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Relationship of plasma interleukin-6 and its genetic variants with hypertension in Hong Kong Chinese

Short title: IL-6 and hypertension

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Abstract

BACKGROUND: Interleukin-6 (IL-6) plays a central role in inflammation, insulin resistance, and atherogenesis. We investigated the associations of plasma IL-6 and its genetic variants with hypertension in both cross-sectional and prospective study designs.

METHODS: Plasma IL-6 was measured in 648 normotensive and 294 hypertensive subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS)-2 in 2000-2004 and three tagging SNPs in the IL-6 gene were genotyped. Among subjects normotensive in CRISPS-2 (baseline), 515 subjects were followed up in CRISPS-3 in 2005-2008 and 100 of them had developed hypertension.

RESULTS: At baseline, plasma IL-6 correlated with systolic blood pressure (r=0.128, P<0.001). Hypertensive subjects had significantly higher plasma IL-6 after adjusting for age and sex (geometric mean [95% CI] = 0.60 [0.54-0.65] vs 0.47 [0.44-0.50] ng/l, P=0.021). In multiple logistic regression, higher plasma IL-6 was associated with hypertension in women (P=0.011), but not in men. The SNP rs1800796 was associated with plasma IL-6 (β=-0.109, P=0.001). However, this SNP was not associated with hypertension or blood pressure at baseline. Among subjects normotensive in CRISPS-2, plasma IL-6 was not associated with the development of hypertension in CRISPS-3.

CONCLUSION: The SNP rs1800796 affected plasma IL-6 with a small effect size. Elevated plasma IL-6 is associated with prevalent hypertension in women, but not incident hypertension, suggesting that hypertension could be caused by other factors that might elevate plasma IL-6.

Introduction

Obesity is known to be associated with both inflammation and hypertension. Excess adipose tissues can secrete proinflammatory cytokines, leading to insulin resistance and increased hepatic production of CRP.[1] Interleukin-6 (IL-6) is a pro-inflammatory cytokine, which plays a central role in chronic inflammation, insulin resistance, and atherogenesis.[1] One of the major sites for IL-6 secretion is visceral fat, which may explain the relationship between visceral fat and systemic inflammation in abdominally obese subjects.[2] IL-6 also plays a regulatory role in hepatic production of C-reactive protein (CRP) and fibrinogen,[3] which are often elevated in the plasma of hypertensive subjects.[4,5] Plasma IL-6 is associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in apparently healthy men.[6] Some of the pro-atherosclerotic and pro-inflammatory effects of CRP are mediated partly by increased production of IL-6.[7] Moreover, IL-6 can enhance myocyte hypertrophy and left ventricular remodelling.[8] On the other hand, elevated blood pressure could also lead to vascular inflammation and hence elevated levels of inflammatory markers.[9] Vasoactive peptides, such as angiotensin II, can also increase IL-6 expression leading to vascular inflammation.[10] Therefore, whether plasma IL-6 can predict the development of hypertension is unclear. Conventional cardiovascular risk factors such as age and HDL cholesterol are associated with both hypertension and plasma IL-6,[6,11] which may thus confound the relationship between hypertension and plasma IL-6, leading to inconsistent findings in previous prospective studies, mainly in Caucasians.[11,12]

There are some studies suggesting that genetic variants in the gene encoding IL-6 (*IL6*) may affect the risk of hypertension.[13-15] We previously demonstrated that a single nucleotide polymorphism (SNP), rs1800796 (-572C>G), could influence plasma fibrinogen[16] and fibrinogen may play a role in the development of hypertension in Hong Kong Chinese.[4]

Previously, we studied the only SNP in the 940 bp fragment of the *IL6* promoter present in our population, rs1800796, and found that it was not associated with hypertension.[16] However, the relationship of plasma IL-6 with this SNP and incident hypertension was unknown. Moreover, there may be some other SNPs in *IL6*, located in exons, introns and the more upstream regions that may affect plasma IL-6 and modulate the risk of hypertension. In the present study, we therefore investigated whether tagging SNPs in *IL6* may influence plasma IL-6 and whether plasma IL-6 is related to hypertension and predicts its development.

Methods

Subjects

The Hong Kong Cardiovascular Risk Factor Prevalence Study is a population-based prospective cohort study of cardiovascular risk factors in Hong Kong Chinese.[17,18] In 1995-1996 (CRISPS-1), a random sample of 2,895 Hong Kong Chinese subjects, representative of the general population, was recruited. In 2000-2004, 1,944 subjects were followed up in CRISPS-2 and detailed reasons for non-participation have been described previously.[17,18] Among the 1,944 subjects from the CRISPS-2 cohort, plasma IL-6 was measured in 942 subjects, 49.3% of whom were women who were randomly selected after stratification for sex. The study protocol was approved by the Ethics Committee of the University of Hong Kong and the Institutional Review Board of the Hong Kong West Cluster of Hospitals. Written informed consent was obtained from all participants.

SNPs selection

Tagging SNPs in *IL6* were selected from the HapMap Han Chinese (Phase II data, release 22). There were four tagging SNPs that captured all the 6 SNPs from 5kb region upstream to 2kb downstream of the gene (position 22,728,345-22,740,141, GenBank accession number

NC_000007) with $r^2 \geq 0.9$ and minor allele frequency (MAF) ≥0.05 (Figure 1 & Supplementary Table S1). Genotyping was performed using the MassARRAY system (Sequenom, San Diego, CA) with the iPLEX™ assay. After genotyping, the SNP rs2069852 showed significant deviation from Hardy-Weinberg equilibrium (HWE) among all subjects (*P*=0.002) and normotensive subjects (*P*=0.017), and was excluded from analysis. The remaining three SNPs captured 5 (83.3%) out of 6 genotyped SNPs in the HapMap Han Chinese.

