Published in final edited form as:
*Am J Cardiol*. 2008 June 15; 101(12): 1683–1688. doi:10.1016/j.amjcard.2008.02.052.

# Cholesterol Ester Transfer Protein, Interleukin 8, Peroxisome Proliferator Activator Receptor Alpha and Toll-Like Receptor 4 Genetic Variations and Risk of Incident Non-Fatal Myocardial Infarction and Ischemic Stroke

**Daniel A. Enquobahrie, MD, PhD**[a,b], **Nicholas L. Smith, MPH, PhD**[a,b,f], **Joshua C. Bis, MS, PhD**[a,d], **Cara L. Carty, MS**[a,b], **Kenneth M. Rice, PhD**[a,c], **Thomas Lumley, PhD**[a,c], **Lucia A. Hindorff, MPH, PhD**[a,b], **Rozenn N. Lemaitre, MPH, PhD**[a,c], **Michelle A. Williams, DSc**[b], **David S. Siscovick, MD, MPH**[a,b,d], **Susan R. Heckbert, MD, PhD**[a,b,g], and **Bruce M. Psaty, MD, PhD**[a,b,d,e,g]

a*Cardiovascular Health Research Unit, University of Washington, Seattle, WA*

b*Department of Epidemiology, University of Washington, Seattle, WA*

c*Department of Biostatistics, University of Washington, Seattle, WA*

d*Department of Medicine, University of Washington, Seattle, WA*

e*Department of Health Services, University of Washington, Seattle, WA*

f*Seattle Epidemiologic Research and Information Center for the Department of Veterans Affairs Office of Research and Development, Seattle, WA*

g*Center for Health Studies, Group Health, Seattle, WA.*

## Abstract

Variations in candidate genes participating in oxidative stress, inflammation and their interactions are potentially associated with diseases of atherosclerotic origin. We investigated independent and joint associations of variations in cholesterol ester transfer protein (*CETP*), interleukin 8 (*IL8*), peroxisome proliferator activator receptor alpha (*PPARA*) and toll-like receptor 4 (*TLR4*) genes with incident non-fatal myocardial infarction (MI) or ischemic stroke. In a population-based case-control study, cases (848 MI and 368 ischemic stroke) and controls (2682) were recruited from postmenopausal women and hypertensive men/women who were members of Group Health in Western Washington State. Common tag single nucleotide polymorphisms (n=34) representing gene-wide variations were selected from gene sequencing data using pairwise linkage disequilibrium. Haplotypes were inferred using a modified expectation maximization algorithm. Multivariate logistic regression evaluated individual haplotype and SNP-disease associations in log-additive models. Global haplotype tests assessed overall gene-disease associations. Logic regression was used to evaluate gene-gene interactions. False discovery rates and permutation tests were used for multiple testing adjustment in evaluating independent associations and interactions respectively. Overall, gene-wide variations in *PPARA* and *TLR4* genes were associated with MI. The minor allele of the

Corresponding Author: Daniel A. Enquobahrie, Harvard School of Public Health, Building 2, Room #322, 655 Huntington Avenue, Boston, MA 02115, Office Phone: (617) 432 7153, Office Facsimile: (617) 432 2435, E-mail: denquoba@hsph.harvard.edu.

*PPARA* SNP, *rs4253623*, was associated with a higher risk of MI (odds ratio: 1.25, 95%CI: 1.08–1.46) while the minor allele of the *TLR4* SNP, *rs1927911*, was associated with a lower risk of MI (odds ratio: 0.88, 95%CI: 0.77–0.99). No within gene or gene-gene interaction was associated with MI or ischemic stroke risk. Potential SNP-disease associations identified in the current study are novel.

