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Lack of Association Between the Trp719Arg Polymorphism in Kinesin-Like Protein-6 and Coronary Artery Disease in 19 Case-Control Studies

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Objectives We sought to replicate the association between the kinesin-like protein 6 (KIF6) Trp719Arg polymorphism (rs20455), and clinical coronary artery disease (CAD).

Background Recent prospective studies suggest that carriers of the 719Arg allele in *KIF6* are at increased risk of clinical CAD compared with noncarriers.

Methods The *KIF6* Trp719Arg polymorphism (rs20455) was genotyped in 19 case-control studies of nonfatal CAD either as part of a genome-wide association study or in a formal attempt to replicate the initial positive reports.

Results A total of 17,000 cases and 39,369 controls of European descent as well as a modest number of South Asians, African Americans, Hispanics, East Asians, and admixed cases and controls were successfully genotyped. None of the 19 studies demonstrated an increased risk of CAD in carriers of the 719Arg allele compared with noncarriers. Regression analyses and fixed-effects meta-analyses ruled out with high degree of confidence an increase of ≥2% in the risk of CAD among European 719Arg carriers. We also observed no increase in the risk of CAD among 719Arg carriers in the subset of Europeans with early-onset disease (younger than 50 years of age for men and younger than 60 years of age for women) compared with similarly aged controls as well as all non-European subgroups.

Conclusions The *KIF6* Trp719Arg polymorphism was not associated with the risk of clinical CAD in this large replication study. (J Am Coll Cardiol 2010;56:1552–63) © 2010 by the American College of Cardiology Foundation

Recent prospective observational studies suggest an association between the Trp719Arg single nucleotide polymorphism (SNP) in kinesin-like protein 6 (rs20455) and the development of clinical coronary artery disease (CAD) (1–5). Carriers of the 719Arg alleles were found to have a modest increase in the risk in the Atherosclerosis Risk in Communities study (ARIC) (hazard ratio [HR] assuming log additive model: 1.11; 95% confidence interval [CI]: 1.02 to 1.21, p = 0.02) (1), the CHS (Cardiovascular Health Study) (HR assuming log dominant model: 1.29; 95% CI: 1.1 to 1.52, p = 0.005) (5), and the WHS (Women's Health Study) (HR assuming log dominant model: 1.24; 95% CI: 1.04 to 1.46, p = 0.01) (4).

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An increase in the risk of CAD was also observed in the placebo arm of 2 statin trials: the WOSCOPS (West of Scotland Coronary Prevention Study) (odds ratio [OR] for incident CAD events assuming log dominant model: 1.55; 95% CI: 1.14 to 2.09, p = 0.005) and the CARE (Cholesterol and Recurrent Events) trial (HR for recurrent myocardial infarction [MI] assuming log dominant model: 1.50; 95% CI: 1.05 to 2.15, p = 0.03) (3). Curiously, carriers of the 719Arg allele were not at increased risk of CAD or recurrent MI in the pravastatin arms of these 2 trials. Furthermore, in the PROVE IT–TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22) trial, carriers in the pravastatin arm were also not at increased risk of CAD, whereas carriers in the atorvastatin arm had a *decreased* risk compared with noncarriers (adjusted HR: 0.65; 95% CI: 0.48 to 0.88; p = 0.005) (2). The discrepant results between the 2 arms of these 3 statin trials were deemed a consequence of a differential effect of genotype on the benefit derived from the use of statins with carriers benefiting to a larger degree from statin therapy than noncarriers (2,3). Because carriers randomized to the more potent statin were actually at decreased risk compared with noncarriers in the PROVE IT–TIMI 22 trial, it was hypothesized that the degree of incremental benefit from statin use among carriers was a function of the intensity of lipid-lowering therapy (2).

The results of these initial studies have been used to justify the development of a *KIF6* Trp719Arg variant pharmacogenomic test (Statincheck) (6). This test is currently being marketed to health care professionals as an aid to identifying subjects at high risk of incident or recurrent CAD events who stand to gain the most from the use of statins (6,7). However, in recent genome-wide association studies for CAD and/or MI, SNPs at the *KIF6* locus were not among the associations reaching genomewide significance (8–12). Furthermore, in the ongoing Ottawa Heart

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Genomics Study, no association was found between the *KIF6* Trp719Arg SNP and angiographically defined CAD in a subset of 1,540 cases and 1,455 controls (13). These discrepant results demand examination of this association in additional populations to substantiate the use of the *KIF6* test in the management of subjects at risk of clinical CAD.