Variables of interest

Hypertension was defined as blood pressure ≥140/90 mm Hg, or taking anti-hypertensive medication. Blood pressure was measured in a sitting position three times at 5-minute intervals using an appropriately sized cuff and a mercury sphygmomanometer by a trained nurse after the subjects had sat for ≥10 minutes as described previously.[17,18] Venous blood was taken from a forearm in the morning after overnight fasting for routine clinical biochemical assays and plasma samples were stored at -70°C until the time of assay. Plasma IL-6 was measured in duplicate using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (Bender MedSystems GmbH, Vienna, Austria). The kit had a sensitivity of 0.02 pg/ml, intra-assay coefficient of variation of 6.9% and inter-assay coefficient of variation of 8.0%. Plasma high-sensitivity CRP and adiponectin were measured as described previously.[19] Details on the measurement of other clinical parameters such as fasting glucose, 2-h glucose after oral glucose tolerance test, fasting insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and fibrinogen had been described previously.[16-19] Data on alcohol drinking, smoking, and history of hypertension were obtained by

standardized interviewing using a questionnaire. Regular drinking was defined as consumption of alcoholic drinks at least once a week.

Statistical analysis

Subject characteristics were compared using SPSS 15.0 (SPSS Inc., Chicago, IL). Variables with skewed distribution, such as triglycerides, fasting glucose, 2-h glucose, fasting insulin, HOMA-IR, plasma adiponectin, CRP, and IL-6, were log-transformed before analysis. Variables were used as covariates in the multiple regression analysis if they were likely to affect the risks of hypertension, or correlated with plasma IL-6. For variables that were highly correlated such as body mass index (BMI) and waist circumference, only one was entered into the regression model. Haploview (version 4.1) was used to estimate pairwise linkage disequilibrium (LD) and select the tagging SNPs.[20] Single variant analysis was performed under the assumption of a dominant model of inheritance for the minor allele as there were few subjects who were homozygous for the minor alleles. Using Genetic Power Calculator[21], our study of 294 hypertensive subjects and 648 normotensive subjects had 80% power to detect an odds ratio (OR) of 1.53 for an allele frequency of 0.15 at the 5% significance level in a dominant inheritance model, assuming a disease prevalence of 31.2%. Correction for multiple testing of three SNPs was performed by the SNP spectral decomposition method (SNPSpD).[22] Under this method, the effective number of independent marker loci (M_{effLi}) was 2 and the experimental-wide significance threshold to keep type 1 error rate at 5% was 0.0253. Correction for testing of multiple phenotypes was not performed as the phenotypes tested were closely related to each other.

Results

Clinical characteristics and genotyping

Among 1,944 subjects in CRISPS-2, plasma IL-6 was measured in 942 subjects, who were randomly selected after stratification for sex, 49.3% of whom were women. There was no significant difference in age between those with (n=942) and without (n=1,002) plasma IL-6 measurement among all subjects, men, or women. There was no significant difference in waist circumference, triglycerides, fasting glucose, 2-h glucose, fasting insulin, HOMA-IR, fibrinogen, and proportion of regular drinking between those with and without plasma IL-6 measurement after adjusting for sex. However, subjects with plasma IL-6 measurement had lower BMI, LDL cholesterol and CRP, but higher HDL cholesterol, adiponectin, and prevalence of smoking and hypertension after adjusting for sex (all P<0.05). Table 1 shows the baseline clinical characteristics of the 294 hypertensive and 648 normotensive subjects in CRISPS-2. Supplementary Table S2 shows the baseline clinical characteristics of the subjects by sex. Women had significantly lower BMI, waist circumference, SBP, DBP, triglycerides, and fasting glucose, but higher HDL cholesterol, fibrinogen, and plasma adiponectin. Women also had much lower proportions of current smoking and regular drinking than men.

The three SNPs, rs17147230, rs1800796, and rs2069837, were all genotyped successfully in ≥99.9% of the subjects with MAFs of 0.430, 0.207, and 0.158 respectively. These SNPs did not show significant deviation from HWE among all subjects or among case- and control-specific subgroups (P>0.08). The pairwise LD (r^2) between rs17147230 and rs1800796, rs1800796 and rs2069837, and rs17147230 and rs2069837 were 0.166, 0.680, and 0.093 respectively.

Association of plasma IL-6 with hypertension at baseline

At baseline, plasma IL-6 correlated with age (r=0.174, P<0.001), BMI (r=0.121, P<0.001), waist circumference (r=0.123, P<0.001), SBP (r=0.128, P<0.001), log-transformed triglycerides (r=0.077, P=0.019), HDL cholesterol (r=-0.100, P=0.002), log-transformed CRP (r=0.254, P<0.001), and fibrinogen (r=0.236, P<0.001). The correlation of plasma IL-6 with SBP remained significant after excluding subjects on anti-hypertensive medication (r=0.083, P=0.020) or when blood pressures in treated subjects were adjusted by adding 10/5 mm Hg (r=0.140, P<0.001).[23] Figure 2 shows the mean SBP according to plasma IL-6 tertiles. Plasma IL-6 did not differ significantly between subjects who were or were not current smokers (geometric mean [95% CI] = 0.54 [0.48-0.61] vs 0.49 [0.47-0.53] ng/l, P=0.192). It was not related to other clinical characteristics shown in Table 1 (all P≥0.05). Among hypertensive subjects, plasma IL-6 did not differ between subjects with (n=170) and without (n=124) anti-hypertensive medication (P=0.120 after adjusting for age and sex).

As shown in Table 1, plasma IL-6 was higher in hypertensive subjects at baseline (P=0.021). In an unadjusted model, the association of plasma IL-6 with hypertension was significant in women (P<0.001), but not in men (P=0.466) in sex-specific analysis (P for interaction=0.001). In multiple logistic regression analysis, higher plasma IL-6 was independently associated with hypertension in women only (Table 2). The sex-interaction in the association of plasma IL-6 with hypertension remained significant after adjusting for the main effects of all the covariates (P=0.010). No significant interaction between plasma IL-6 and other covariates was found in the full adjustment model. Similar results were obtained if BMI was replaced by waist circumference in the regression model (data not shown) or if BMI was removed from the model (P=0.627 and 0.003 in men and women for IL-6 respectively).

Association of SNPs with plasma IL-6 at baseline

As shown in Figure 3, the SNP rs1800796 was significantly associated with plasma IL-6 at baseline after adjusting for age and sex (β=-0.090, *P*=0.005). The presence of the minor G allele was associated with a plasma IL-6 level 14.6 (95% CI: 4.6-23.5)% lower. In multiple linear regression analysis, rs1800796 was independently associated with plasma IL-6 (Table 3). There was no significant sex-interaction after adjusting for the main effects of all the covariates (*P*=0.427). Similar results were obtained if BMI was replaced by waist circumference or was removed in the regression model (*P*=0.001 for rs1800796 in both cases). Among the clinical characteristics shown in Table 1, the presence of the minor G allele of rs1800796 was also associated with higher plasma fibrinogen (mean±SD: 3.08±0.61 g/l for CG+GG vs 2.94±0.60 g/l for CC) after adjusting for age, sex, and plasma IL-6 (*P*<0.001), but not other characteristics.

