APPENDIX

Supplementary Materials

Cohort descriptions

*Studies which had not previously reported on data regarding presence of MI in subjects with CAD. Updated and full individual level data were obtained from the other cohorts.

The Atherosclerotic Disease VAscular functioN and genetiC Epidemiology study (ADVANCE): Detailed descriptions of the eligibility criteria and the source population for all groups in the multi ethnic ADVANCE study are provided elsewhere.[1-4] Briefly, between October 28, 2001 and December 31, 2003, 3,179 member of the Kaiser Permanente of Northern California health maintenance organization were enrolled into three CAD groups and two control groups. The case groups included subjects with "early onset" CAD (as defined by a CAD qualifying event captured by the electronic databases at any time after Jan 1, 1999 including MI, 3 angina with at least one angiographic stenosis of >50%, or revascularization procedure in men 18 to 45 or women 18 to 55 years of age at the time of the event), incident stable exertional angina at an older age, and incident nonfatal MI at an older age. The young control group included *de novo* recruited subjects between the ages of 30 and 45 for men or 30 and 55 for women as well as a subset of 479 participants in the same age range from the Coronary Artery Risk Development in Young Adults (CARDIA) study originally recruited at the Oakland field center and attending the study's Year 15 examination in 2000–2001. Older controls included men and women aged 60–69 years at the time of identification in June, 2001. All controls were free of clinical CAD, cerebrovascular disease and peripheral arterial disease at the time of recruitment. Thus, the total study sample of the ADVANCE study included 3658 subjects.

None of the control groups were included in this study. Detailed angiographic data was also not available for this study. Among the "early onset" case group, we excluded 54 cases that self-reported a CAD event at a time that was 2 or more calendar years earlier than the event captured in the Kaiser electronic databases. Similar exclusions were not needed for the older onset case groups because of the manner these cases were ascertained which increased our confidence that the qualifying even captured by the Kaiser electronic databases was the first ever clinical CAD event (either angina with no prior MI vs. MI). "Early onset" cases were classified as MI cases for this study if they had evidence of increased cardiac enzymes at the time of their qualifying event. All other "early onset" cases were classified as CAD and either had undergone a revascularization procedure or an angiogram which documented at least one 50% stenosis.

All subjects provided information on their own race/ethnicity, the race/ethnicity of their parents and the race/ethnicity of their grandparents as well as everyone's place of birth. This information was used to classify participants into one of the following racial/ethnic groups: white/European, black/African American, Hispanic, South Asian, East Asian, Pacific Islander, Native American or admixed among at least two of the above categories.

Participants were considered ethnically admixed when they did not report that his or her grandparents were all white, all African American, all East Asian, all South Asian, all Hispanic, all Native American, or all Native Hawaiian/Pacific Islander. All cases classified as admixed were excluded from this study to minimize the potential for population stratification. Lastly, we excluded all Pacific Islanders and Native Americans because of the very small number of cases (<10).

All ADVANCE subjects were genotyped at the SNPs of interest in this study as previously described using TAQMAN™[4].

1. Go, A.S., Iribarren, C., Chandra, M., Lathon, P.V., Fortmann, S.P., Quertermous, T. and Hlatky, M.A. (2006) Statin and beta-blocker therapy and the initial presentation of coronary heart disease. Ann. Intern. Med., 144, 229–238.

2. Taylor-Piliae, R.E., Norton, L.C., Haskell, W.L., Mahbouda, M.H., Fair, J.M., Iribarren, C., Hlatky, M.A., Go, A.S. and Fortmann, S.P. (2006) Validation of a new brief physical activity survey among men and women aged 60–69 years. Am. J. Epidemiol., 164, 598–606.

3. Iribarren, C., Go, A.S., Husson, G., Sidney, S., Fair, J.M., Quertermous, T., Hlatky, M.A. and Fortmann, S.P. (2006) Metabolic syndrome and early-onset coronary artery disease: is the whole greater than its parts? J. Am. Coll. Cardiol., 48, 1800–1807.

4. Assimes TL, Knowles JW, Basu A, et al. Susceptibility locus for clinical and subclinical coronary artery disease at chromosome 9p21 in the multi-ethnic ADVANCE study. Hum Mol Genet 2008;17:2320-8.

Coronary Disease Cohort Study (CDCS)*: From July 2002, patients admitted to either Christchurch Hospital or Auckland City Hospital (New Zealand) were recruited into the Coronary Disease Cohort Study (CDCS), which aimed to assess clinical, neurohormonal, imaging, and genetic measurements as biomarkers of cardiovascular prognosis in coronary heart disease. Patients were recruited according to the following inclusion criteria: ischemic discomfort plus one or more of ECG change (ST-segment depression or elevation of ≥.5 mm, T-wave inversion of ≥3 mm in ≥3 leads, or left bundle branch block), elevated levels of cardiac markers, a history of coronary disease, age of ≤65 years, and a history of diabetes or vascular disease. Patients were excluded from the study if they had a severe comorbidity that limited their life expectancy to <3 years. The study intentionally included a broad spectrum of age, both sexes, significant subgroups with the important antecedent risk factors, and disease processes such as hypertension and diabetes. Although recruitment was ongoing, the subcohort reported in this study included the first 1054 patients of which 767 were recruited on admission to Christchurch Hospital and 287 patients to Auckland City Hospital. Anthropometric and clinical characteristics were recorded at planned follow-up clinic visits, and clinical events were recorded from questionnaires, patient notes, and National Health Information Services and hospital

Patient Management System databases. Median follow-up was 4.0 years (range, 0.2 to 6.9 years). The study conformed to the principles outlined in the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, and was approved by the New Zealand Multi-region Ethics Committee. Each participating patient provided written informed consent.

(Description adapted from: Ellis KL, Pilbrow AP, Frampton CM, et al. A common variant at chromosome 9p21.3 is associated with age of onset of coronary disease but not subsequent mortality. *Circ Cardiovasc Genet* .2010;3:286-293)

The Cleveland Clinic GeneBank Study (Cleveland GB): The Cleveland Clinic GeneBank study is an angiographic study of ~10,000 subjects that has been used previously for discovery and replication of novel genes and risk factors for atherosclerotic CVD in Cleveland, Ohio, USA. Briefly, subject recruitment into GeneBank occurred between 2001 and 2006 and provides an ongoing focus for analyzing the association of biochemical and genetic factors with coronary atherosclerosis in a consecutive cohort of stable patients undergoing elective diagnostic cardiac evaluation. Enrollment criteria included stable patients undergoing elective coronary angiography without known myocardial infarction at time of enrollment and ability to give informed consent. Extensive clinical, demographic, laboratory and angiographic data were collected from electronic medical record, and longitudinal follow-up data confirmed with source documentation with adjudication of all major adverse cardiac events. Ethnicity information was self-reported. Fasting blood was drawn and plasma, serum, and DNA were isolated. Lipid profile and complete metabolic panel were assayed on all samples.

CAD cases (N=2471) were those subjects with angiographic evidence of CAD (AngCAD cases), defined as ≥50% stenosis in one or more coronary vessels, at the time of enrollment into GeneBank. The distribution of those cases with prior history of MI (AngCADMI+; N=1332) and those without history of MI (AngCADMI-; N=1079) was approximately equal. Specifically, an AngCADMI+ subject was defined as having angiographic evidence or history of CAD as well as positive history of MI at GeneBank enrollment whereas an AngCADMI- subject was defined as having angiographic evidence or history of CAD but no history of MI at enrollment. Control subjects (N=202) were intentionally selected to be older (>50 years old) and exhibited no or minimal evidence of CAD upon angiography. Genome-wide data was obtained from the Affymetrix Genome-Wide Human SNP Array 6.0 platform (genotyped at the University of Ottawa).

CATHGEN*: The study participants were enrolled at Duke University Medical Center (Durham, NC) through the CATHGEN bio-repository, consisting of subjects greater than 18 years of age, recruited sequentially through the cardiac catheterization laboratories from 2001-2011. Biological samples and extensive clinical, angiographic, and longitudinal follow-up data were collected on all subjects consenting to participation. Blood samples were obtained from the femoral artery at initiation of the procedure. CAD cases were

defined as individuals with greater than 50% stenosis in one or more epicardial vessels. MI cases were defined as those having a history of MI (by self-report and corroborated by review of medical records), or having suffered an MI during the study follow-up period. For this meta-analysis only Caucasian samples were used. This study was approved by the Duke University Medical Center Institutional Review Board on Human Subjects and all subjects gave written informed consent.

Genotyping of RS10757278 was performed in 2 laboratories, either at the DeCode Laboratories as described in Helgadottir et al. (1) or in the Center for Human Genetics laboratories using standard quality control procedures (2). Allele and genotype frequencies for RS10757278 were compared between sample sets genotyped by the two labs and found to be similar.

Controls from this cohort, defined as those with no vessels with greater than 50% stenosis, no history of MI prior or subsequent to the index cardiac catheterization, no history of percutaneous or surgical coronary revascularization procedure; no subsequent percutaneous or surgical coronary revascularization procedures.

1. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, Jonasdottir A, Sigurdsson A, Baker A, Palsson A, Masson G, Gudbjartsson D, Magnusson K, Andersen K, Levey AI, Backman VM, Matthiasdottir S, Jonsdottir T, Palsson S, Einarsdottir H, Gunnarsdottir S, Gylfason A, Vaccarino V, Hooper W, Reilly MP, Granger CB, Austin H, Rader DJ, Shah SH, Auyyumi AA, Gulcher JR, Thorgeirsson G, Thorsteinsdottir U, Kong A, Stefansson K. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007;316:1491-3. PMID 17478679.
2. Sutton BS, Crosslin DR, Shah SH, Nelson SC, Bassil A, Hale AB, Haynes C, Goldschmidt-Clermont, PJ, Vance JM, Kraus WE, Gregory SG, Hauser ER. Comprehensive genetic analysis of the platelet activating factor acetylhydrolase (PLA2G7) gene and cardiovascular disease in case/control and family datasets. Human Molecular Genetics, 2008, 17(9), 1318-1328.