In this paper, we report association analyses for the *KIF6* Trp719Arg SNP in 19 case-control studies of CAD that have recently genotyped this SNP using various genotyping platforms either as part of a genome-wide association study or in a formal attempt to replicate the initial positive reports (1–5).

Abbreviations and Acronyms

CAD = coronary artery disease
CI = confidence interval
HR = hazard ratio
MI = myocardial infarction
OR = odds ratio
SNP = single nucleotide polymorphism

Methods

Study populations. We included subjects participating in 19 different case-control studies of CAD conducted around the world: the ADVANCE (Atherosclerotic Disease, VAscular functioN, and genetiC Epidemiology) study (14) of northern California; the AMI Gene Study/Dortmund Health (15) in Germany; the CATHGEN Research Project (16) in North Carolina; the deCODE CAD study (10) in Iceland; the National FINRISK study (17) in Finland; the sibling GeneSTAR (Genetic Study of Atherosclerosis Risk) (18) in Maryland; the GerMIFS I and Ger-MIFS II (German Myocardial Infarction Family) studies (8) in Germany; the HARPS (Heart Attack Risk in Puget Sound) (19) study in Washington state; the international INTERHEART study (20,21) coordinated by the Population Health Research Institute of McMaster University in Hamilton, Ontario, Canada; the IFS (Irish Family Study) (22) in Ireland; the MDC (Malmo Diet and Cancer) study in Sweden, the MEDSTAR (Medstar/Washington Hospital Center) (10) study in Washington, DC; the MGH PCAD (Massachusetts General Hospital Premature CAD) study (24) in Boston, Massachusetts; the MAHI (Mid-America Heart Institute) study (25,26) in Kansas City, Missouri; the PennCATH (University of Pennsylvania Medical Center Cardiac catheterization cohort) study (10) in Philadelphia, Pennsylvania; the REGICOR (Registre Gironi' del Cor) (27) study in Girona, Spain; the VHS (Verona Heart Study) (28) in Verona, Italy; and the WTCCC CAD (Wellcome Trust Case Control Consortium study of Coronary Artery Disease) (11) in the United Kingdom. All participants gave written informed consent, and local ethics committees approved all studies.

Investigators from several of these 19 studies are members of consortia with a primary interest of identifying novel genetic determinants of CAD through the use of genome-wide association study technology. The consortia include the PennCATH/MedStar consortium composed of the PennCATH and MedStar studies, the Myocardial Infarction Genetics consortium (MIGen) (12) composed of the HARPS, REGICOR, MGH PCAD, FINRISK, and MDC studies; the Cardiogenics consortium (8) composed of the WTCCC, GerMIFS I, GerMIFS II studies; and the CARDIoGRAM (Coronary ARtery Disease Genome-wide Replication And Meta-analysis) consortium composed of the ADVANCE study, the deCODE study, and the Penn/MedStar, MIGen, and CARDIOGENICS consortia.

Each study used standard criteria to identify cases with MI established by international organizations during the 1990s or more recently. Although some of these studies restricted their enrollment of cases to subjects with at least 1 MI, others included cases diagnosed with clinically significant coronary atherosclerosis without MI. Elevation of cardiac markers (creatine kinase-MB or troponin) and symptoms and/or electrocardiography suggestive of cardiac ischemia were typical criteria used to identify MI cases. Non-MI cases included subjects with angina and confirmatory test results for ischemia, unstable angina based on symptoms, and electrocardiographic changes without elevation of cardiac enzymes, and revascularization procedures that may have occurred in the presence or absence of symptoms. For the current analyses, both CAD cases with and without a diagnosis of MI were considered to emulate the outcome used in the majority of the prospective studies published to date (1–5).

Most of the studies either only enrolled subjects of European descent or restricted their genotyping efforts to Europeans. However, in addition to Europeans, the ADVANCE study genotyped a modest number of African Americans, Hispanics, East Asians, and admixed individuals; the CATHGEN and GeneSTAR studies genotyped a modest number of African Americans; and the INTERHEART study genotyped a large number of South Asians.