### Keywords

genetic risk factors; myocardial infarction; ischemic stroke

## Introduction

Oxidative stress, inflammation and their interactions are potentially associated with diseases of atherosclerotic origin.[1] Cholesterol ester transfer protein (CETP), interleukin 8 (IL8), peroxisome proliferator activator receptor alpha (PPARA) and toll like receptor 4 (TLR4) have been shown to play roles in oxidative stress, inflammation and cardiovascular diseases (CVD).[2–6] Supporting evidence for these functions has led investigators to hypothesize the role of their genetic counterparts in diseases such as myocardial infarction (MI) and stroke. Previous studies evaluating associations between genetic variations in *CETP*, *IL8*, *PPARA* and *TLR4* with MI and/or stroke reported inconsistent findings warranting further comprehensive replication studies.[5, 7–10] Most studies did not evaluate whole gene variations and were typically conducted among patients with demonstrated atherosclerotic lesions to assess disease progression and outcome. Further, no population-based study to our knowledge investigated global interactions between these four genes in relation to MI and/or stroke. We conducted a large population-based case-control study to evaluate independent and joint associations of gene-wide variation in *CETP*, *IL8*, *PPARA* and *TLR4* with non-fatal incident MI or ischemic stroke.

## Methods

The study was part of a group of case-control studies that investigate risk factors associated with cardiovascular diseases. Study participants (Table 1) were selected from members of Group Health, a large integrated health care organization in Washington State. Cases were nonhypertensive postmenopausal women and hypertensive (pharmacologically treated) men and women, 30–79 years of age who suffered incident non-fatal MI or ischemic stroke during the study period (January 1, 1995 through December 31, 2002). Cases were identified using computerized discharge diagnoses from Group Health facilities and from claims databases. The index date for cases was the date of admission for the first MI or ischemic stroke. Incident events of MI were verified based on symptoms, electrocardiogram findings, enzyme levels, and physician diagnosis and treatment. Ischemic stroke diagnosis was verified using history of rapid onset of a neurologic deficit that persisted at least 24 hours or evidence of infarction on brain imaging studies or at surgery. Cases whose index event was a complication of a procedure were not eligible for the study. Controls were a stratified random sample of Group Health members selected from enrollment files based on person-time and were frequency matched to MI cases by age (within decade), sex, treated hypertension status and calendar year of index date. The index date for controls was a random computer generated date within the matching calendar year. Both cases and controls were alive at time of recruitment and had at least 4 visits to a Group Health provider prior to the index date. After exclusion of both cases and controls with previous history of either MI or stroke, a total of 1216 cases (848 MI and 368 IS) and 2682 controls were available for the study. Study eligibility, participant characteristics and risk factor information was collected by medical record review and telephone interview. Information was collected only for the period before the index date. A

venous blood sample was collected from subjects and DNA was extracted from white blood cells using standard phenol extraction methods. Group Health Human Subjects Review Committee approved the study and all participants provided written informed consent.

Single nucleotide polymorphisms (SNPs) were identified by genomic resequencing by Seattle Program for Genomic Applications (for *CETP*, *IL8* and *PPARA*) or Innate Immunity (for *TLR4*). Subsets of gene-specific tag-SNPs that describe common (minor allele frequencies ≥ 5%) gene-wide variation in European Americans and African Americans were selected using LDselect software that is based on linkage disequilibrium (threshold of correlation $r^2 = 0.64$).[11] A total of 50 tag-SNPs (16 *CETP*, 5 *IL8*, 9 *PPARA* and 20 *TLR4*) representing gene-wide variations were selected for genotyping. Genotyping for 44 of the 50 tag-SNPs (13 *CETP*, 5 *IL8*, *7 PPARA* and 19 *TLR4*) was successfully performed by Illumina® with a GoldenGate custom panel using BeadArray® technology. Among these tag-SNPs, 99.9 % of nucleotide pairs were called. After dropping 10 tag-SNPs that were rare (<2.5% minor allele frequency) in the study population, a total of 34 SNPs (13 *CETP*, 3 *IL8*, *6 PPARA* and 12 *TLR4*) remained for investigation (Table 2). We used *Phase v2.0* to infer haplotypes.[12] Haplotype uncertainty was estimated using the diplotype probability. Within a gene, haplotypes with frequencies less than 2.5% among controls were grouped together in a gene-specific haplotype bin assigned "Others". A total of 23 (6 *CETP*, 4 *IL8*, 6 *PPARA* and 7 *TLR4*) common haplotypes were investigated.