Association of SNPs with hypertension at baseline

None of the SNPs were significantly associated with hypertension at baseline (*P*>0.05, Supplementary Table 3). Among subjects not taking anti-hypertensive medication, none of the SNPs were significantly associated with SBP or DBP at baseline (*P*>0.05, Supplementary Table S3). Similar insignificant results were obtained when blood pressures in treated subjects were adjusted by adding 10/5 mmHg (data not shown).[23] Sex-specific analysis also did not reveal a significant association of SNPs with prevalent hypertension, SBP or DBP (*P*>0.05, Supplementary Table S3).

Follow-up study in CRISPS-3

Among 648 subjects normotensive in CRISPS-2, 515 subjects were followed up in CRISPS-3 in 2005-2008 after a median interval of five years and 100 of them had developed hypertension in CRISPS-3 (Table 4). Baseline plasma IL-6 did not differ significantly

between subjects with or without incident hypertension. Sex-specific analysis also did not reveal a significant difference. Among 294 subjects hypertensive in CRISPS-2, 213 subjects were followed up in CRISPS-3 and 189 subjects remained hypertensive in CRISPS-3. Among 415 subjects normotensive at both visits and 189 subjects hypertensive at both visits, plasma IL-6 level was higher in hypertensive subjects, especially in women. Supplementary Table S4 shows the baseline characteristics of these subjects. However, none of the SNPs were significantly associated with hypertension or blood pressure (both adjusting for and removing those taking anti-hypertensive medication) among subjects who were either normotensive or hypertensive at both CRISPS-2 and CRISPS-3 (data not shown).

Discussion

Elevated plasma levels of inflammatory markers are often associated with cardiovascular diseases and mortality in prospective studies.[8,24,25] In some studies, the association is even stronger for plasma IL-6 than other inflammatory markers such as CRP and tumor necrosis factor (TNF)-α.[8,25] As hypertension is a risk factor for cardiovascular diseases and mortality, plasma IL-6 may also be associated with hypertension or blood pressure. The association of plasma IL-6 with blood pressure and prevalent hypertension in our cross-sectional study supports the concept of chronic inflammation in hypertension.[4-6,11,12] The association tended to be stronger in women with significant sex-interaction. This is consistent with the higher prevalence of cardiovascular risk factors in women with hypertension.[26] This gender difference is also observed in other inflammatory markers, such as fibrinogen.[4] In this study sample, the proportion of current smoking was much higher in men than women. Interestingly, smoking could interact with genetic variants in *IL6* on the circulating levels of inflammatory markers.[27] This may contribute partly to the difference in the association of

plasma IL-6 with hypertension and the strikingly different OR for the association of smoking with hypertension between men and women.

In fact, there are two prospective studies on the role of IL-6 in the prediction of the development of hypertension,[11,12] but their findings are inconsistent. In the Women's Health Study, the association of plasma IL-6 with incident hypertension was no longer significant after further adjusting for conventional cardiovascular risk factors and plasma CRP.[11] In the Multi-Ethnic Study of Atherosclerosis (MESA), plasma IL-6 was significantly associated with incident hypertension even after adjusting for conventional cardiovascular risk factors.[12] However, compared to the Women's Health Study, plasma CRP was not used as a covariate in the adjustment model in the MESA study. In our study, baseline plasma IL-6 did not differ significantly between subjects with or without new-onset hypertension at follow-up in a simple model after adjusting for age, sex, and follow-up duration. Therefore, our study supports the findings from the Women's Health Study that plasma IL-6 could not predict the future risk of hypertension and hypertension may be caused by other factors that elevate plasma IL-6. In the Copenhagen City Heart Study, plasma CRP and fibrinogen, acute phase proteins downstream of IL-6 in the inflammatory cascade and markers of chronic inflammation, were not associated with blood pressure.[28]

As hypertension is a complex disease and environmental factors also play an important role in its development, we studied the association of genetic variants with hypertension or blood pressure related traits, which were less likely to be confounded by environmental factors. The SNP rs1800796, which was significantly associated with plasma IL-6, was not associated with hypertension or blood pressures in our study. This may be due to its small effect size on plasma IL-6. Nevertheless, together with the negative association of plasma IL-6 with

incident hypertension, our finding also does not support a major direct role of IL-6 in the development of hypertension.

The SNP rs1800796 possesses functional effects in the luciferase reporter gene assay.[29] In our study, the minor G allele of rs1800796 was significantly associated with lower plasma IL-6 but higher plasma fibrinogen. It may seem contradictory as both elevated plasma IL-6 and fibrinogen are associated with higher risks of hypertension[4,6,11,12] and cardiovascular adverse events.[8,24,25] In fact, endogenous IL-6 also has anti-inflammatory properties in the regulation of pro-inflammatory cytokines, such as TNF-α and IL-1β, in both local and systemic acute inflammatory responses.[30] Similarly, a genetic variant, rs8192284, in the IL-6 receptor gene (*IL6R*) is associated with lower plasma IL-6, but higher risk for the metabolic syndrome in Southern Chinese.[31] It is interesting that the G allele of rs1800796 was associated with lower plasma IL-6 in our and other studies,[32,33] but higher plasma IL-6 in some other studies.[34,35] Nevertheless, this discrepancy in the allelic effect on plasma IL-6 in the literature does not detract from the final conclusion of the present study regarding the association of a genetic variant in *IL6* with its plasma level.

In this study, genetic variants in *IL6* are not associated with hypertension or any blood pressure related traits. This is consistent with our previous case-control study in Hong Kong Chinese, showing no significant association of rs1800796 with hypertension.[16] Similarly, rs1800795 in *IL6* was not associated with incident hypertension in a prospective cohort of Caucasian women.[36] In fact, genetic variants in *IL6* have been shown to be associated with type 2 diabetes,[35,37] ischemic stroke,[38] and coronary heart disease.[13,39] Therefore, IL-6 may be more related to these diseases than hypertension. The elevated plasma IL-6 in hypertension

may be partly due to the presence of these closely related diseases or their underlying risk factors.