Feldkirch/Austria (Drexel et al)*: The study included two large cohorts of consecutive patients undergoing coronary angiography for the evaluation of suspected or established stable CAD. The first cohort (referred to as cohort 1) consists of 671 consecutive Caucasian patients who underwent coronary angiography from September 1999 through October 2000; the second cohort (referred to as cohort 2) comprises 940 consecutive Caucasian patients who underwent coronary angiography from August 2005 through December 2007. In both study cohorts, information on conventional cardiovascular risk factors such as a history of smoking, hypertension, or diabetes was obtained by a standardized interview. Height and weight were recorded and body mass index (BMI) was calculated as body weight (kg)/height (m)2. Type 2 diabetes mellitus (T2DM) was diagnosed according to WHO criteria [1] and hypertension according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

was defined as arterial blood pressure greater than or equal to either 140mm Hg systolic or 90mm Hg diastolic. In both study populations, coronary angiography was performed with the Judkins technique, and significant CAD was diagnosed in the presence of coronary stenoses with lumen narrowing of at least 50%, as described previously. The Ethics Committee of the Medical University of Innsbruck approved the presented study, and all participants gave written informed consent.

1. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–53.

(Description adapted from: Muendlein A, Saely CH, Rhomberg S, et al. Evaluation of the association of genetic variants on the chromosomal loci 9p21.3, 6q25.1, and 2q36.3 with angiographically characterized coronary artery disease. *Atherosclerosis*. 2009; 205:174-180)

Emory Cardiovascular Biobank (EmCB) consists of 3492 consecutive patients enrolled prior to undergoing elective or emergent cardiac catheterization across three Emory Healthcare sites, since 2003 in Atlanta, GA, USA. Patients aged 20-90 years were interviewed to collect information on demographic characteristics, medical history and behavioral (lifestyle) habits. Risk factor prevalence was determined by physician diagnosis and/or treatment for hypertension, hyperlipidemia and diabetes. Smoking was classified as non-smoker or "ever smoked" if there was a lifetime history of smoking at least 100 cigarettes. Medical records were reviewed to confirm self-reported history of MI and other conditions as well as to document previous angiographic findings and prior coronary revascularization. Two operators, evaluated all coronary angiograms by visual estimation of luminal narrowing in multiple segments based on a modified form of the AHA/ACC classification of the coronary tree. Full details of coronary angiography phenotyping have been described previously.[1]

Genotyping for all samples was carried out at deCODE genetics in Reykjavik, Iceland, as part of ongoing collaborative studies, with rs10757278 chosen as the representative SNP for the 9p21 region based on our group's prior work. All single SNP (rs10757278) genotyping was carried out using the Centaurus (Nanogen) platform.15 The quality of each Centaurus SNP assay was evaluated by genotyping each assay on the Caucasian (CEU) samples and comparing the results with the HapMap data. All assays had mismatch rate <0.5%. The study was approved by the Institutional Review Board at Emory University, Atlanta, GA. All subjects provided written informed consent at the time of enrollment.

1. Patel, R.S., et al., The chromosome 9p21 risk locus is associated with angiographic severity and progression of coronary artery disease. Eur Heart J, 2010. 31(24): p. 3017-23.

Japan and Korea (Hinohara et al)* Japanese cases (n=629, age: 59.3±10.1) consisted of 579 MI and 50 AP cases were recruited from Kitasato University Hospital and related hospitals. Korean cases (n=828, age: 61.2±11.1) consisted of 461 MI and 367 AP cases were recruited from Samsung Medical Center. The diagnosis of CAD was based on the standard criteria as described previously (Hohda et al. 2003). Severity of coronary atherosclerosis was classified according to the number of coronary vessels with significant stenosis (angiographic luminal stenosis > 50%) as 0, 1, 2 or 3 vessel disease (VD). Informed consent was given from each participant and the study was approved by the Ethics Review Boards of Medical Research Institute of Tokyo Medical and Dental University, Kitasato University School of Medicine, and Samsung Medical Center.

(Description adapted from: Hinohara, K., et al., Megakaryoblastic leukemia factor-1gene in the susceptibility to coronary artery disease. *Hum Genet.* 2009; 126(4): 539-547.)

The Intermountain Heart Collaborative Study (IHCS): Since 1994, patients undergoing coronary angiography within Intermountain Healthcare tertiary care hospitals including Intermountain Medical Center (IMC), LDS Hospital, and McKay-Dee Hospital have been recruited to the IHCS (http://clinicaltrials.gov/ct2/show/NCT00406185). Subjects provide written informed consent to bank DNA and plasma samples. As of December 2011, over 20,000 patients were enrolled. The population of this region is primarily Caucasian, ethnically homogeneous but outbred and highly representative of US Caucasians. With angiographic data, medical history, full clinical diagnosis information, and longitudinal follow-up data, a wealth of CVD traits are available. Presence of CAD is defined as one-, two-, or three-vessel disease of ≥70% stenosis and no-CAD controls are defined as <10% stenosis in all coronary arteries. Incident CVD events and death are ascertained through electronic medical records from all hospitalizations (single provider system) and from local, state, and US death records.

INVEST-Genetic Substudy*: INVEST compared adverse CV outcomes in 22 576 hypertensive patients with CAD randomized to 2 different antihypertensive treatment strategies. Details of the methods and main outcomes were previously reported. Briefly, patients with CAD and essential hypertension requiring drug therapy who were aged >50 years were eligible. Patients were randomly assigned to a verapamil SR- or an atenolol-based treatment strategy. Details on addition of study drugs and dose titration are published elsewhere. The INVEST Genetic Substudy (INVEST-GENES) cohort consisted of 5979 patients from 213 sites in the United States who provided genomic DNA samples and additional written informed consent for genetic studies.

INVEST-GENES Case-Control Samples*: The INVEST-GENES case-control sample, a subset of INVESTGENES cohort, included all 304 patients (cases) who experienced a primary outcome event during the trial (first occurrence of all-cause death, nonfatal MI, or nonfatal stroke) and frequency-matched controls (who did not experience death, MI, or stroke)

matched on age (decades), sex, and race/ethnicity (defined as self-identified race/ethnicity as recorded by the physician).

1. Pepine CJ, Handberg EM, Cooper-DeHoff RM, Marks RG, Kowey P, Messerli FH, Mancia G, Cangiano JL, Garcia-Barreto D, Keltai M, Erdine S, Bristol HA, Kolb HR, Bakris GL, Cohen JD, Parmley WW. A calcium antagonist vs a non-calcium antagonist hypertension treatment strategy for patients with coronary artery disease. The International Verapamil-Trandolapril Study (INVEST): a randomized controlled trial. JAMA.2003;290:2805–2816.

2. Pepine CJ, Handberg-Thurmond E, Marks RG, Conlon M, Cooper-DeHoff R, Volkers P, Zellig P. Rationale and design of the International Verapamil SR/Trandolapril Study (INVEST): an Internet-based randomized trial in coronary artery disease patients with hypertension. J Am Coll Cardiol. 1998;32:1228 –1237.

Munich/Germany (Hoppmann et al)* The study included 2,028 patients with ischemic symptoms or evidence of myocardial ischemia in the presence of ≥50% de novo stenosis located in native coronary vessels. Patients were treated with percutaneous coronary intervention and sirolimus- (Cypher, Cordis, Johnson & Johnson Company, Warren, New Jersey) or paclitaxel-eluting (Taxus, Boston Scientific, Natick, Massachusetts) stent implantation. An oral loading dose of 600 mg of clopidogrel was administered to all patients at least 2 h before the intervention. During the procedure, patients were given intravenous aspirin, heparin, or bivalirudin; glycoprotein IIb/IIIa inhibitor usage was at the discretion of the operators. After the intervention, all patients received 200 mg/day aspirin indefinitely, 150 mg clopidogrel for the first 3 days (or until discharge) followed by 75 mg/day for at least 6 months and other cardiac medications (including beta-blockers, angiotensin-converting enzyme inhibitors, statins) according to the judgment of the patient's physician. After stenting, patients remained in the hospital for at least 48 h. Re-hospitalization for repeat angiography was scheduled between 6 and 8 months or earlier if noninvasive evaluation or clinical presentation suggested the presence of ischemia. Clinical follow-up by office visit or direct telephone call to the patient was scheduled at 36 months. The study protocol was approved by the institutional ethics committee responsible for both participating centers, Deutsches Herzzentrum München and 1. Medizinische Klinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. All subjects gave their written, informed consent for participation in the study. The reported investigations were in accordance with the principles of the Declaration of Helsinki.

(Description adapted from: Hoppmann P, erl A, Türk S, et al. No association of chromosome 9p21.3 variation with clinical and angiographic outcomes after placement of drug-eluting stents. *JACC: Cariovascular Interventions.* 2009. 2(11):1149-55)

PennCath: PennCath is a University of Pennsylvania (U. Penn) Medical Center based coronary angiographic study that has been used previously for replication of novel genes

and risk factors for atherosclerotic CVD and type-2 diabetes. Briefly, PennCath, recruited between July 1998 and March 2003, provides an ongoing focus for analyzing the association of biochemical and genetic factors with coronary atherosclerosis in a consecutive cohort of patients undergoing cardiac catheterization and coronary angiography. A total of 3,850 subjects provided written informed consent in a Penn Institutional Review Board approved protocol. Enrollment criteria included any clinical indication for cardiac catheterization and ability to give informed consent. The following data were extracted from the medical record; age, gender, self-reported race/ethnicity, past medical (including diabetes, hypertension, dyslipidemia, prior MI and cardiac events), social, family and medication history, cardiovascular risk factors, physical exam including vital signs, weight and height (for BMI). Ethnicity information was self-reported. Coronary angiograms were scored at the time of procedure by the interventional cardiologist. Blood was drawn in a fasting state, DNA (buffy coats) and plasma was isolated, and lipoproteins and glucose were assayed on all samples.