Specific global gene-disease associations were evaluated separately for MI and ischemic stroke using a logistic regression model that included all common haplotypes of the gene and the most common haplotype as a referent. The null hypothesis that no single haplotype odds ratio is different from 1 was used as the global test of association. This global Wald test is not affected by the choice of the referent group. Odds ratios represent the odds of an event associated with each additional copy of the haplotype relative to an additional copy of the referent haplotype. Diplotype probabilities were used as a weight in analyses clustered on participant identifier. The false discovery rate (FDR) correction, q-value, was used for multiple testing (8 global gene tests) correction.[13–14] Since genes evaluated were candidate genes previously described, a q-value below 0.20, corresponding to a 20% false discovery rate (FDR), was selected as a threshold for acceptable level of significance.[13] In a secondary analysis, separate SNP-specific logistic models were fit for each outcome separately. These models included a single variable for each SNP (additive effect of SNPs coded 0, 1 and 2 for minor allele copies). We did not correct for multiple testing in these analyses.

Logic regression, a method of identifying significant combinations among all 34 SNPs associated with higher or lower risk of MI or ischemic stroke, was used to evaluate both within gene and gene-gene interactions.[15] Logic regression identifies Boolean combinations of binary predictors using an adaptive algorithm that selects a combination that minimizes the residual sum of squares or the deviance. Model selection was optimized using cross-validation while multiple testing adjusted significance was evaluated using permutations in the logic regression algorithm. We conducted sensitivity analyses by restricting participants to only whites (to account for population stratification) and also by combining MI and ischemic stroke cases into a larger case group (to increase power). Further, we conducted a sensitivity analysis to assess potential heterogeneity of SNP-disease associations by gender, diabetes status, smoking, obesity and alcohol intake. All confidence intervals were calculated at the 1-alpha = 95% level. All models were adjusted for age, sex, race, index year and treated hypertension status.

## Results

Among 3898 study participants included in this study, 41% were male and 91% were white (Table 1). Global gene variation of *PPARA* was associated with risk of MI (global Wald test

p-value=0.033; false discovery rate=13%) (Table 3). Each additional copy of the *PPARA-C* haplotype was associated with an 82% higher MI risk compared with an additional copy of the referent haplotype. Each additional copy of *rs4253623* (part of the *PPARA-C* haplotype) minor allele was associated with a 25% higher MI risk (Adj. OR: 1.25, 95%CI: 1.08–1.46, p-value: 0.004). Similarly, global gene variation of *TLR4* was associated with MI risk (global Wald test p-value=0.008; false discovery rate=6%). Each additional copy of the *TLR4-D* haplotype was associated with a 40% lower risk of MI compared with an additional copy of the referent haplotype. Minor allele of *rs1927911*, part of the *TLR4-D* haplotype, was associated with a 12% lower MI risk (Adjusted OR: 0.88, 95%CI: 0.77–0.99, p-value=0.043). The global *CETP* gene and MI risk association test was marginal (global Wald test p-value=0.083). However, no common haplotype or SNP of *CETP* was associated with MI risk. *IL8* gene-wide variation was not associated with risk of MI. Global variations in *CETP, IL8, PPARA* or *TLR4* were not associated with ischemic stroke. However, in secondary analysis, minor alleles of two CETP SNPs, *rs12720922* and *rs9939224*, were associated with a similar 24% (Adjusted OR: 1.24, 95%CI: 1.03–1.51, p-value=0.026) and 26% (Adjusted OR: 1.26, 95%CI: 1.05–1.51, p-value= 0.025) higher ischemic stroke risk respectively.

In logic regression evaluation of associations of MI and ischemic stroke, separately, with combinations of all 34 SNPs of the 4 genes that were considered, signal test p-values were greater than 0.10 suggesting absence of sufficient evidence to support interactions between evaluated SNPs in MI or ischemic stroke. Results of our sensitivity analyses (by restricting participants to only whites and by combining both case groups) were similar to results already described. We also did not find significant effect modification of associations by gender, diabetes status, smoking, obesity and alcohol intake beyond what would have been expected by chance.

