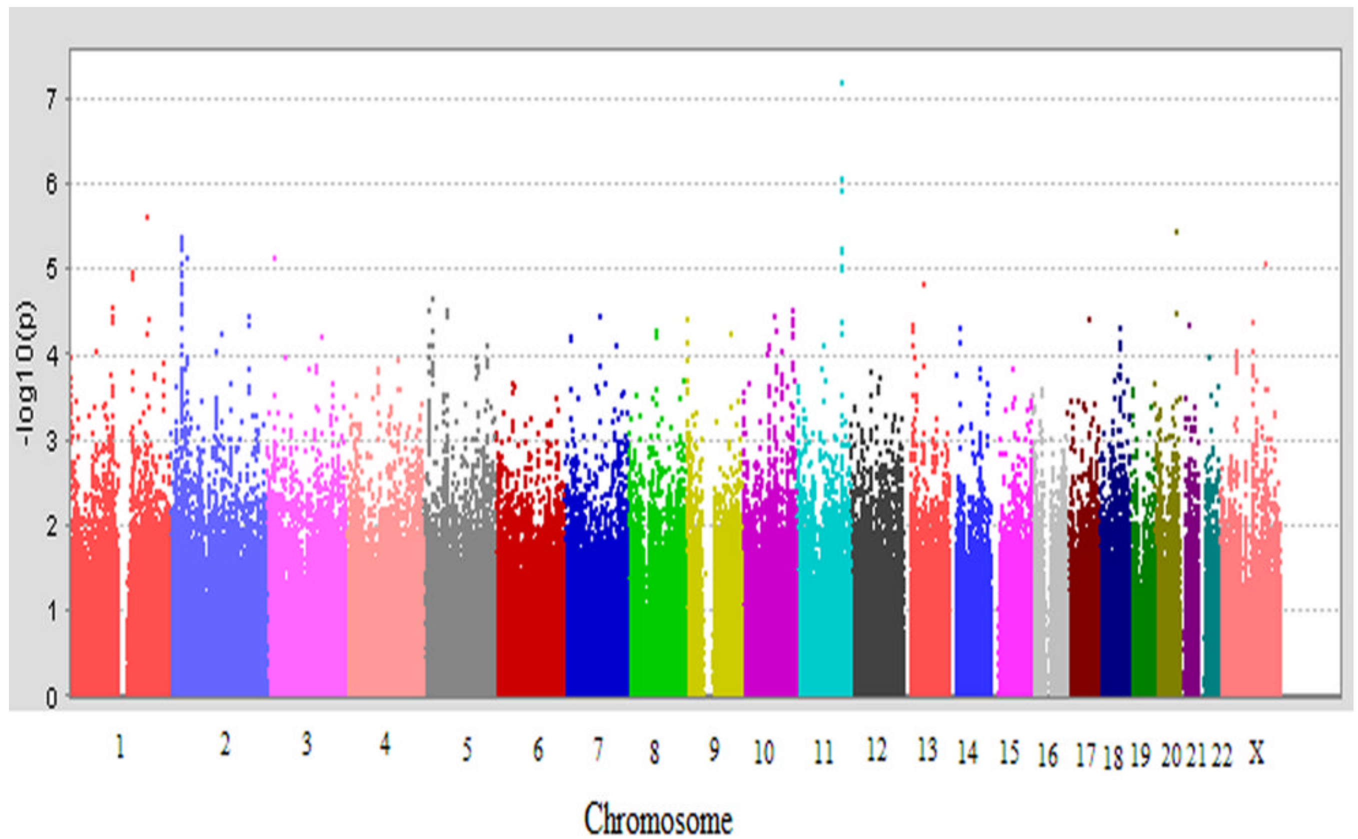

Figure 1. Genome-wide scan of plasma IL-18 levels in the Nurses' Health Study

The analysis was adjusted for age, BMI, and diabetes status. Each point represents a SNP from the 704,409 SNPs remaining after quality control filters. Different bands are used to differentiate SNPs on consecutive autosomal and X chromosomes.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