PennCath (N=1,401 Caucasians) composed of controls (N=468) who on coronary angiography showed no or minimal (<10% stenosis of any vessel) evidence of CAD and angiographic CAD (AngCAD) cases (N=933) with one or more coronary vessels with ≥50% stenosis. AngCAD cases were equally selected for cases with MI (AngCADMI+ cases; N=470) and cases without history or presentation with MI (AngCADMI- cases; N=463). Controls were aged over 40 in men and 45 in women. Cases were selected to be young ≤60 for males and ≤65 for females.

MedStar: The MedStar study, conducted by the Cardiovascular Research Institute of the MedStar Health Research Institute, is a Washington Hospital Center based angiographic study of 1,500 subjects specifically designed for biomarker and genetic association studies of acute and chronic coronary atherosclerosis. Briefly, the MedStar study is a cross sectional study of coronary atherosclerosis in a consecutive cohort of selected patients undergoing cardiac catheterization at Washington Hospital Center between August 2004 and March 2007. All subjects were enrolled in a MedStar Health Research Institute Institutional Review Board approved protocol and all subjects gave written informed consent. Enrollment criteria included any clinical indication for cardiac catheterization and ability to give informed consent. The following data was extracted from the medical record; age, gender, race/ethnicity, past medical, social, family and medication history, cardiovascular risk factors (diabetes, smoking, and hypertension), physical exam including vital signs, weight and height (for BMI), and cardiovascular findings. Ethnicity information was self-reported. Coronary angiograms were scored on the day by the interventional cardiologist who performed the procedure and reviewed by a second cardiologist. Blood was drawn in a 12-hour fasting state (except in those with acute MI), at the time of the initial catheter insertion prior to the administration of any contrast dye for plasma, for serum separation and Buffy coat DNA isolation.

MedStar (N=1,322 Caucasians) composed of controls (N=447) who on coronary angiography showed no evidence of CAD and CAD (AngCAD) cases (N=875) with one or

more coronary vessels with ≥50% stenosis divided into AngCADMI+ cases (N=421) and AngCADMI- cases (N=454). Controls were aged over 45 in men and women. Cases were selected to be young with age at diagnosis of CAD ≤55 for males and ≤60 for females. MedStar genotyping was performed with PennCath samples at U. Penn. Limited data from the MedStar GWAS has been used previously for replication of novel genes for atherosclerotic CVD and its risk factors.

(Adapted from the supplementary material in: Reilly, M.P., et al., Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet*, 2011. 377(9763): p. 383-92.)

POPGEN*: The CHD patients comprised a population-representative collection of unrelated Germans. They were recruited from Schleswig-Holstein, the northernmost region in Germany, through the population-based biobank popgen (1). In the recruitment area, all coronary angiograms of any of the five cardiac catheterization laboratories were screened. Study subjects were described before (2) and were required to have coronary catheterization demonstrating significant CHD (at least a 70% stenosis in one major epicardial coronary vessel). 1,104 cases had a diagnosed disease onset <55 years, of whom a subset of 596 individuals had suffered a myocardial infarction. The majority (90.3%) had a history of severe CHD and had undergone a coronary revascularization procedure (percutaneous coronary intervention or coronary artery bypass grafting). Written informed consent was obtained from all participants and the recruitment and the experimental protocols were approved by the institutional ethics review board and data protection authorities.

1. Krawczak M, Nikolaus S, von Eberstein H, Croucher PJ, El Mokhtari NE, et al. (2006) PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. Community Genet 9: 55–61.
2. Schaefer AS, Richter GM, Groessner-Schreiber B, Noack B, Nothnagel M, El Mokhtari NE, Loos BG, Jepsen S, Schreiber S. Identification of a Shared Genetic Susceptibility Locus for Coronary Heart Disease and Periodontitis. PLoS Genet. 2009 Feb;5(2):e1000378. Epub 2009 Feb 13.

PROCARDIS*: Ascertainment criteria for PROCARDIS probands were MI or symptomatic acute coronary syndrome (SACS), on the assumption that the latter represents a similar pathological process according to modified World Health Organisation diagnostic criteria before the age of 66 y. [1] Diagnosis of MI required documentation of two or more of: (a) typical ischemic chest pain, pulmonary edema, syncope or shock; (b) development of pathological Q-waves and/or appearance or disappearance of localized ST-elevation followed by T-wave inversion in two or more standard electrocardiograph leads; (c) increase in concentration of serum enzymes consistent with MI (e.g. creatine kinase more than twice the upper limit of normal). Diagnosis of SACS required documentation of hospitalization for one of the following indications: (a) unstable angina diagnosed by

typical ischemic chest pain at rest associated with reversible ST-depression in two or more standard electrocardiograph leads; (b) thrombolysis for suspected MI (as indicated by localized ST-elevation in two or more standard electrocardiograph leads) even without later development of T-wave inversion, Q-waves, or a significant enzyme rise; or (c) emergency revascularization (i.e. during same admission) following presentation with typical ischemic chest pain at rest. Probands completed questionnaires in order to recruit affected siblings with a range of CAD diagnoses at age < 66 y (MI, SACS, chronic stable angina, or intervention for coronary revascularization), who were then invited to participate in the study if their diagnoses were confirmed. Parents and up to four unaffected siblings per family were recruited wherever possible to augment the recovery of linkage phase information. Informative families were recruited in Germany, Italy, Sweden, and the United Kingdom; 99.5% of the study participants reported having a white European ancestry. The protocol was approved by the Ethics Committees of the participating institutions and all participants gave written, informed consent.

1. ISFC/WHO (1979) Nomenclature and criteria for diagnosis of ischemic heart disease. Report of the Joint International Society and Federation of Cardiology/World Health Organization task force on standardization of clinical nomenclature. Circulation 59: 607–609

(Description adapted from: Farrall M., Green F.R., Peden J.F., Olsson P.G., Clarke R., Hellenius M.L., Rust S., Lagercrantz J., Franzosi M.G., Schulte H., et al. Genome-wide mapping of susceptibility to coronary artery disease identifies a novel replicated locus on chromosome 17. PLoS Genet. 2006;2:e72.)

The Southampton Atherosclerosis Study (SAS)*: Consecutive Caucasian patients undergoing interventional or diagnostic coronary angiography were recruited in the Wessex Cardiothoracic Unit, Southampton General Hospital. Demographic and clinical data were recorded including age, gender, weight, height, occupation, smoking habit and number of cigarettes consumed per day by each smoker, the presence or absence of hyperlipidemia (defined as cholesterol >5.2 mmol/l and/or triglyceride >3 mmol/l), current medications particularly the use of statins and fibrates, the presence or absence of hypertension (defined as diastolic blood pressure > 95 mmHg and/or systolic blood pressure > 160 mmHg), the presence or absence of type 1 or type 2 diabetes, the presence or absence of previous myocardial infarction, and the presence or absence of coronary heart disease in first degree relatives under 65 years of age. All coronary angiograms were assessed by one consultant cardiologist. A total of 1178 patients had angiographically documented CAD as having >50% diameter stenosis in at least one major epicardial coronary artery. Among them, 479 had >50% stenosis in one coronary artery, 397 had 50% stenosis in two coronary arteries, and 302 had >50% stenosis in three coronary arteries. Among the 1178 patients with angiographic CAD, 639 had had an MI diagnosed according to standard clinical criteria with electrocardiographic and enzymatic changes.

SK-CAD, South Korea (Shen et al)*: Case-control association study involving 611 unrelated cases (63.7±10.1 years old) that were randomly selected from patients admitted to the Samsung Medical Center (Seoul, South Korea) with a diagnosis of CAD. Koreans represent one of the world's most ethnically and genetically homogeneous populations and are ideal for case-control association studies. As such, all subjects in this study were of the same ethnicity (Korean). The diagnostic criteria for CAD are as described previously and include ≥70% luminal narrowing in at least one vessel by coronary angiography, percutaneous coronary angioplasty, coronary artery bypass graft, and MI. The diagnosis of MI was based on typical chest pain of >30 minutes duration, characteristic electrocardiographic patterns of acute MI, and significant elevation of cardiac enzymes such as creatine kinase myocardial band "CK-MB". For the control group, 294 volunteers (60.1±11.0 years old) were selected from individuals who were admitted to Samsung Medical Center for evaluation by coronary angiography for reasons other than CAD, mainly valvular heart disease, but were found to have no detectable stenosis. Thus, the controls were in general good health and determined to have no CAD. Blood was drawn after an overnight fast but within 48 hours of admission to the hospital and before coronary angiography or percutaneous coronary intervention. Fasting concentrations of total cholesterol, triglyceride, and blood sugar were measured at the Department of Laboratory Medicine at Samsung Medical Center. Hypertension was defined as systolic blood pressure of >140 mm Hg or diastolic blood pressure >90 mm Hg, and diabetes was defined as ongoing therapy of diabetes or a fasting blood sugar of >126 mg/dL. The local institutional review board is on human subject research approved this study, and written informed consent was obtained from all participants. The investigation conformed to the principles outlined in the Declaration of Helsinki.