## Discussion

We demonstrated associations of global *PPARA* and *TLR4* genetic variations with MI risk. The *PPARA rs4253623* SNP was associated with increased MI risk while the *TLR4 rs1927914* SNP was associated with decreased MI risk. *PPARA* and *TLR4* genetic variations were not associated with ischemic stroke risk. Global *CETP* and *IL8* genetic variations were not associated with either MI or ischemic stroke. No significant interaction between SNPs of the 4 genes (*CETP, IL8, PPARA* and *TLR4*) in MI or IS was apparent in our study.

Investigators have previously reported associations between *PPARA* and *TLR4* gene variations with cardiovascular diseases. Two *PPARA* SNPs, one in exon 5 (*rs1800206*) and another in intron 7 (*rs4253778* in partial allelic association with *rs1800206*) were associated with risk of non-fatal MI.[7] We did not directly genotype a non-synonymous *PPARA* SNP or the intron SNPs (*rs4253778* or *rs1800206*). The *PPARA* SNP, *rs4253765*, in linkage disequilibrium with SNP *rs4253778,* was not associated with either MI or ischemic stroke in our study. The *PPARA* SNP, *rs4253623*, associated with MI risk in the current study has not been described before. The *TLR4* non-synonymous SNP, *rs4986790*, has been associated with a 37–61% decrease in risk of acute coronary events such as MI in several case control studies and a meta analysis,[16–18] though several other studies reported no associations.[8,19–20] Similarly, we did not find a reduced risk of MI associated with that particular SNP in our study. Lin et al found an association of a SNP in intron 1 of the *TLR4* gene (A119C), with risk of IS in an Asian study population.[21] We did not genotype the A119C SNP directly or a SNP in linkage disequilibrium with this SNP. The intron 1 SNP, *rs1927911,* part of the *TLR4-D* haplotype associated with lower risk of MI in our study or the SNPs tagged by it have not been previously described.

Reports of associations between *CETP* variations and cardiovascular diseases are inconsistent.[9–10, 22–24] The B allele of the *CETP*/TaqIB polymorphism (*rs708272*) was associated with reduced CHD risk in some,[9, 22] but not other studies.[23] Two tag-SNPs (*rs17231506* and *rs1800775*) in linkage disequilibrium with the *rs708272* SNP were not associated with risk of MI in our study. The –A allele of the *CETP* gene C629A SNP in the promoter region (in linkage disequilibrium with a non-synonymous SNP *rs5882* and SNP *rs708272*) was associated with decreased cardiovascular death.[24] The assay for *rs289716* SNP, in significant LD with *rs5882* in our panel, failed and thus we did not genotype that SNP in our population. Thompson et al investigated 9 *CETP* SNPs that span the whole gene and found that only SNP *rs1800774* was associated with history of MI.[10] We did not replicate that finding. The marginal global *CETP* gene and MI association in our study is potentially due to the marginal higher risk associated with having a copy of an uncommon haplotype. The 2 *CETP* SNPs, *rs708273* and *rs12720922*, associated with ischemic stroke in our secondary analysis were not described before. Further, there was no global *CETP* gene and IS association weakening the evidence for the associations.

Although IL8 chemokine has been associated with a number of overlapping functions in inflammation mediation and growth promotion in vascular cells known to be involved in cardiovascular risk development[3] and the protein has been associated with increased risk of cardiovascular disease such as unstable angina and acute MI,[25–26] *IL8* haplotypes and/or SNPs were not associated with risk of MI or ischemic stroke in our study. No previous study has investigated *IL8* SNPs or haplotypes in relation to MI or stroke.

Potential reasons for inconsistencies between specific SNP-disease association findings reported in our study compared with others include differences in SNP minor allele frequencies, linkage disequilibrium structure and disease status (fatal/non-fatal) among study populations. Genetic variants associated with fatal events or short-term mortality would be under-represented in our case-control study. Study power may be an issue in our interaction analysis where we adjusted for multiple testing. Most of our study participants were white and our results may not be generalizable to other racial groups. Finally, since we did not measure biomarkers in the current study, further studies that investigate associations between genetic variations, counterpart proteins, intermediates and disease status are needed to clarify mechanisms of associations. In conclusion, in this population-based case control study, we reported associations of *PPARA* and *TLR4* genetic variation with risk of incident non-fatal MI. We also identified novel specific SNP-disease associations that need further investigations.