Our study has the advantages of investigating the relationship between IL-6 and hypertension using both cross-sectional and prospective study designs as well as genotype data. However, the major limitation of this study is the small sample size, which limits the power to identify the risk factors of incident hypertension and detect small effect size of genetic variants that are usually encountered in recent genome-wide association studies of hypertension.[40] The association of SNPs with blood pressure and that of plasma IL-6 with incident hypertension may become significant if a larger cohort study were used. As subjects were recruited from the general population, hypertensive subjects were older than normotensive subjects. There was also a high percentage of anti-hypertensive medication treatment among hypertensive subjects (57.8%) in our population-based cohort although plasma IL-6 was not related to anti-hypertensive medication treatment. Moreover, as hypertension is a polygenic disease, our study only investigated SNPs in *IL6*, but not those in genes encoding other inflammatory markers, which may interact with SNPs in *IL6*. The single measurement of plasma IL-6 may not be sufficient to reflect chronic IL-6 level and inflammation status.

In conclusion, plasma IL-6 is elevated in hypertension, but cannot predict incident hypertension. Although plasma IL-6 is affected by the SNP rs1800796, this SNP is not associated with hypertension or blood pressure, suggesting that hypertension is caused by other factors that elevate plasma IL-6.

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Disclosure

The authors declared no conflict of interest.

References

1. Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* 2003;24:278-301.
2. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes* 2007;56:1010-1013.
3. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989;242:237-239.
4. Ong KL, Tso AW, Cherny SS, Sham PC, Lam KS, Jiang CQ, Thomas GN, Lam TH, Cheung BM. A genetic variant in the gene encoding fibrinogen beta chain predicted development of hypertension in Chinese men. *Thromb Haemost* 2010;103:728-735.
5. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA* 2003;290:2945-2951.
6. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. *Hypertension* 2001;38:399-403.
7. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, Dhillon B, Mickle DA. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation* 2002;105:1890-1896.
8. Vasan RS, Sullivan LM, Roubenoff R, Dinarello CA, Harris T, Benjamin EJ, Sawyer

DB, Levy D, Wilson PW, D'Agostino RB; Framingham Heart Study. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. *Circulation* 2003;107:1486-1491.

9. Virdis A, Ghiadoni L, Plantinga Y, Taddei S, Salvetti A. C-reactive protein and hypertension: is there a causal relationship? *Curr Pharm Des* 2007;13:1693-1698.
10. Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. *Circulation* 2000;101:1372-1378.
11. Sesso HD, Wang L, Buring JE, Ridker PM, Gaziano JM. Comparison of interleukin-6 and C-reactive protein for the risk of developing hypertension in women. *Hypertension* 2007;49:304-310.
12. Lakoski SG, Cushman M, Siscovick DS, Blumenthal RS, Palmas W, Burke G, Herrington DM. The relationship between inflammation, obesity and risk for hypertension in the Multi-Ethnic Study of Atherosclerosis (MESA). *J Hum Hypertens* 2011;25:73-79.
13. Humphries SE, Luong LA, Ogg MS, Hawe E, Miller GJ. The interleukin-6 -174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. *Eur Heart J* 2001;22:2243-2252.
14. Tanaka C, Mannami T, Kamide K, Takiuchi S, Kokubo Y, Katsuya T, Kawano Y, Miyata T, Ogihara T, Tomoike H. Single nucleotide polymorphisms in the interleukin-6 gene associated with blood pressure and atherosclerosis in a Japanese general population. *Hypertens Res* 2005;28:35-41.
15. Nakajima T, Ota N, Yoshida H, Watanabe S, Suzuki T, Emi M. Allelic variants in the interleukin-6 gene and essential hypertension in Japanese women. *Genes Immun*

1999;1:115-119.

16. Wong LY, Leung RY, Ong KL, Cheung BM. Plasma levels of fibrinogen and C-reactive protein are related to interleukin-6 gene -572C>G polymorphism in subjects with and without hypertension. *J Hum Hypertens* 2007;21:875-882.

17. Cheung BM, Wat NM, Man YB, Tam S, Thomas GN, Leung GM, Cheng CH, Woo J, Janus ED, Lau CP, Lam TH, Lam KS. Development of diabetes in Chinese with the metabolic syndrome: a 6-year prospective study. *Diabetes Care* 2007;30:1430-1436.

18. Cheung BM, Wat NM, Man YB, Tam S, Cheng CH, Leung GM, Woo J, Janus ED, Lau CP, Lam TH, Lam KS. Relationship between the metabolic syndrome and the development of hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2). *Am J Hypertens* 2008;21:17-22.

19. Ong KL, Li M, Tso AW, Xu A, Cherny SS, Sham PC, Tse HF, Lam TH, Cheung BM, Lam KS; Investigators of the Hong Kong Cardiovascular Risk Factor Prevalence Study. Association of genetic variants in the adiponectin gene with adiponectin level and hypertension in Hong Kong Chinese. *Eur J Endocrinol* 2010;163:251-7.

20. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-265.

21. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003;19:149-50

22. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004;74:765-769.

23. Cui JS, Hopper JL, Harrap SB. Antihypertensive treatments obscure familial contributions to blood pressure variation. *Hypertension* 2003;41:207-210.

24. Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6

and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. *JAMA* 2001;286:2107-2113.

25. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events: results from the Health ABC study. *Circulation* 2003;108:2317-2322.
26. Ong KL, Tso AW, Lam KS, Cheung BM. Gender difference in blood pressure control and cardiovascular risk factors in Americans with diagnosed hypertension. *Hypertension* 2008;51:1142-1148.
27. Sunyer J, Forastiere F, Pekkanen J, Plana E, Kolz M, Pistelli R, Jacquemin B, Brüske-Hohlfeld I, Pitsavos Ch, Bellander T, Koenig W, Peters A; AIRGENE Study Group. Interaction between smoking and the interleukin-6 gene affects systemic levels of inflammatory biomarkers. *Nicotine Tob Res* 2009;11:1347-1353.
28. Asferg C, Jensen JS, Marott JL, Appleyard M, Møgelvang R, Jensen GB, Jeppesen J. Markers of inflammation and hemodynamic measurements in obesity: Copenhagen City Heart Study. *Am J Hypertens* 2009;22:451-456.
29. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. *J Clin Endocrinol Metab* 2003;88:255-259.
30. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, Achong MK. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest* 1998;101:311-320.
31. Jiang CQ, Lam TH, Liu B, Lin JM, Yue XJ, Jin YL, Cheung BM, Thomas GN. Interleukin-6 receptor gene polymorphism modulates interleukin-6 levels and the

metabolic syndrome: GBCS-CVD. *Obesity (Silver Spring)* 2010;18:1969-1974.

32. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD, Humphries SE. Interleukin-6 gene -174g>c and -572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *Arterioscler Thromb Vasc Biol* 2001;21:1458-1463.
33. Mälarstig A, Wallentin L, Siegbahn A. Genetic variation in the interleukin-6 gene in relation to risk and outcomes in acute coronary syndrome. *Thromb Res* 2007;119:467-473.
34. Sanderson SC, Kumari M, Brunner EJ, Miller MA, Rumley A, Lowe GD, Marmot MG, Humphries SE. Association between IL6 gene variants -174G>C and -572G>C and serum IL-6 levels: interactions with social position in the Whitehall II cohort. *Atherosclerosis* 2009;204:459-64.
35. Koh SJ, Jang Y, Hyun YJ, Park JY, Song YD, Shin KK, Chae JS, Kim BK, Ordovas JM, Lee JH. Interleukin-6 (IL-6) -572C-->G promoter polymorphism is associated with type 2 diabetes risk in Koreans. *Clin Endocrinol (Oxf)* 2009;70:238-244.
36. Conen D, Cheng S, Steiner LL, Buring JE, Ridker PM, Zee RY. Association of 77 polymorphisms in 52 candidate genes with blood pressure progression and incident hypertension: the Women's Genome Health Study. *J Hypertens* 2009;27:476-483.
37. Huth C, Heid IM, Vollmert C, Gieger C, Grallert H, Wolford JK, Langer B, Thorand B, Klopp N, Hamid YH, Pedersen O, Hansen T, Lyssenko V, Groop L, Meisinger C, Döring A, Löwel H, Lieb W, Hengstenberg C, Rathmann W, Martin S, Stephens JW, Ireland H, Mather H, Miller GJ, Stringham HM, Boehnke M, Tuomilehto J, Boeing H, Möhlig M, Spranger J, Pfeiffer A, Wernstedt I, Niklason A, López-Bermejo A, Fernández-Real JM, Hanson RL, Gallart L, Vendrell J, Tsiavou A, Hatziagelaki E, Humphries SE, Wichmann HE, Herder C, Illig T. IL6 gene promoter polymorphisms

and type 2 diabetes: joint analysis of individual participants' data from 21 studies. *Diabetes* 2006;55:2915-2921.

38. Tong Y, Wang Z, Geng Y, Liu J, Zhang R, Lin Q, Li X, Huang D, Gao S, Hu D, Li Y, Cheng J, Lu Z. The association of functional polymorphisms of IL-6 gene promoter with ischemic stroke: analysis in two Chinese populations. *Biochem Biophys Res Commun* 2010;391:481-485.
39. Basso F, Lowe GD, Rumley A, McMahon AD, Humphries SE. Interleukin-6 -174G>C polymorphism and risk of coronary heart disease in West of Scotland coronary prevention study (WOSCOPS). *Arterioscler Thromb Vasc Biol* 2002;22:599-604.
40. Delles C, McBride MW, Graham D, Padmanabhan S, Dominiczak AF. Genetics of hypertension: from experimental animals to humans. *Biochim Biophys Acta* 2010;1802:1299-1308.

Figure legend

Figure 1 A schematic diagram of *IL6*. Exons and introns are represented by boxes and thin lines respectively with their sizes indicated above. The MAFs of the tagging SNPs in HapMap Han Chinese are indicated in the brackets.

Figure 2 Mean SBP in subjects according to the sex-specific plasma IL-6 tertiles at baseline. For all subjects, blood pressures in treated subjects were adjusted by adding 10/5 mm Hg.[17] Error bars indicate standard errors. The cut-off values of tertiles 1, 2 and 3 in men and women respectively are <0.438 ng/l and <0.380 ng/l, 0.438-0.763 ng/l and 0.380-0.683 ng/l, and ≥0.764 ng/l and ≥0.784 ng/l.

Figure 3 Association of SNPs with plasma IL-6 at baseline. Error bars indicate standard errors of geometric means. Subjects homozygous for the minor allele were grouped with heterozygotes for comparison with those homozygous for the major allele to increase the sample size, and *P* values were adjusted for age and sex. **P* values that can pass multiple testing correction ($P<0.0253$).

Table 1 Clinical characteristics of 942 subjects in CRISPS-2 (baseline)

Characteristics	Normotensive (*n*=648)	Hypertensive (*n*=294)
Age (years)	49.9 ± 11.0	59.2 ± 10.7‡
Women (%)	50.5	46.6
BMI (kg/m^2)	23.3 ± 3.2	25.2 ± 3.3‡
Waist circumference (cm)	78.0 ± 9.7	84.7 ± 9.1‡
SBP (mm Hg)[b]	115.2 ± 11.3	147.4 ± 14.5‡
DBP (mm Hg)[b]	72.8 ± 8.2	89.3 ± 10.5‡
Triglyceride (mmol/l)[a]	1.11 (1.07-1.16)	1.49 (1.41-1.58)‡
HDL cholesterol (mmol/l)	1.40 ± 0.36	1.27 ± 0.37‡
LDL cholesterol (mmol/l)	3.17 ± 0.78	3.34 ± 0.89*
Fasting glucose (mmol/l)[a]	5.13 (5.06-5.20)	5.78 (5.62-5.95)‡
2-h glucose (mmol/l)[a]	6.50 (6.32-6.69)	8.10 (7.72-8.50)‡
Fasting insulin (mIU/l)[a]	6.67 (6.40-6.95)	9.27 (8.66-9.93)‡
HOMA-IR[a]	1.52 (1.45-1.59)	2.38 (2.20-2.58)‡
Fibrinogen (g/l)	2.92 ± 0.58	3.15 ± 0.65†
Adiponectin (mg/l)[a]	7.28 (6.93-7.64)	6.37 (5.91-6.86)‡
CRP (mg/l)[a]	0.54 (0.50-0.58)	0.88 (0.79-0.98)‡
IL-6 (ng/l)[a]	0.47 (0.44-0.50)	0.60 (0.54-0.65)*
men	0.52 (0.48-0.57)	0.55 (0.48-0.63)
women	0.42 (0.38-0.46)	0.65 (0.58-0.73)‡
Current smoking (%)	22.4	14.6‡
Regular drinking (%)	12.2	9.0*

Data are expressed as mean±SD unless otherwise stated.