(Description adapted from: Shen Gong-Qing, Li L, Rao S, et al. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. *Arterioscler Thromb Vasc Biol* . 2008; 28:360-365)

China (Wang et al)*: The study population consisted of 2387 Chinese Han patients undergoing coronary angiography to evaluate suspected or established CAD. Of these, 1278 patients had type 2 diabetes with (n=620) and without (n=658) significant CAD (≥50% luminal diameter narrowing in at least one coronary artery), and 1109 were non-diabetics (545 with and 564 without CAD). Type 2 diabetes was defined as a fasting plasma glucose level of ≥7.0 mmol/L or a non-fasting plasma glucose level of ≥11.1 mmol/L, or taking oral hypoglycemic drugs or receiving parenteral insulin therapy. Patients with type 1 diabetes were identified by measuring C peptide levels and excluded; we also excluded those with chronic viral or bacterial infections, tumors, or immune system disorders. Diagnosis of hypertension was based on the presence of elevated systolic (≥140 mmHg) and/or diastolic (≥90 mmHg) blood pressure, or use of antihypertensive medications in the year before admission. Patients were diagnosed with hyperlipidemia if they had serum levels of total cholesterol (TC) >5.7 mmol/L (220 mg/dl), triglycerides (TG) >1.7 mmol/L (150 mg/dl), low-density lipoprotein cholesterol (LDL-C) >3.64 mmol/L (140 mg/dl), or high-

density lipoprotein cholesterol (HDL-C) <0.91 mmol/L (35 mg/dl). Early-onset CAD was defined as clinical CAD occurring by age ≤55 years in male or ≤60 years in female patients.

(Description adapted from: Wang W, et al. Polymorphism on chromosome 9p21.3 contributes to early-onset and severity of coronary artery disease in non-diabetic and type 2 diabetic patients. *Chin Med J*. 2011;124(1):66-71)

The Wellcome Trust Case-Control Consortium (WTCCC) Study: The WTCCC design and findings for CAD have been described in detail in prior publications(11, 12). For the analysis of CAD vs. CAD-free controls in the present manuscript, we restricted analysis to CAD cases that had undergone coronary artery bypass graft (CABG) surgery or percutaneous coronary revascularization (PCI) indicating significant angiographic CAD. This represented 1,318 AngCAD cases selected from the total 1,926 CAD cases in WTCCC.

(Adapted from the supplementary material in: Reilly, M.P., et al., Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet*, 2011. 377(9763): p. 383-92.)

Supplementary Table 1: Summary of studies with insufficient data for meta-analysis

Study	**Cohort**	**CHD cases n**	**Ethnicity**	**Main findings in the citation**
Thedoraki et al [1]	THISEAS	305	European ancestry	Not reported for 9p21 variants
Chen et al [2]	-	212	Chinese	9p21 variants associated with early-onset CAD

1. Chen Z, Qian Q, Ma G, Wang J, Zhang X, Feng Y, Shen C, Yao Y. A common variant on chromosome 9p21 affects the risk of early-onset coronary artery disease. *Mol Biol Rep*. 2009;36:889-893
2. Theodoraki EV, Nikopensius T, Suhorutsenko J, Peppes V, Fili P, Kolovou G, Papamikos V, Richter D, Zakopoulos N, Krjutskov K, Metspalu A, Dedoussis GV. Fibrinogen beta variants confer protection against coronary artery disease in a Greek case-control study. *BMC Med Genet*. 2010;11:28

Bayesian analysis methodology

The approach allows inference on which (if any), of the SNPs are most likely to be associated with disease through use of a Bayesian model selection algorithm (namely Reversible Jump [1,2]) which allows calculation of a posterior probability that each SNP is associated with disease, as well as effect estimates adjusted for the other SNPs. In particular we obtain a test of association at the locus level, i.e. across all SNPs included in the model, which increases power. For further details on the method we refer the reader to Newcombe et al.[3] This method was used to meta-analyze associations between SNPs from the 9p21 locus with MI and number of stenotic vessels. Three different SNPs from the 9p21 locus were reported by at least one of the studies we identified - rs10757278, rs1333049 and rs2383206. Ideally we would have included separate terms for all three in the Bayesian model, however, rs10757278 and rs1333049 were indistinguishable in HapMap and as such the model had no information to differentiate them - genotypes from these SNPs were pooled into a single variable. The Bayesian model required specification of prior distributions for the SNP effects, and a prior probability that at least one of the SNPs is associated with disease. For each analysis we chose Normal(0,0.4) priors for the log-Odds Ratio – this is a realistically informative prior which suggests that the Odds Ratio is most likely to lie between 0.5 and 2.2. Since we judge these SNPs to be reasonable candidates for association with MI and stenotic vessels we specified a prior probability of 10% that at least one of the SNPs was associated with each disease. Sensitivity analyses were run with alternative choices of 5% and 20%, in which inference was identical. Analyses were run for 10 million iterations to insure convergence, which was checked through inspection of chain plots and running different chains.

1. Green, P.J., *Reversible jump Markov chain Monte Carlo computation and Bayesian model determination.* Biometrika, 1995. 82(4): p. 711-732.

2. Lunn, D.J., J.C. Whittaker, and N. Best, *A Bayesian toolkit for genetic association studies.* Genet Epidemiol, 2006. 30(3): p. 231-47.

3. Newcombe, P.J., et al., *Multilocus Bayesian meta-analysis of gene-disease associations.* Am J Hum Genet, 2009. 84(5): p. 567-80.

Supplementary Table 2: Summarized odd ratio per copy of 9p21 risk allele for angiographic CAD and MI respectively, by Bayesian analysis

	SNP	OR*	95% CI*	Posterior Probability of association with disease**	Bayes Factor for association with disease	Interpretation
All subjects						
1 vessel vs 2 vessels	rs23830206	1.07	(1.02, 1.14)	0.15	3.4	Some evidence that one of the SNPs is associated (posterior probability 29%), but they cannot be distinguished (both SNPs received approximately equal individual posterior probabilities).
	rs10757278/rs1333049	1.07	(1.02, 1.14)	0.14	3.2	
	One or more	-	-	0.29	4.0	
2 vessels vs 3 vessels	rs23830206	1.07	(1.01, 1.13)	0.13	2.8	Some evidence that one of the SNPs is associated (posterior probability 26%), but they cannot be distinguished (both SNPs received approximately equal individual posterior probabilities).
	rs10757278/rs1333049	1.07	(1.02, 1.13)	0.14	3.0	
	One or more	-	-	0.26	3.4	
1 vessel vs Multivessel	rs23830206	1.12	(1.07, 1.17)	0.48	17.7	Extremely strong evidence that one of the SNPs is associated (posterior probability 100%), but they cannot be distinguished (both SNPs received approximately equal individual posterior probabilities).
	rs10757278/rs1333049	1.11	(1.07, 1.17)	0.53	21.5	
	One or more	-	-	1.00	24,219	
CAD/MI+ vs CAD/CAD/MI-	rs23830206	0.98	(0.95, 1.01)	0.01	0.2	No evidence of association at any SNP (99% posterior probability for the null model),
	rs10757278/rs1333049	0.98	(0.95, 1.02)	0.01	0.1	
	One or more	-	-	0.01	0.1	
Non-diabetic only						
1 vessel vs Multivessel	rs23830206	1.13	(1.07, 1.20)	0.39	12	Extremely strong evidence that one of the SNPs is associated (posterior probability 100%).
	rs10757278/rs1333049	1.13	(1.07, 1.19)	0.62	31	The model seemed to favor rs10757278/rs1333049 (posterior probability was 62% vs 39% for rs23830206)
	One or more	-	-	1.00	2,840	
MI	rs23830206	1.00	(0.96, 1.04)	0.01	0.1	No evidence of association at either SNP (97% posterior probability for the null model)
	rs10757278/rs1333049	1.00	(0.96, 1.04)	<0.01	0.1	
	One or more	-	-	0.03	0.1	

*Calculated conditional on the variant being included in the model (SNPs jump in and out of the model as part of the Reversible Jump algorithm, to allow estimation of posterior probabilities), and the other variant excluded from the model (eases interpretation since variants are so highly correlated). **Should be interpreted with regard to the prior probability - these were 0.1 for one or more variants, and 0.05 for each individually.

Supplementary Figure Legends:

Figure S1: Linkage disequilibrium between the 3 9p21 SNPs included in the meta- analysis, based on data from HapMap. Numbers shown in red diamonds are r^2 values (in percentage). r^2 values for rs10757278 and rs1333049 is 1 (100%).

Figure S2: Odds ratios for angiographic CAD as compared to angiographic controls for 9p21 genotype (n=10,428 cases, n=2,722 controls): (A) per copy of risk allele (risk allele versus non-risk allele); (B) risk allele heterozygotes versus non-risk allele homozygotes; (C) risk allele homozygotes versus non-risk allele homozygotes.

Figure S3: Odds ratios for two-vessel CAD as compared with single-vessel CAD per copy of 9p21 risk allele (risk allele versus non-risk allele). D+L = DerSimonian and Laird random effects model, Bayesian = Bayesian model. Note: non-European ancestry cohorts were not included in the Bayesian analysis. Bayesian analysis was unable to distinguish between rs10757278/rs1333049 and rs2383206, for which the marginal effect estimate was nearly identical.

Figure S4: Odds ratios for three-vessel CAD as compared with two-vessel CAD per copy of 9p21 risk allele (risk allele versus non-risk allele). D+L = DerSimonian and Laird random effects model, Bayesian = Bayesian model. Note: non-European ancestry cohorts were not included in the Bayesian analysis. Bayesian analysis was unable to distinguish between rs10757278/rs1333049 and rs2383206, for which the marginal effect estimate was nearly identical.

Figure S5: Odds ratios for association between 9p21 and MI in CAD+ patients, under a genotypic model comparing heterozygote risk carriers versus non risk homozygotes. CAD/MI+ = cases with history of MI and underlying CAD; CAD/MI- = controls without history of MI but with underlying CAD. Subgroup analysis for different definitions of CAD used – clinical, angiographic stenosis >50% or >70%.

Figure S6: Odds ratios for association between 9p21 and MI in CAD+ patients, under a genotypic model comparing homozygote risk carriers versus non risk homozygotes. CAD/MI+ = cases with history of MI and underlying CAD; CAD/MI- = controls without history of MI but with underlying CAD. Subgroup analysis for different definitions of CAD used – clinical, angiographic stenosis >50% or >70%.

Figure S7: Summary odds ratio per copy of 9p21 risk allele for MI in CAD patients, by study size, ethnicity and diabetes status categories.