## Acknowledgment

This study was supported by the following grants from the National Health Lung and BloodInstitute: HL73410, HL60739, HL68639, HL43201, HL74745, HL68986 and 1-T32-HL07902.

The authors are indebted to the participants of the Heart and Vascular Health Studies for their cooperation. They are also grateful for the expertise provided by staff of the Cardiovascular Health Research Unit at the University of Washington.

## References

1. Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis: part I: new genes and pathways. Circulation 2004;110:1868–1873. [PubMed: 15451808]
2. Klerkx AH, El Harchaoui K, van der Steeg WA, Boekholdt SM, Stroes ES, Kastelein JJ, Kuivenhoven JA. Cholesteryl ester transfer protein (CETP) inhibition beyond raising high-density lipoprotein cholesterol levels: pathways by which modulation of CETP activity may alter atherogenesis. Arterioscler Thromb Vasc Biol 2006;26:706–715. [PubMed: 16439711]
3. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. Clin Sci (Lond) 2005;108:205–213. [PubMed: 15540988]

4. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 2000;49:497–505. [PubMed: 11089900]
5. Tai ES, Demissie S, Cupples LA, Corella D, Wilson PW, Schaefer EJ, Ordovas JM. Association between the PPARA L162V polymorphism and plasma lipid levels: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 2002;22:805–810. [PubMed: 12006394]
6. de Kleijn D, Pasterkamp G. Toll-like receptors in cardiovascular diseases. Cardiovasc Res 2003;60:58–67. [PubMed: 14522407]
7. Doney AS, Fischer B, Lee SP, Morris AD, Leese G, Palmer CN. Association of common variation in the PPARA gene with incident myocardial infarction in individuals with type 2 diabetes: A Go-DARTS study. Nucl Recept. 2005 Nov 25;published online
8. Yang IA, Holloway JW, Ye S. Southampton Atherosclerosis Study (SAS) Group. TLR4 Asp299Gly polymorphism is not associated with coronary artery stenosis. Atherosclerosis 2003;170:187–190. [PubMed: 12957699]
9. Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, Lahoz C, Coltell O, Wilson PW, Schaefer EJ. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 2000;20:1323–1329. [PubMed: 10807749]
10. Thompson JF, Durham LK, Lira ME, Shear C, Milos PM. CETP polymorphisms associated with HDL cholesterol may differ from those associated with cardiovascular disease. Atherosclerosis 2005;181:45–53. [PubMed: 15939053]
11. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–120. [PubMed: 14681826]
12. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–989. [PubMed: 11254454]
13. Smith NL, Hindorff LA, Heckbert SR, Lemaitre RN, Marciante KD, Rice K, Lumley T, Bis JC, Wiggins KL, Rosendaal FR, Psaty BM. Association of genetic variations with nonfatal venous thrombosis in postmenopausal women. JAMA 2007;297:489–498. [PubMed: 17284699]
14. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med 1990;9:811–818. [PubMed: 2218183]
15. Kooperberg C, Ruczinski I, LeBlanc ML, Hsu L. Sequence Analysis Using Logic Regression. Genetic Epidemiology 2001;21:S626–S631. [PubMed: 11793751]
16. Ameziane N, Beillat T, Verpillat P, Chollet-Martin S, Aumont MC, Seknadji P, Lamotte M, Lebret D, Ollivier V, de Prost D. Association of the Toll-like receptor 4 gene Asp299Gly polymorphism with acute coronary events. Arterioscler Thromb Vasc Biol 2003;23:e61–e64. [PubMed: 14563652]
17. Holloway JW, Yang IA, Ye S. Variation in the toll-like receptor 4 gene and susceptibility to myocardial infarction. Pharmacogenet Genomics 2005;15:15–21. [PubMed: 15864121]
18. Kolek MJ, Carlquist JF, Muhlestein JB, Whiting BM, Horne BD, Bair TL, Anderson JL. Toll-like receptor 4 gene Asp299Gly polymorphism is associated with reductions in vascular inflammation, angiographic coronary artery disease, and clinical diabetes. Am Heart J 2004;148:1034–1040. [PubMed: 15632890]
19. Zee RY, Hegener HH, Gould J, Ridker PM. Toll-like receptor 4 Asp299Gly gene polymorphism and risk of atherothrombosis. Stroke 2005;36:154–157. [PubMed: 15576653]
20. Norata GD, Garlaschelli K, Ongari M, Raselli S, Grigore L, Benvenuto F, Maggi FM, Catapano AL. Effect of the Toll-like receptor 4 (TLR-4) variants on intima-media thickness and monocyte-derived macrophage response to LPS. J Intern Med 2005;258:21–27. [PubMed: 15953129]
21. Lin YC, Chang YM, Yu JM, Yen JH, Chang JG, Hu CJ. Toll-like receptor 4 gene C119A but not Asp299Gly polymorphism is associated with ischemic stroke among ethnic Chinese in Taiwan. Atherosclerosis 2005;180:305–309. [PubMed: 15910856]
22. Brousseau ME, O'Connor JJ Jr, Ordovas JM, Collins D, Otvos JD, Massov T, McNamara JR, Rubins HB, Robins SJ, Schaefer EJ. Cholesteryl ester transfer protein TaqI B2B2 genotype is associated with higher HDL cholesterol levels and lower risk of coronary heart disease end points in men with