More details on the design of each study including the precise criteria used by each of the 19 studies to ascertain cases and controls can be found in the Online Appendix and related references.

Genotyping. The Trp719Arg polymorphism in kinesin-like protein 6 (rs20455) was directly genotyped in all studies, and no imputation of the genotype was necessary. For the GerMIFS I and WTCCC CAD studies, the genotyping data were extracted from the Affymetrix 500k array (29). For the deCODE, ADVANCE, and GeneSTAR studies, the genotyping data were extracted from the Illumina Infinium HumanHap317/370, 550, and 1M chip arrays, respectively (29). For the GerMIFS II, FINRISK, HARPS, MDC, MGH PCAD, PennCATH, Medstar, and REGICOR studies, the genotyping data were extracted from the Affymetrix 6.0 array (29). For the AMI Gene, IFS, INTERHEART, and MAHI studies, the SNP was genotyped using the iPLEX MassARRAY platform (Sequenom) platform (29). Finally, for CATHGEN and VHS, the SNP

Table 1 Study Design of Case-Control Studies Included in the Meta-Analysis of the Trp719Arg Polymorphism (rs20455) in Kinesin-Like Protein-6 and Basic Demographic Characteristics of Participants Stratified by Case-Control Status

Study	n*	Country of Study	Ascertainment Scheme	Qualifying Event	Age (yrs)/Sex Criterion	Age, yrs (Mean ± SD)	% Female	% Non-European
ADVANCE								
Cases	505	U.S.	Population- based	CAD	Men ≤45 or women ≤55	45.4 ± 6.5	61.5	45.7
Controls	514	U.S.	Population- based	—	—	45.6 ± 5.7	61.3	39.5
AMI Gene								
Cases	793	Germany	Hospital-based	MI	Men <65	52.2 ± 8.2	0.0	0.0
Controls	1,121	Germany	Hospital-based	—	—	52.6 ± 13.7	53.1	0.0
CATHGEN								
Cases	1,575	U.S.	Hospital-based	MI	≥18 men or women	61.9 ± 11.9	28.9	17.6
Controls	970	U.S.	Hospital-based	—	≥18 men or women	56.6 ± 11.8	52.4	24.7
deCODE								
Cases	4,313	Iceland	Population- based	CAD	No age criterion	68.9 ± 12.1	30.8	0.0
Controls	24,952	Iceland	Population- based	—	—	49.2 ± 21.7	63.2	0.0
FINRISK								
Cases	167	Finland	Drawn from population-based cohort	MI	Men ≤50 or women <60	47.1 ± 6.2	33.5	0.0
Controls	172	Finland	Nested case-cohort	—	—	47.1 ± 6.0	31.4	0.0
GeneSTAR								
Cases	378	U.S.	Hospital-based	CAD	Men and women <60	46.9 ± 7.0	26.2	24.6
Controls	2,652	U.S.	Unaffected siblings of cases	—	—	47.2 ± 13.1	58.2	40.5
GerMIFS I								
Cases	722	Germany	Hospital-based	MI	Men ≤60 or women <65	50.2 ± 7.9	32.5	0.0
Controls	1,643	Germany	Population-based	—	—	62.5 ± 10.1	50.5	0.0
GerMIFS II								
Cases	1,126	Germany	Hospital-based	MI	Men ≤60 or women ≤65	51.3 ± 7.6	20.3	0.0
Controls	1,277	Germany	Hospital-based	—	—	51.2 ± 11.9	47.9	0.0
HARPS								
Cases	505	U.S.	Community-based	MI	Men ≤50 or women ≤60	46.0 ± 6.9	51.1	0.0
Controls	559	U.S.	Community-based	—	—	45.2 ± 7.3	55.5	0.0
INTERHEART (Europeans)								
Cases	789	Several†	Hospital-based	MI	No age criterion	61.6 ± 12.3	29.0	0.0
Controls	859	Several†	Hospital- and community-based	—	—	61.2 ± 12.2	30.7	0.0
INTERHEART (South Asians)								
Cases	1,092	Several‡	Hospital-based	MI	No age criterion	51.4 ± 10.8	10.1	100.0
Controls	1,187	Several‡	Hospital- and community-based	—	—	49.8 ± 11.0	9.1	100.0
IFS								
Cases	482	Northern Ireland	Hospital-based	MI	Men ≤55 or women ≤60	46.0 ± 6.3	20.1	0.0
Controls	622	Northern Ireland	Older siblings of cases	—	—	55.2 ± 8.0	55.2	0.0
MDC								
Cases	86	Sweden	Drawn from population-based cohort	MI	Men ≤50 or women ≤60	48.5 ± 4.4	41.9	0.0
Controls	99	Sweden	Nested case-cohort	—	—	48.7 ± 4.6	42.4	0.0