Figure S8: Funnel plot and L'Abbe plot for analysing publication bias and heterogeneity in the meta-analysis of 9p21 association with MI in CAD patients (Allelic Model)

Figure S1: Linkage disequilibrium between the three 9p21 SNPs included in the meta-analysis

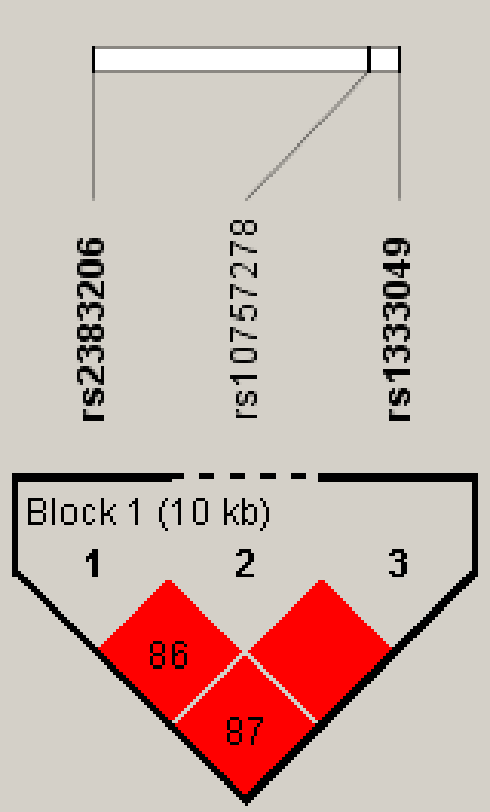
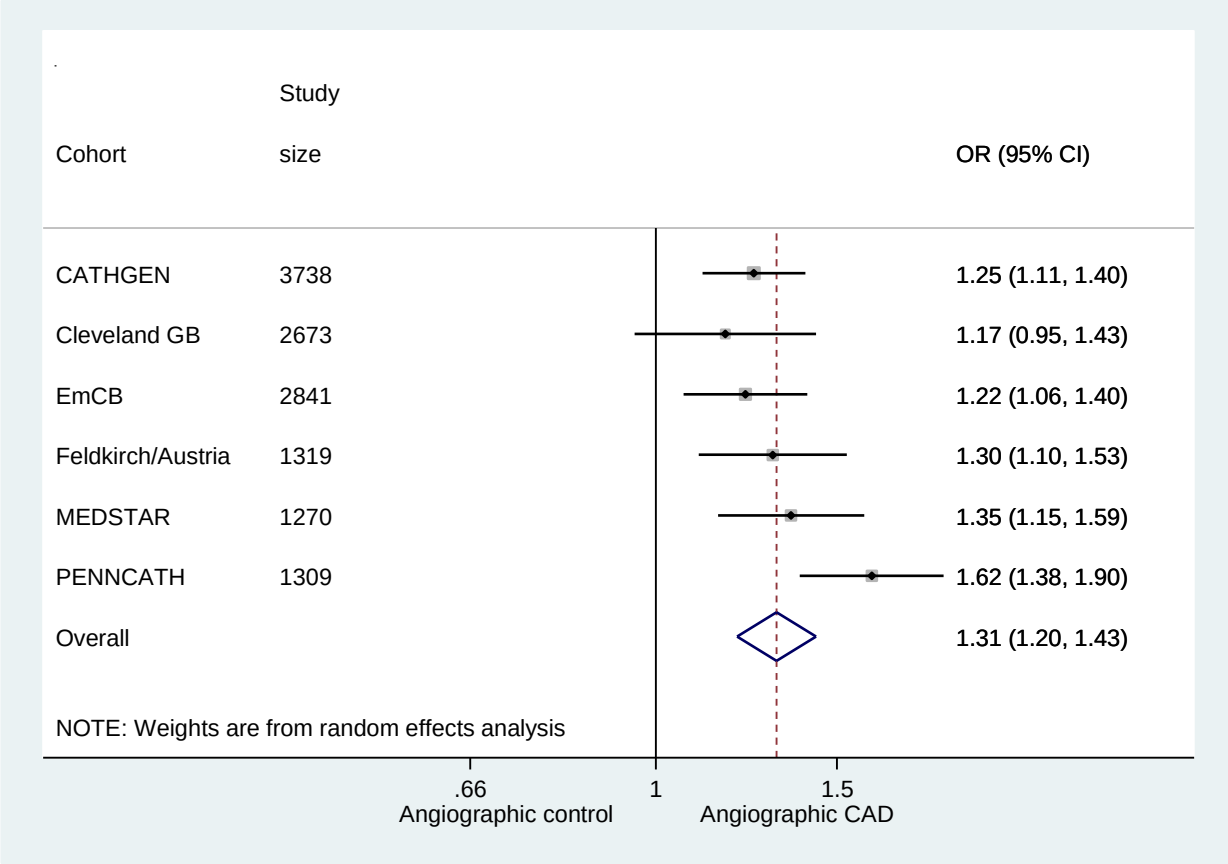
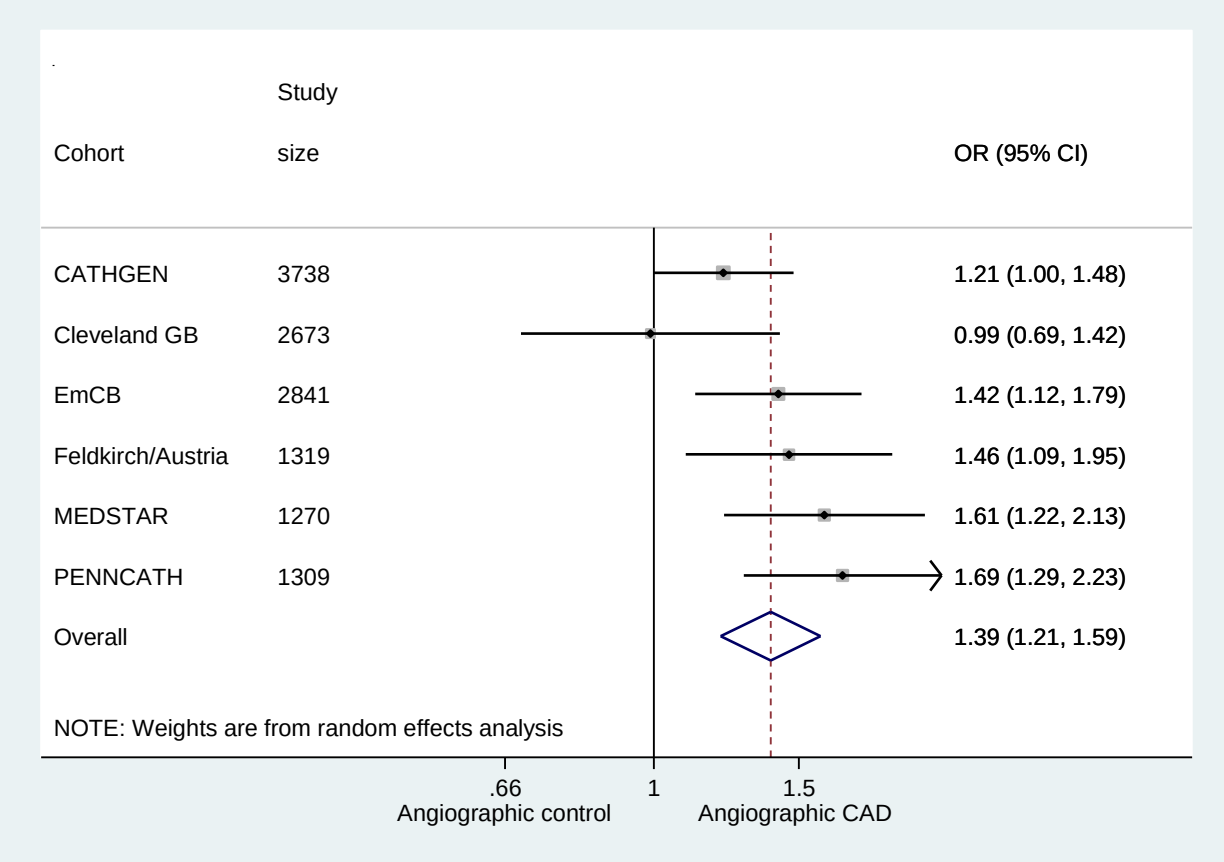


Figure S2A: Association between 9p21 and angiographic CAD versus angiographic controls (Allelic Model)