HDL deficiency: Veterans Affairs HDL Cholesterol Intervention Trial. Arterioscler Thromb Vasc Biol 2002;22:1148–1154. [PubMed: 12117730]

23. Arca M, Montali A, Ombres D, Battiloro E, Campagna F, Ricci G, Verna R. Lack of association of the common TaqIB polymorphism in the cholesteryl ester transfer protein gene with angiographically assessed coronary atherosclerosis. Clin Genet 2001;60:374–380. [PubMed: 11903340]
24. Blankenberg S, Rupprecht HJ, Bickel C, Jiang XC, Poirier O, Lackner KJ, Meyer J, Cambien F, Tiret L. AtheroGene Investigators. Common genetic variation of the cholesteryl ester transfer protein gene strongly predicts future cardiovascular death in patients with coronary artery disease. J Am Coll Cardiol 2003;41:1983–1989. [PubMed: 12798569]
25. Kanda T, Hirao Y, Oshima S, Yuasa K, Taniguchi K, Nagai R, Kobayashi I. Interleukin-8 as a sensitive marker of unstable coronary artery disease. Am J Cardiol 1996;77:304–307. [PubMed: 8607415]
26. Boekholdt SM, Peters RJ, Hack CE, Day NE, Luben R, Bingham SA, Wareham NJ, Reitsma PH, Khaw KT. IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Arterioscler Thromb Vasc Biol 2004;24:1503–1508. [PubMed: 15178568]

**Table 1**

Study population characteristics

| Characteristics | Controls (n=2682) | Cases | |
|---|---|---|---|
| | | Myocardial infarction (n=848) | Ischemic stroke (n=368) |
| **Men** | 41.6 % | 42.8 % | 31.4 % |
| **Mean Age (years)** | 65.2 | 65.8 | 68.5 |
| **Whites** | 91.1 % | 91.1 % | 90.8 % |
| **Blacks** | 3.8 % | 3.7 % | 6.0 % |
| **Other** | 5.1 % | 5.1 % | 3.2 % |
| **Smoker** | | | |
| **Never/Non smoker** | 46.2 % | 37.1 % | 50.8 % |
| **Former** | 43.8 % | 44.6 % | 36.5 % |
| **Current** | 10.0 % | 18.3 % | 12.7 % |
| **Treated Hypertension** | 73.1 % | 71.6 % | 71.4 % |
| **Treated Diabetes Mellitus** | 11.4 % | 24.1 % | 24.3 % |
| **Mean Body Mass Index ($kg/m^2$)** | 29.5 | 30.1 | 30.0 |