*P<0.05, †P<0.01, and ‡P<0.001 for normotensive versus hypertensive subjects after adjusting for age and sex, except for age (adjusted for sex only), sex (for age only) and sex-specific IL-6 level (for age only).

[a]Data are expressed as geometric mean (95% CI) due to skewed distributions.

[b]Subjects on anti-hypertensive medication were excluded (*n*=170).

Table 2 Multiple logistic regression analysis for hypertension at baseline

Parameters	**Men (*n*=416)**		**Women (*n*=415)**	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
Age (years)	1.07 (1.04-1.09)	<0.001*	1.08 (1.05-1.11)	<0.001*
BMI (kg/m^2)	1.12 (1.02-1.23)	0.017*	1.11 (1.01-1.21)	0.024*
2-h glucose (mmol/l)[a]	1.41 (1.08-1.84)	0.011*	0.90 (0.65-1.23)	0.501
HOMA-IR[a]	1.04 (0.72-1.52)	0.827	1.70 (1.18-2.46)	0.004*
Current smoking	0.48 (0.28-0.83)	0.008*	3.00 (0.88-10.29)	0.080
IL-6 (ng/l)[a]	0.92 (0.72-1.18)	0.517	1.49 (1.10-2.00)	0.009*
Nagelkerke r^2	0.299		0.358	

Triglycerides (log-transformed), HDL cholesterol, adiponectin (log-transformed), and regular drinking were also included in the model but they were not significant factors in all subjects, men or women (P>0.05).

[a]ORs are expressed in term of per SD of log-transformed unit.

*P<0.05.

Table 3 Multiple linear regression analysis for plasma IL-6 (log-transformed) at baseline (*n*=925)

Parameters	**β**	***P***
Age (years)	0.099	0.007*
CRP (mg/l, log-transformed)	0.161	<0.001*
Fibrinogen (g/l)	0.158	<0.001*
Presence of minor G allele of rs1800796	-0.108	0.001*
r^2	0.103	

Sex, BMI, triglycerides (log-transformed), SBP, and HDL cholesterol were also included in the model but they were not significant factors (*P*>0.05).

*$P<0.05$.

Table 4 Baseline characteristics of 515 normotensive subjects in CRISPS-2 according to the hypertension status in CRISPS-3

Characteristics	**Normotensive (*n*=415)**	**Hypertensive (*n*=100)**	***P*[b]**
Age (years)	47.0 ± 9.6	55.7 ± 9.5	<0.001
Women (%)	52.3	46.0	0.487
BMI (kg/m^2)	23.0 ± 2.9	24.1 ± 3.8	0.002
SBP (mm Hg)	112.4 ± 10.2	124.8 ± 8.7	<0.001
DBP (mm Hg)	71.9 ± 8.1	77.0 ±7.4	<0.001
IL-6 (ng/l)[a]	0.44 (0.41-0.48)	0.51 (0.45-0.59)	0.355
men	0.49 (0.44-0.56)	0.56 (0.47-0.67)	0.624
women	0.40 (0.36-0.46)	0.46 (0.37-0.57)	0.401

Data are expressed as mean±SD or geometric mean (95% CI) unless otherwise stated.

[a]Data are expressed as geometric mean (95% CI) due to skewed distributions.

[b]Adjusted for baseline age, sex, and follow-up duration.

Figure 1

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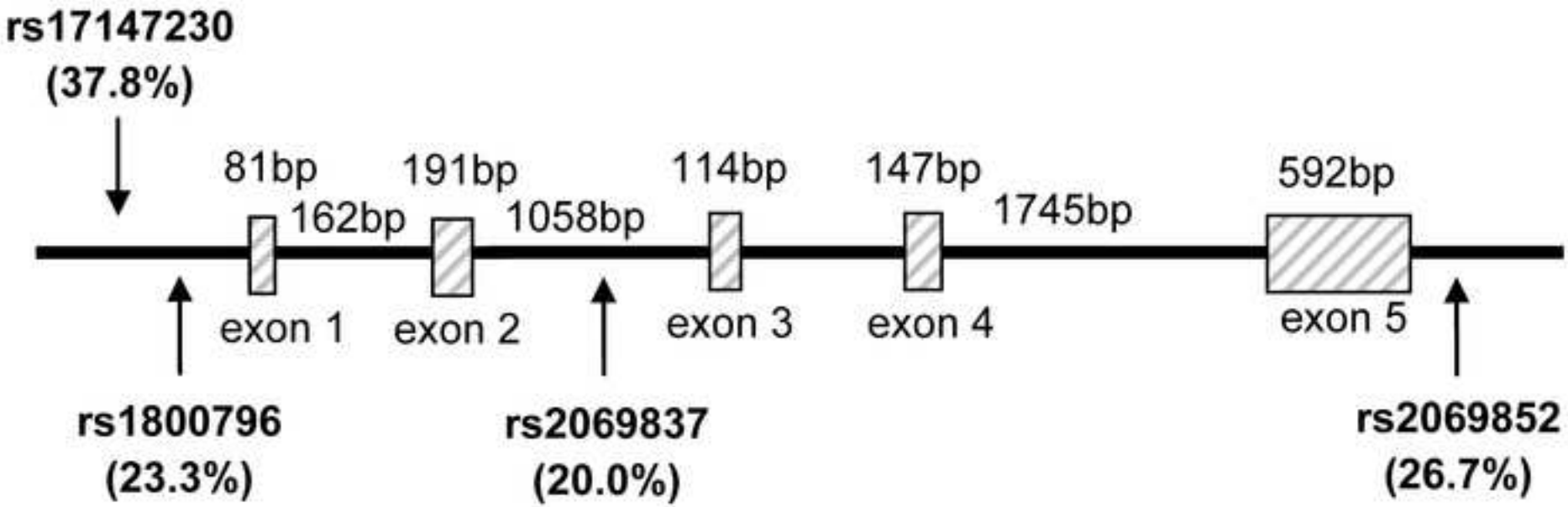