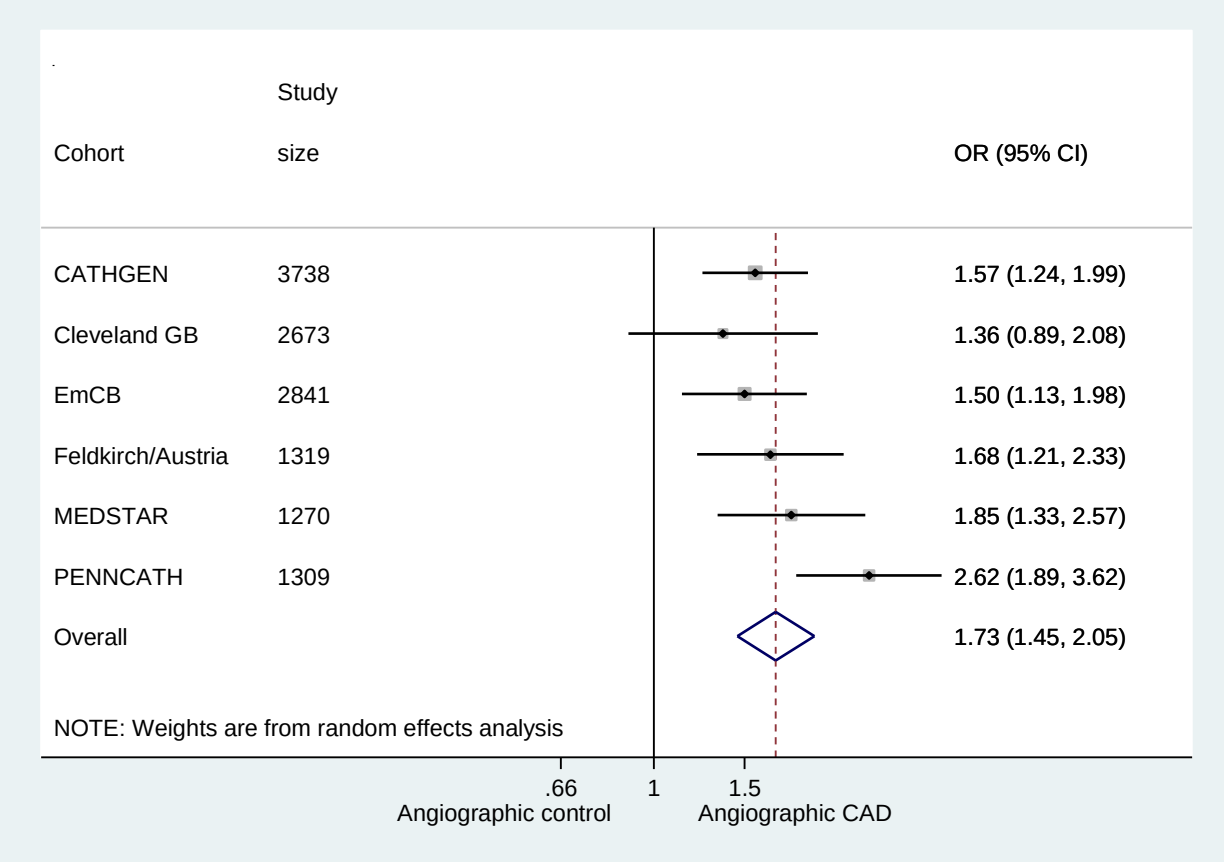
Test of OR=1 : z= 6.01, p < 0.0001

Figure S2B: Association between 9p21 and angiographic CAD versus angiographic controls (Genotypic Model – Heterozygote Risk v Homozygote Non Risk)



Test of OR=1: z= 4.59, p < 0.0001

Figure S2C: Association between 9p21 and angiographic CAD versus angiographic controls (Genotypic Model – Homozygote Risk versus Homozygote Non Risk)



Test of OR=1: z= 6.18, p < 0.0001

Figure S3: Association between 9p21 and two-vessel disease as compared with one-vessel disease (Allelic Model)

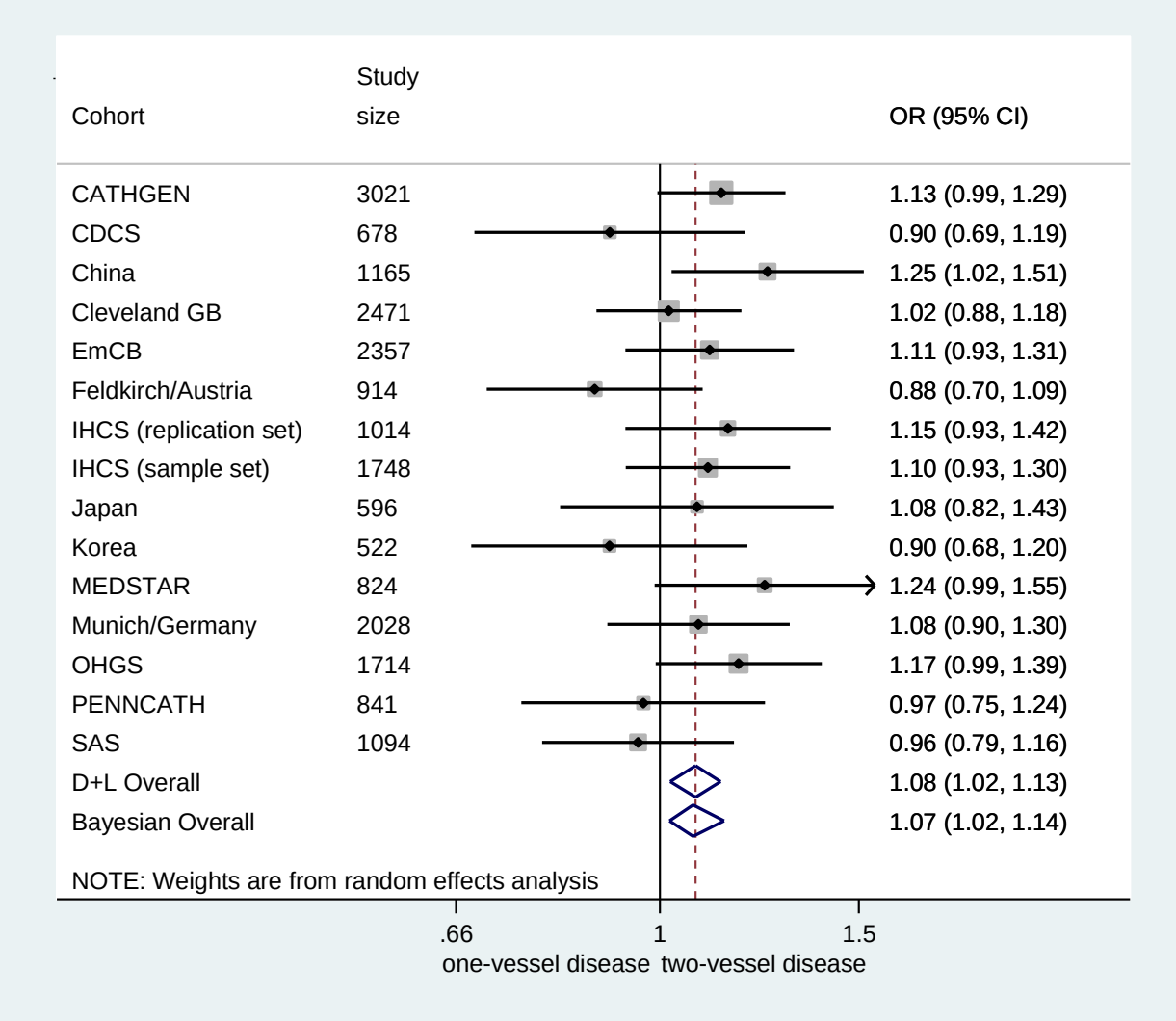


Figure S4: Association between 9p21 and three-vessel disease as compared with two-vessel disease (Allelic Model)