**Table 2**

Single nucleotide polymorphisms (SNPs) and haplotypes

| SNPs | | | | Haplotypes | | |
|---|---|---|---|---|---|---|
| **Gene** | **Reference number** | **Position*** | **MAF**** | **Haplotype ID** | **Structure***** | **Freq**** |
| ***CETP*** | *rs17231506* | 5'F | 30 % | *CETP-A* | CGCA**C**GGTC**A**CCT | 20 % |
| | *rs4783961* | 5'F | 49 % | *CETP-B* | **TA**C**C**TGGTCG**T**CT | 14 % |
| | *rs17245715* | 5'F | 10 % | *CETP-C* | C**A**CATA**TT**CG**T**CT | 6 % |
| | *rs1800775* | 5'F | 49 % | *CETP-D* | CG**TC**TGGTCGCCT | 7 % |
| | *rs708273* | In2 | 30 % | *CETP-E* | **TA**C**C**TGGTCGC**GT** | 6 % |
| | *rs12720922* | In2 | 18 % | *CETP-Others*§ | ---------- | 47 % |
| | *rs9939224* | In2 | 21 % | | | |
| | *rs11076174* | In2 | 8 % | | | |
| | *rs5883* | Ex9S | 5 % | | | |
| | *rs4784744* | In10 | 35 % | | | |
| | *rs1800774* | In12 | 35 % | | | |
| | *rs289742* | 3'F | 13 % | | | |
| | *rs12720917* | 3'F | 15 % | | | |
| ***IL8*** | *rs4073* | 5'F | 48 % | *IL8-A* | **A**TC | 51 % |
| | *rs2227307* | In1 | 47 % | *IL8-B* | T**GT** | 42 % |
| | *rs2227543* | In3 | 42 % | *IL8-C* | T**G**C | 3 % |
| | | | | *IL8-Others*§ | ----- | 3 % |
| ***PPARA*** | *rs4253623* | In2 | 13 % | *PPARA-A* | **G**GG**CA**T | 29 % |
| | *rs135543* | In2 | 29 % | *PPARA-B* | A**A**GTGT | 22 % |
| | *rs4253655* | In2 | 16 % | *PPARA-C* | **GA**G**C**G**C** | 11 % |
| | *rs4253699* | In2 | 20 % | *PPARA-D* | **G**G**A**TGT | 9 % |
| | *rs4253701* | In2 | 10 % | *PPARA-E* | A**A**GTGT | 6 % |
| | *rs4253765* | In6 | 19 % | *PPARA-Others*§ | ---------- | 23 % |
| ***TLR4*** | *rs2770150* | 5'F | 27 % | *TLR4-A* | **G**CTGCACCGAGT | 27 % |
| | *rs1927914* | 5'F | 36 % | *TLR4-B* | ACTGCACCGAGT | 22 % |
| | *rs7864330* | In1 | 6 % | *TLR4-C* | ACTGCACC**C**AGT | 14 % |
| | *rs1927911* | In1 | 28 % | *TLR4-D* | A**TT**A**A**CACCGAGT | 7 % |
| | *rs11536878* | In3 | 12 % | *TLR4-E* | A**TT****AA**ACCG**G**GT | 6 % |
| | *rs4986790* | Ex4N | 6 % | *TLR4-F* | A**TG**GC**GT**CGAG**C** | 5 % |
| | *rs4986791* | Ex4N | 5 % | *TLR4-Others*§ | ---------- | 18 % |
| | *rs7869402* | Ex4U | 4 % | | | |
| | *rs11536889* | 3'F | 15 % | | | |
| | *TLR4-17050* | 3'F | 16 % | | | |
| | *TLR4-17723* | 3'F | 5 % | | | |
| | *rs1927906* | 3'F | 10 % | | | |

*SNP position descriptions: F=flanking, Ex=exon, In=intron, N=non-synonymous, U= untranslated.

**SNP minor allele frequencies (MAF) and probability-weighed haplotype frequencies among controls.

***Haplotype structures in order of SNP list.

§Others, a haplotype group that includes all other haplotypes with frequencies less than 5%.