Figure 2

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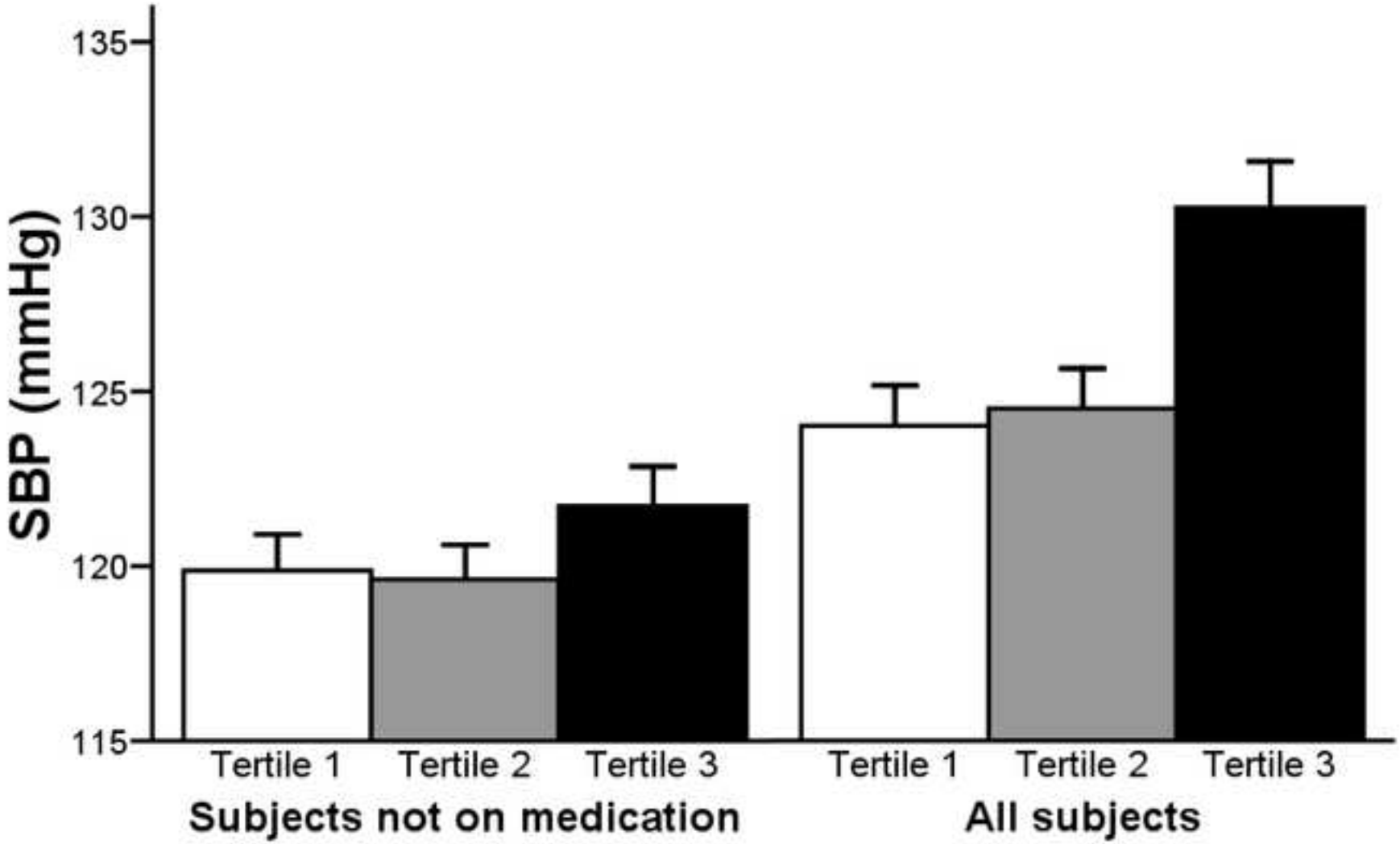

Figure 3

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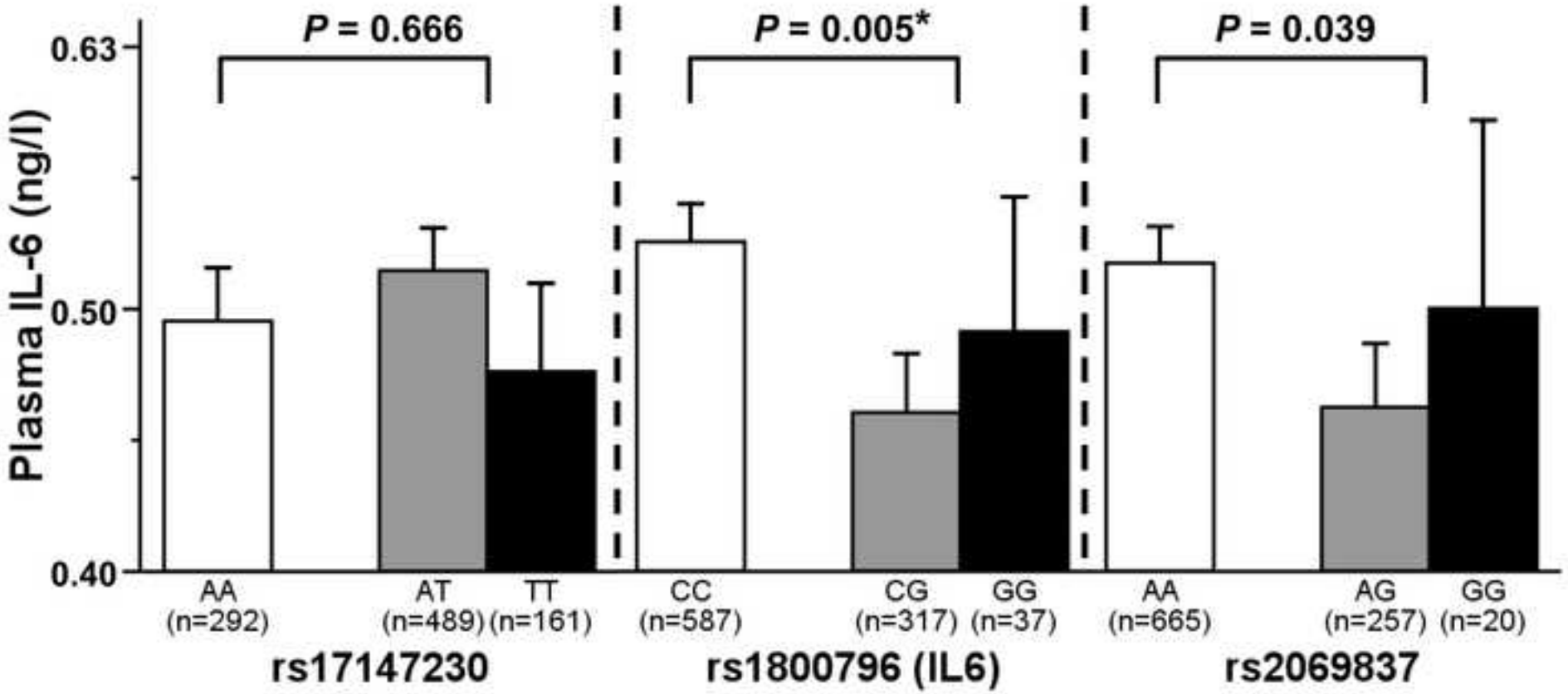