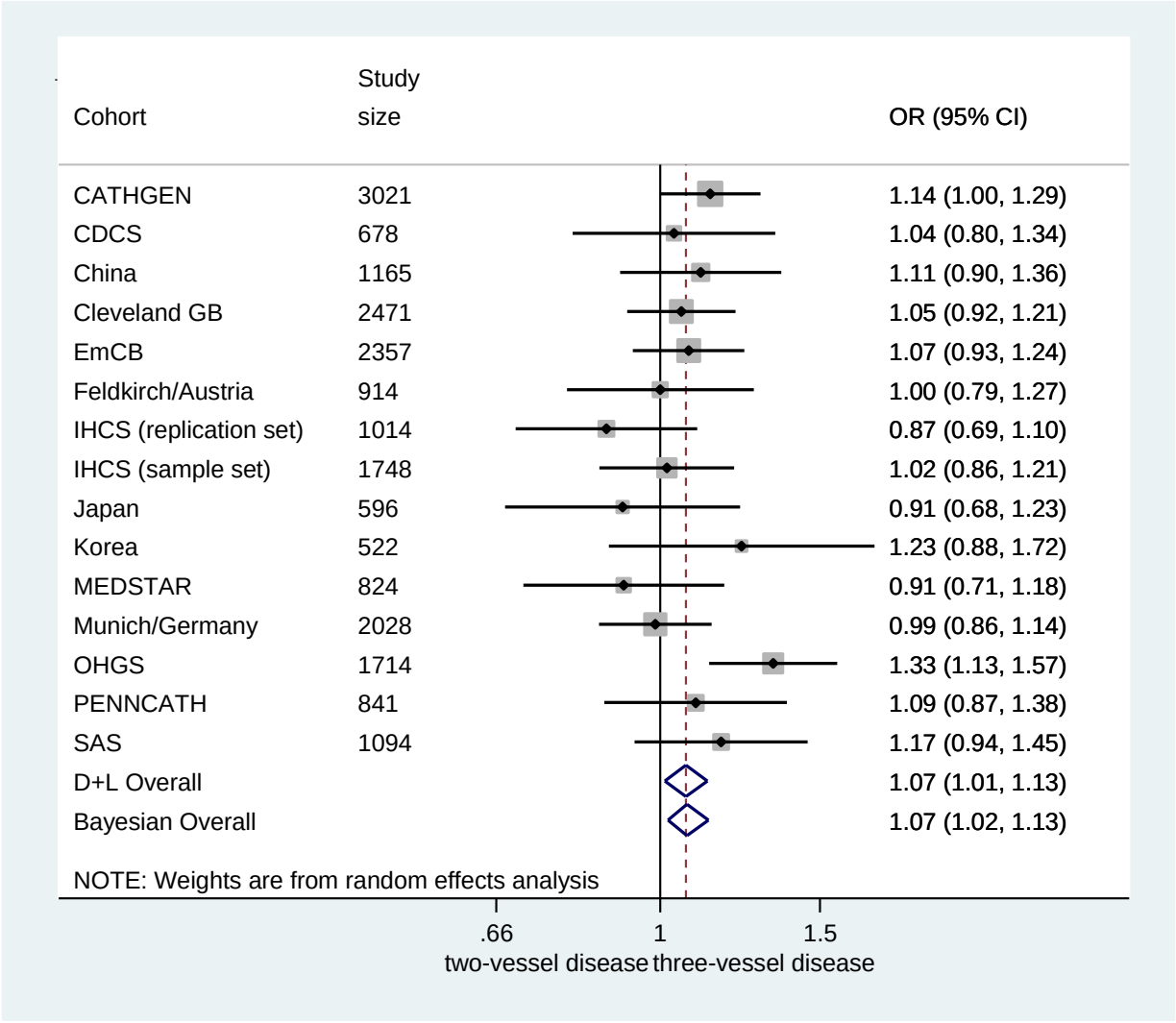
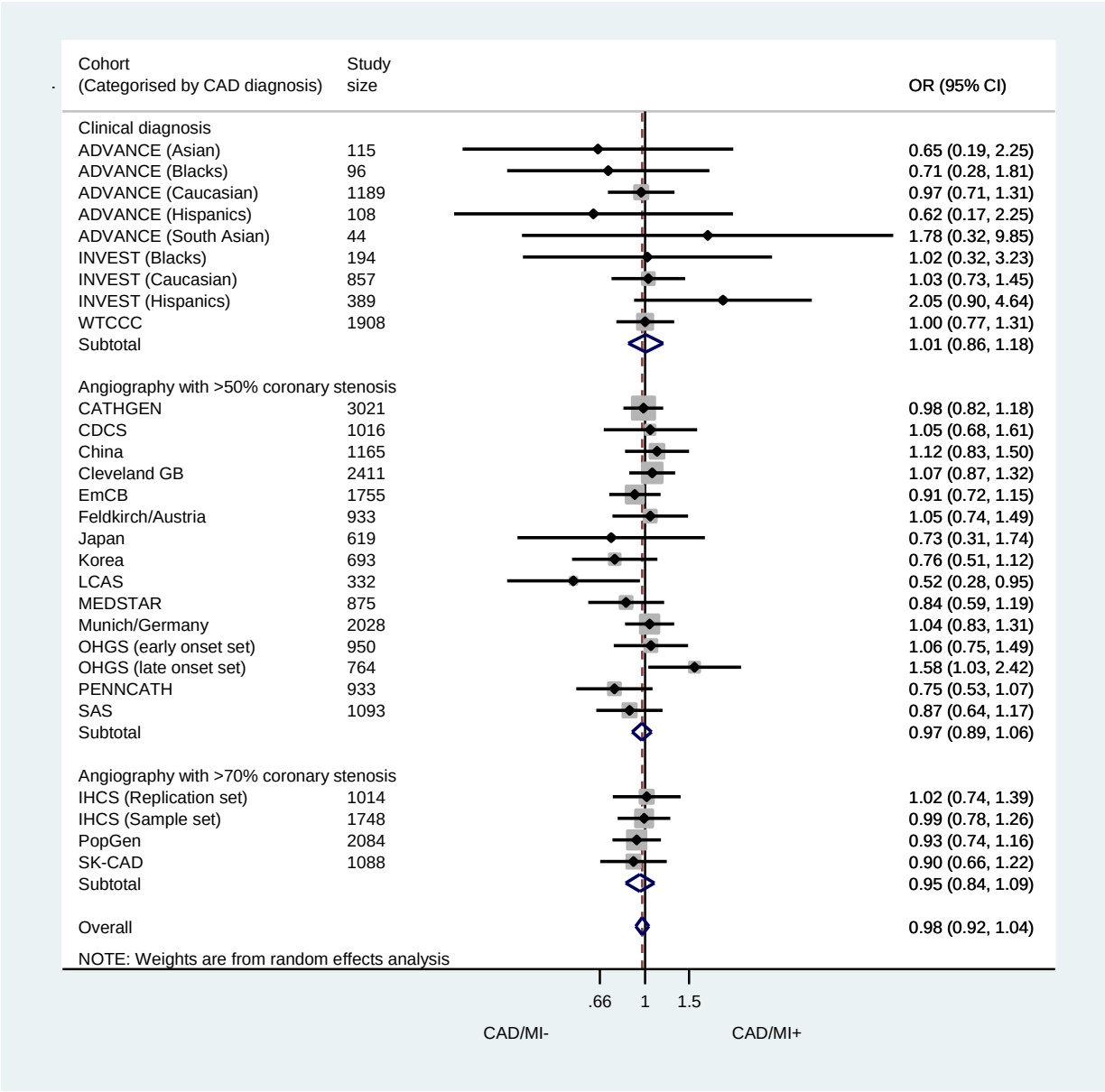
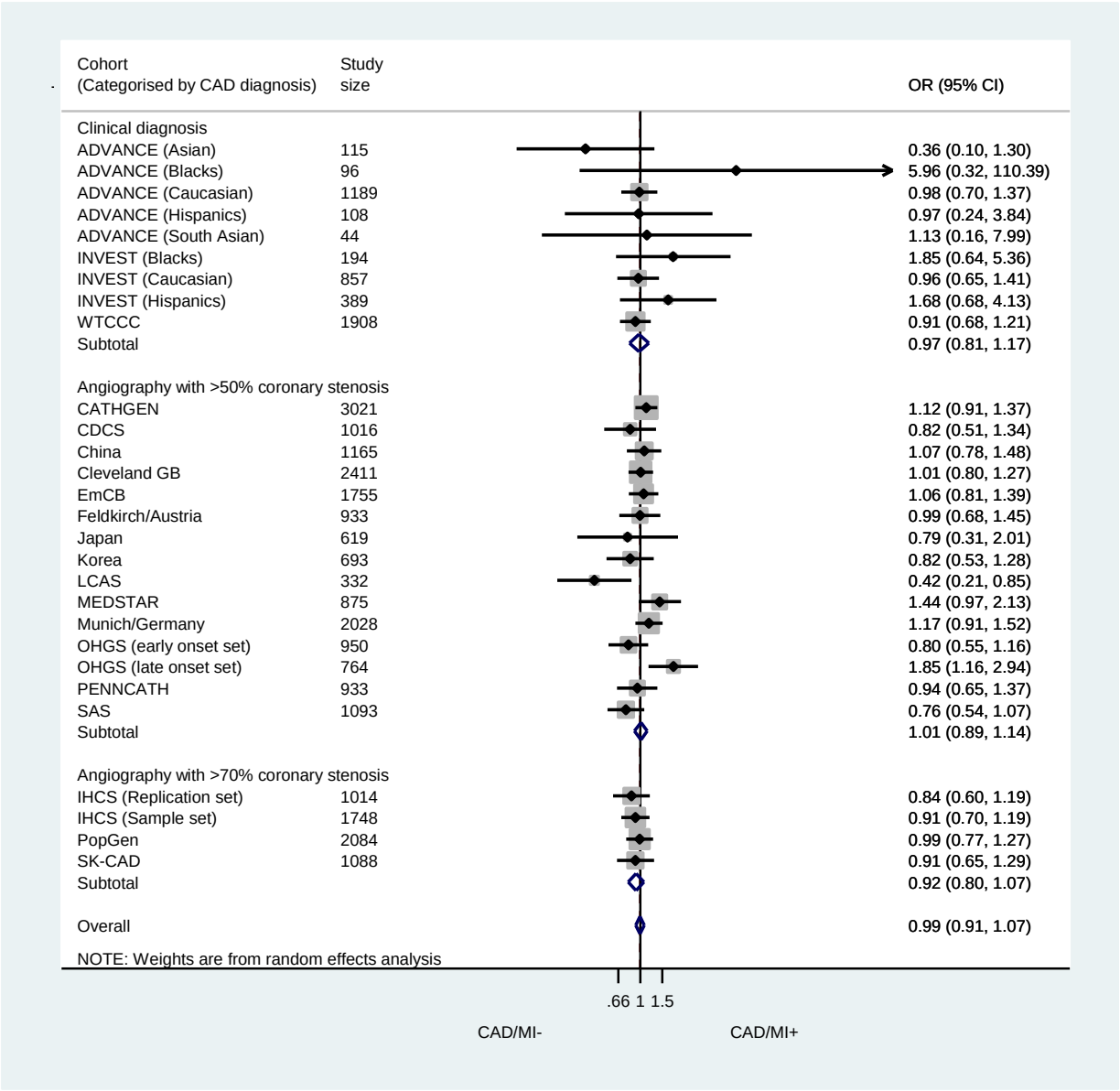


Figure S5: Association between 9p21 and MI in patients with underlying CAD (Genotypic Model - Heterozygote Risk versus Homozygote Non Risk).



Significance test(s) of OR=1
Clinical diagnosis z= 0.08 p = 0.936
Angiography with >50 z= 0.66 p = 0.510
Angiography with >70 z= 0.69 p = 0.491
Overall z= 0.80 p = 0.425