## Table 3

Global gene and haplotype associations with MI and ischemic stroke

| Genes (haplotypes) | Freq (%)** Control/MI/IS | Myocardial infarction (MI) OR** (95%CI) | Ischemic stroke OR** (95%CI) |
|---|---|---|---|
| **CETP** | | | |
| ***CETP-A*** | 20/18/19 | Referent | Referent |
| ***CETP-B*** | 14/16/13 | 1.19 (0.97–1.45) | 0.84 (0.64–1.10) |
| ***CETP-C*** | 6/5/7 | 0.92 (0.69–1.22) | 0.94 (0.65–1.35) |
| ***CETP-D*** | 7/6/5 | 0.95 (0.70–1.27) | 0.77 (0.52–1.14) |
| ***CETP-E*** | 6/7/6 | 1.29 (0.99–1.68) | 1.02 (0.68–1.49) |
| ***CETP-Others*** | 47/48/50 | 1.21 (0.99–1.46) | 0.87 (0.68–1.15) |
| | **Global Wald test (p-value)§** | 0.083 | 0.621 |
| | **False discovery rate (q-value)** | 22% | >50% |
| **IL8** | | | |
| ***IL8-A*** | 51/53/52 | Referent | Referent |
| ***IL8-B*** | 42/41/42 | 0.92 (0.78–1.07) | 0.98 (0.78–1.22) |
| ***IL8-C*** | 3/3/3 | 0.99 (0.64–1.54) | 0.79 (0.44–1.40) |
| ***IL8-Others*** | 3/2/4 | 1.08 (0.51–2.30) | 1.18 (0.54–2.61) |
| | **Global Wald test (p-value)§** | 0.734 | 0.812 |
| | **False discovery rate (q-value)** | >50% | >50% |
| **PPARA** | | | |
| ***PPARA-A*** | 29/28/27 | Referent | Referent |
| ***PPARA-B*** | 22/20/21 | 1.00 (0.80–1.25) | 1.03 (0.84–1.27) |
| ***PPARA-C*** | 11/19/13 | **1.82 (1.20–2.77)** | 1.20 (0.92–1.57) |
| ***PPARA-D*** | 9/9/7 | 1.08 (0.88–1.33) | 0.83 (0.61–1.12) |
| ***PPARA-E*** | 6/5/7 | 0.99 (0.75–1.32) | 1.25 (0.83–1.89) |
| ***PPARA-Others*** | 23/19/25 | 1.15 (0.79–1.68) | 1.08 (0.63–1.87) |
| | **Global Wald test (p-value)§** | 0.033 | 0.393 |
| | **False discovery rate (q-value)** | 13% | >50% |
| **TLR4** | | | |
| ***TLR4-A*** | 27/29/26 | Referent | Referent |
| ***TLR4-B*** | 22/21/21 | 0.89 (0.74–1.07) | 0.94 (0.72–1.24) |
| ***TLR4-C*** | 14/13/15 | 0.86 (0.67–1.09) | 1.08 (0.79–1.49) |
| ***TLR4-D*** | 7/5/6 | **0.60 (0.44–0.82)** | 0.95 (0.60–1.48) |
| ***TLR4-E*** | 6/6/9 | 0.82 (0.61–1.10) | 1.36 (0.90–2.06) |
| ***TLR4-F*** | 5/7/5 | 1.15 (0.82–1.62) | 0.98 (0.62–1.54) |
| ***TLR4-Others*** | 18/19/19 | **0.67 (0.48–0.93)** | 0.88 (0.54–1.43) |
| | **Global Wald test (p-value)§** | 0.008 | 0.788 |
| | **False discovery rate (q-value)** | 6% | >50% |

* Probability-weighed haplotype frequencies among controls/myocardial infarction (MI) cases/ischemic stroke (IS) cases.

** Odds ratios (95%CI) of logistic additive models comparing each haplotype to the referent haplotype adjusted for matching factors (age, sex, race, index year and hypertension status).

§ Global Wald test p-value for overall association of gene with disease.