Supplementary Table S1 Tagging SNP selection

SNP	Chromosome position (NC_000007)	Location in gene	MAF (CHB HapMap)	Alleles (Major : Minor)	Tagging SNP	r^2
rs17147230	22728701	5' near gene	0.378	A:T	rs17147230	1.00
rs1800796	22732771	5' near gene	0.233	C:G	rs1800796	1.00
rs2069837	22734552	intron 2	0.2	A:G	rs2069837	1.00
rs1524107	22734744	intron 2	0.25	T:C	rs1800796	0.94
rs2066992	22734774	intron 2	0.244	T:G	rs1800796	0.94
rs2069852	22738785	3' near gene	0.267	A:G	rs2069852	1.00

Supplementary Table S2 Clinical characteristics of 942 subjects in CRISPS-2 (baseline) by sex

Characteristics	**Men (*n*=478)**	**Women (*n*=464)**
Age (years)	53.3 ± 12.0	52.3 ± 11.5
BMI (kg/m^2)	24.2 ± 3.3	23.5 ± 3.3†
Waist circumference (cm)	84.1 ± 9.3	75.9 ± 9.0‡
Hypertension (%)	32.8	29.5
SBP (mm Hg)[b]	123.1 ± 14.4	117.5 ± 18.5‡
DBP (mm Hg)[b]	78.0 ± 9.8	72.8 ± 10.6†
Triglyceride (mmol/l)[a]	1.34 (1.28-1.41)	1.11 (1.06-1.16)‡
HDL cholesterol (mmol/l)	1.24 ± 0.35	1.47 ± 0.36‡
LDL cholesterol (mmol/l)	3.28 ± 0.81	3.17 ± 0.82
Fasting glucose (mmol/l)[a]	5.44 (5.34-5.54)	5.21 (5.11-5.32)†
2-h glucose (mmol/l)[a]	6.91 (6.66-7.17)	6.93 (6.70-7.17)
Fasting insulin (mIU/l)[a]	7.30 (6.94-7.68)	7.49 (7.10-7.91)
HOMA-IR[a]	1.76 (1.67-1.87)	1.74 (1.63-1.85)
Fibrinogen (g/l)	2.93 ± 0.63	3.05 ± 0.58†
Adiponectin (mg/l)[a]	5.97 (5.63-6.33)	8.21 (7.79-8.65)‡
CRP (mg/l)[a]	0.67 (0.61-0.73)	0.59 (0.54-0.65)
IL-6 (ng/l)[a]	0.53 (0.49-0.57)	0.48 (0.44-0.52)
Current smoking (%)	35.1	4.3‡
Regular drinking (%)	18.9	3.5‡

Data are expressed as mean±SD unless otherwise stated.

*P<0.05, †P<0.01, and ‡P<0.001 for men versus women subjects after adjusting for age, except for age (no adjustment).

[a]Data are expressed as geometric mean (95% CI) due to skewed distributions.

[b]Subjects on anti-hypertensive medication were excluded (*n*=86 in men and 84 in women).

Supplementary Table S3 *P* values for the association of SNPs with hypertension and blood pressure at baseline in CRISPS-2

SNP	*P*		
	All	**Men**	**Women**
Hypertension (*n*=942)			
rs17147230	0.737	0.574	0.216
rs1800796	0.432	0.993	0.222
rs2069837	0.242	0.714	0.145
SBP (*n*=772)*			
rs17147230	0.736	0.523	0.874
rs1800796	0.120	0.047	0.618
rs2069837	0.241	0.064	0.741
DBP (*n*=772)*			
rs17147230	0.685	0.433	0.671
rs1800796	0.204	0.271	0.419
rs2069837	0.186	0.151	0.447

All *P* values were adjusted for age and sex (except in sex-specific analysis). Subjects homozygous for the minor allele were grouped with heterozygotes for comparison with those homozygous for the major allele.

*Subjects on anti-hypertensive medication were excluded (*n*=170).

Supplementary Table S4 Baseline characteristics of 415 subjects normotensive at both CRISPS-2 and CRISPS-3, and 189 subjects hypertensive at both CRISPS-2 and CRISPS-3

Characteristics	**Normotensive (*n*=415)**	**Hypertensive (*n*=189)**	***P*[c]**
Age (years)	47.0 ± 9.6	57.8 ± 9.6	0.173
Women (%)	52.3	47.6	0.758
BMI (kg/m^2)	23.0 ± 2.9	25.4 ± 3.4	<0.001
SBP (mm Hg)[a]	112.4 ± 10.2	150.7 ± 13.5	<0.001
DBP (mm Hg)[a]	71.9 ± 8.1	89.8 ±11.9	<0.001
IL-6 (ng/l)[b]	0.44 (0.41-0.48)	0.60 (0.54-0.68)	0.005
men	0.49 (0.44-0.56)	0.56 (0.48-0.67)	0.564
women	0.40 (0.36-0.46)	0.65 (0.56-0.75)	0.001

Data are expressed as mean±SD or geometric mean (95% CI) unless otherwise stated.

[a]Subjects on anti-hypertensive medication were excluded (*n*=112).

[b]Data are expressed as geometric mean (95% CI) due to skewed distributions.

[c]Adjusted for baseline age and sex.