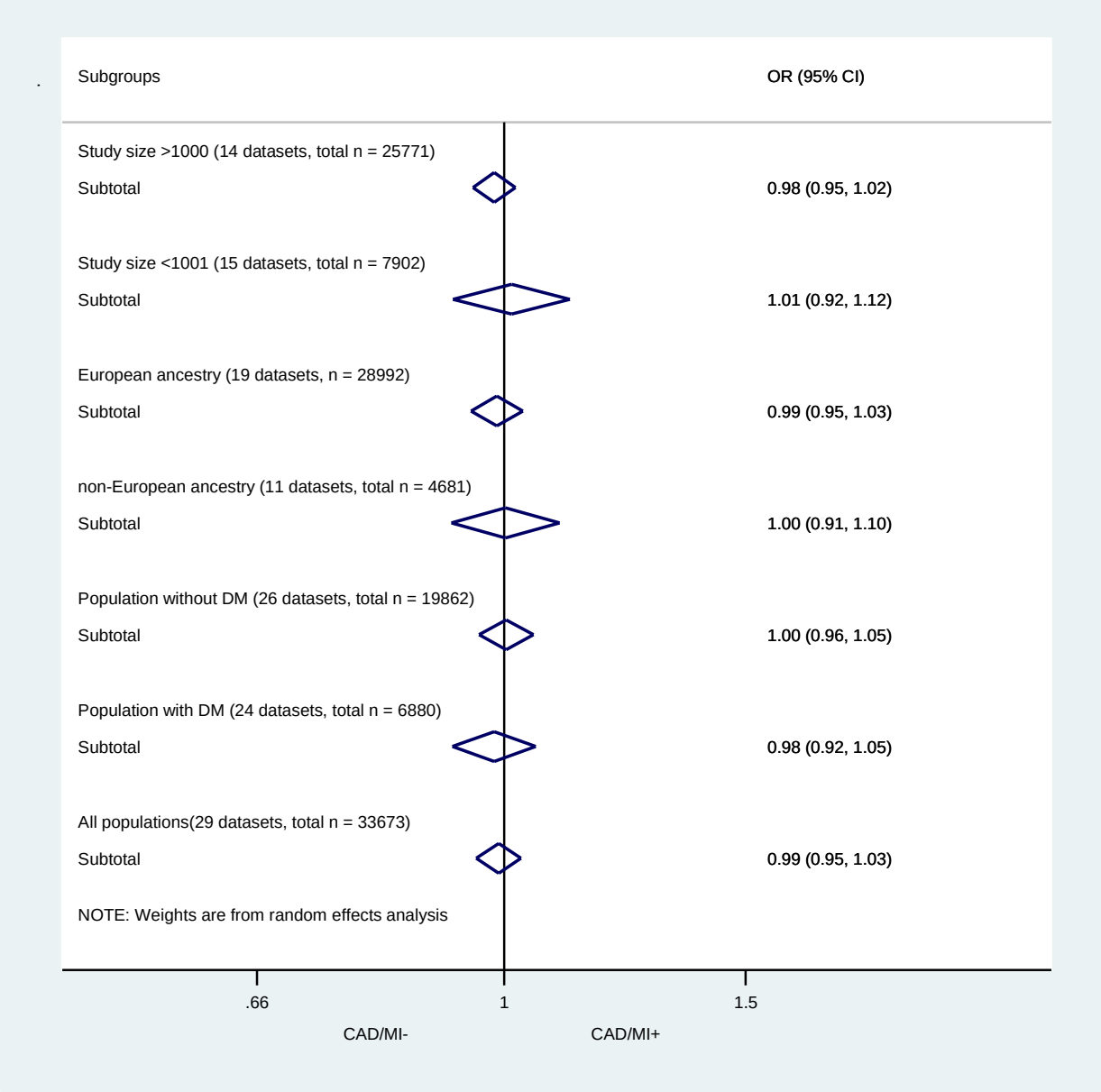
Figure S6: Association between 9p21 and MI in patients with underlying CAD (Genotypic Model - Homozygote Risk versus Homozygote Non Risk)



Significance test(s) of OR=1

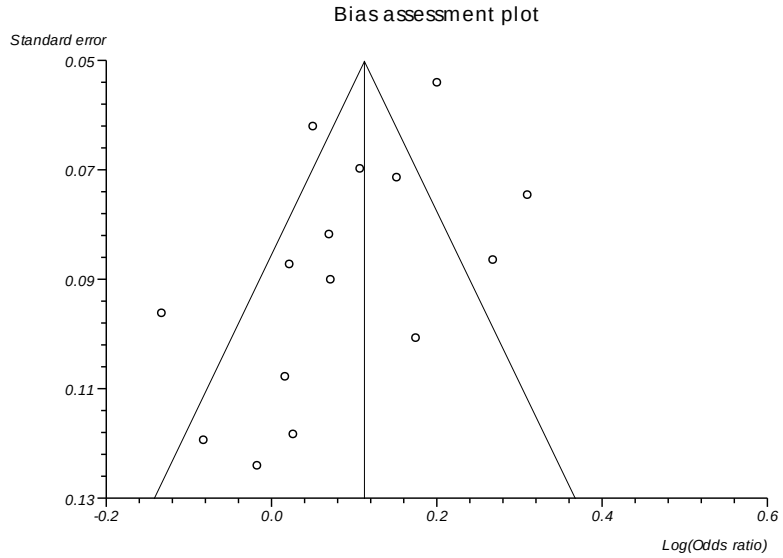
Clinical diagnosis z= 0.29 p = 0.776
Angiography with >50 z= 0.12 p = 0.904
Angiography with >70 z= 1.09 p = 0.277
Overall z= 0.27 p = 0.789

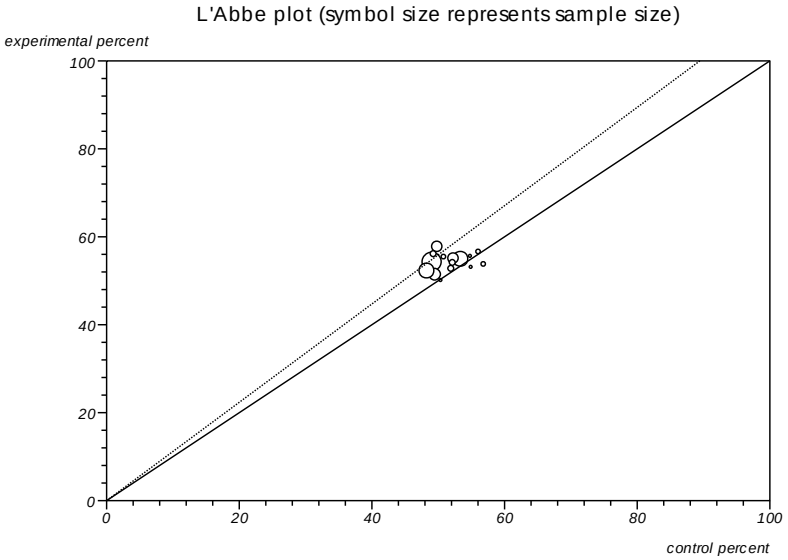
Figure S7: Summary odds ratios per copy of 9p21 risk allele for MI in CAD patients, by subgroups



DM = Diabetes mellitus

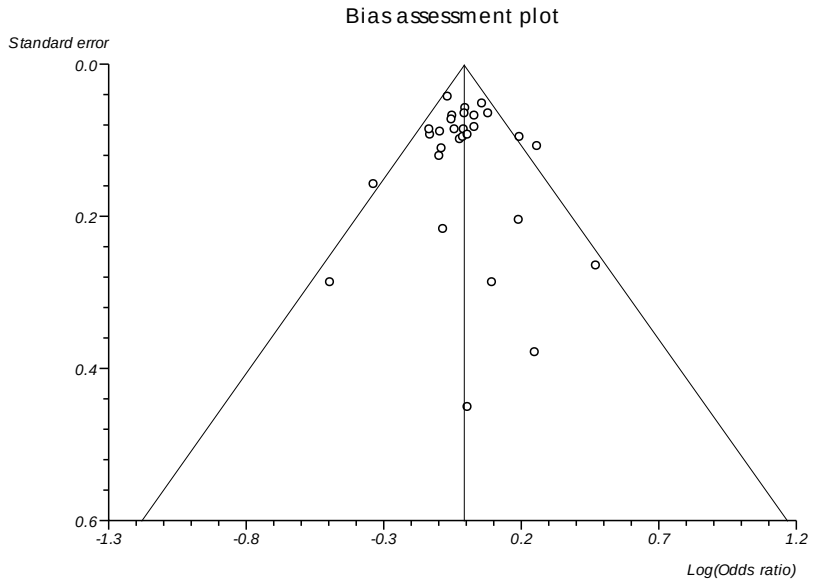
Figure S8. Funnel plot and L'Abbe plot for analysing publication bias and heterogeneity in the meta-analysis of 9p21 association with angiographic CAD burden (Allelic Model)

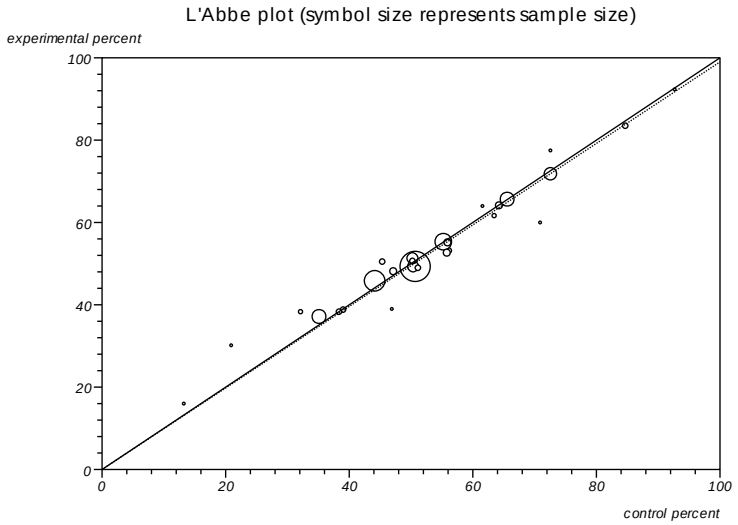




Random effects (DerSimonian-Laird)
Pooled odds ratio = 1.103227 (95% CI = 1.038376 to 1.172127)
Chi^2 (test odds ratio differs from 1) = 10.101659 (df = 1) P = 0.0015

Bias indicators
Begg-Mazumdar: Kendall's tau = -0.352381 P = 0.059
Egger: bias = -2.842009 (95% CI = -5.796918 to 0.1129) P = 0.0581
Horbold-Egger: bias = -2.839311 (92.5% CI = -5.495802 to -0.18282) P = 0.0591
Figure S9. Funnel plot and L'Abbe plot for analysing publication bias and heterogeneity in the meta-analysis of 9p21 association with MI in CAD patients (Allelic Model)





Random effects (DerSimonian-Laird)
Pooled odds ratio = 0.990373 (95% CI = 0.95385 to 1.028295)
Chi^2 (test odds ratio differs from 1) = 0.254602 (df = 1), P = 0.6139

Bias indicators
Begg-Mazumdar: Kendall's tau = -0.019704, P = 0.8672
Egger: bias = 0.096736 (95% CI = -0.898515 to 1.091987), P = 0.8434
Horbold-Egger: bias = 0.09212 (92.5% CI = -0.811716 to 0.995956), P = 0.8517