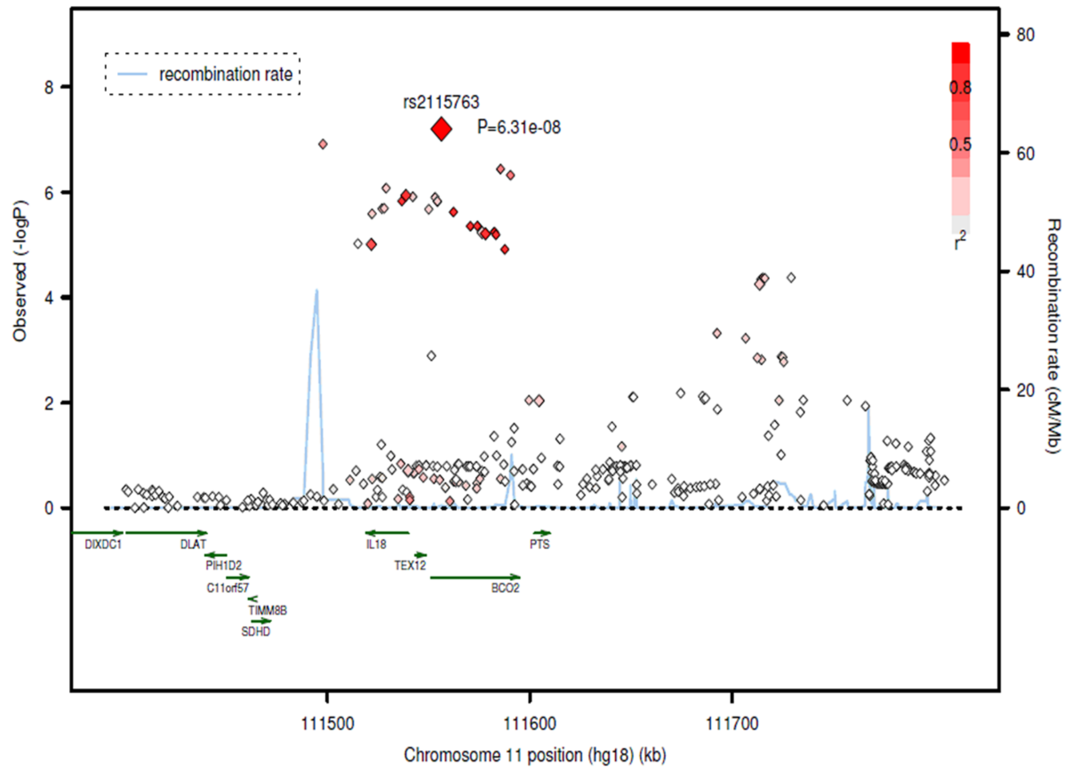

Figure 2. Association signals in and adjacent to *IL18* gene region on chromosome 11

SNPs included in this panel included all SNPs (82 genotyped and 241 imputed SNPs) within the region from 111400 kb to 111810 kb on chromosome 11. The vertical axis representing the $-\log_{10}P$ values. Recombination rates in this region were plotted in the background in light blue. Pairwise LDs between rs2115763 and other SNPs were estimated using HapMap LD data. The color from light red to deep red represents the strength of LD.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 1

Top SNPs for plasma IL-18 levels (pg/mL)*

SNPs	Chrom	Position (kb)†	Alleles (A1/A2)	Gene	Function	MAF‡	HWE§	NHS		WGHS		Combine	Mean (SD) of IL-18 levels//		
								Beta	*P*	Beta	*P*	*P*	A1/A1	A1/A2	A2/A2
rs2115763	11	111556.4	A/T	*BCO2*	intron	0.33	0.85	0.09	6.31E-08	0.09	0.02	3.88E-09	299.8(139.6)	330.0(175.8)	354.1(145.7)
rs1834481	11	111529.0	C/G	*IL18*	intron	0.24	0.60	−0.09	8.38E-07	−0.13	0.003	1.02E-08	334.7(69.3)	303.7(143.8)	279.6(121.3)
rs7106524	11	111538.8	G/A	*IL18*	intron	0.35	0.32	0.08	1.15E-06	0.10	0.009	3.22E-08	304.1(148.6)	325.9(168.7)	352.3(150.1)
rs12420140	11	111576.5	G/A	*BCO2*	intron	0.27	0.39	−0.08	5.83E-06	−0.09	0.02	3.98E-07	336.3(170.6)	302.5(142.9)	297.9(138.9)
rs4935984	11	111578.1	G/A	*BCO2*	intron	0.33	0.95	0.07	6.16E-06	0.09	0.02	4.47E-07	304.2(146.6)	329.7(172.7)	346.7(141.6)
rs10891329	11	111515.1	C/T	IL18	3′ near gene	0.32	0.66	0.07	9.52E-06	--	--	--	306.8(149.4)	325.2(170.0)	354.2(151.7)
rs5744280	11	111521.7	G/A	*IL18*	intron	0.32	0.30	0.07	9.83E-06	0.10	0.01	3.90E-07	307.2(148.4)	325.9(170.1)	351.9(151.6)

*IL-18 levels were log-transformed for linear regression model adjusted for age, diabetes status, and BMI.

†Position based on NCBI build 36.3.

‡MAF, minor allele frequency in NHS.

§HWE, deviation from Hardy-Weinberg equilibrium *P* value in NHS.

//A1: Major allele, A2: Minor allele;

Mean ± SD of IL-18 levels were values in NHS.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Multiple Linear Regression Statistics of SNPs Retained by the Forward Selection Algorithm

	Model 1*			Model 2†		
Variables	**Beta (s.e.)**	***P* value**	**Variance explained (%)‡**	**Beta (s.e.)**	***P* value**	**Variance explained (%)‡**
Age (years)	0.004 (0.001)	4.9E-03	0.50	0.004 (0.001)	4.8E-03	0.54
BMI (kg/m^2)	0.01 (0.002)	6.0E-07	1.67	0.01 (0.002)	3.2E-07	1.80
Diabetes status	0.098 (0.023)	3.2E-05	1.16	0.094 (0.023)	5.4E-05	1.10
rs2115763	0.087 (0.016)	6.4E-08	2.0	0.065 (0.017)	0.0002	0.96
rs1834481	--	--	--	−0.066 (0.019)	0.0006	0.79

*Independent variables included age, BMI, diabetes status (yes/no), and rs2115763.

†Based on model 1, rs1834481 was further included in the model as an independent variable.

‡Measured by R^2, 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.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table 3

Associations between Haplotypes of SNPs rs2115763 and rs1834481 and IL-18 levels

Haplotype				
rs2115763	**rs1834481**	**Frequency**	**Beta**	***P* value**
T	C	0.33	0.088	1.12E-07
A	G	0.24	−0.094	4.05E-07
A	C	0.43	−0.01	0.51

Global *P* value = 3.85E-09.