Continued on next page

Table 1 Continued

Study	n*	Country of Study	Ascertainment Scheme	Qualifying Event	Age (yrs)/Sex Criterion	Age, yrs (Mean ± SD)	% Female	% Non-European
MedStar								
Cases	875	U.S.	Hospital-based	CAD	Men and women <65	48.9 ± 6.4	18.2	0.0
Controls	447	U.S.	Hospital-based	—	Men and women ≥45	59.8 ± 8.9	48.8	0.0
MGH PCAD								
Cases	204	U.S.	Hospital-based	MI	Men ≤50 or women ≤60	47.0 ± 6.1	29.9	0.0
Controls	260	U.S.	Hospital-based	—	—	53.8 ± 11.1	33.5	0.0
MAHI								
Cases	807	U.S.	Hospital-based	MI	No age criterion	61.5 ± 12.7	32.1	0.0
Controls	637	U.S.	Outpatients	—	—	60.7 ± 12.4	39.0	0.0
PennCATH								
Cases	933	U.S.	Hospital-based	CAD	Men and women ≤66	52.7 ± 7.6	24.3	0.0
Controls	468	U.S.	Hospital- based	—	Men and women ≥45	61.7 ± 9.6	51.7	0.0
REGICOR								
Cases	312	Spain	Hospital-based	MI	Men ≤50 or women ≤60	45.9 ± 5.8	20.2	0.0
Controls	317	Spain	Drawn from community-based cohort	—	—	46.0 ± 5.6	21.5	0.0
VHS								
Cases	1,106	Italy	Hospital-based	CAD	No age criterion	61.4 ± 10.0	20.4	0.0
Controls	383	Italy	Hospital-based	—	—	58.0 ± 12.3	35.0	0.0
WTCCC CAD								
Cases	1,922	U.K.	Community-based	CAD	Men and women <66	49.3 ± 7.9	20.2	0.0
Controls	2,933	U.K.	Community-based	—	—	44.7 ± 9.3	49.2	0.0

*Total number of subjects with successful genotyping of rs20455. †Predominantly from Europe. ‡Predominantly from India, Pakistan, Bangladesh, and Sri Lanka.

ADVANCE = Atherosclerotic Disease, VAscular functioN, and genetiC Epidemiology; AMI Gene = AMI Gene Study/Dortmund Health; CAD = coronary artery disease; CATHGEN = CATHGEN Research Project; deCODE = deCODE genetics CAD study; FINRISK = National Finrisk study; GeneSTAR = Genetic Study of Atherosclerosis Risk; GerMIFS I and GerMIFS II = German Myocardial Infarction Family studies I and II; HARPS = Heart Attack Risk in Puget Sound; IFS = Irish Family Study; INTERHEART = international INTERHEART study coordinated by Population Health Research Institute of McMaster University; MAHI = Mid-America Heart Institute; MDC = Malmo Diet and Cancer; MedStar = Washington Hospital Center/MedStar angiographic CAD study; MGH PCAD = Massachusetts General Hospital of Premature CAD study; MI = myocardial infarction; PennCATH = University of Pennsylvania Medical center angiographic CAD study; REGICOR = Registre Gironı' del Cor study; U.K. = United Kingdom; U.S. = United States; VHS = Verona Heart Study; WTCCC CAD = Wellcome Trust Case Control Consortium study of CAD.

was genotyped using the Centaurus platform (29). All genotype data generated from the various platforms passed extensive quality control measures including tests of Hardy-Weinberg equilibrium ($p > 0.001$ in controls).

Clinical measures. The age at onset of clinical CAD (for cases) and sex for cases and controls were documented in all studies. The presence of other traditional risk factors was documented in most but not all studies. In the deCODE study, risk factor data other than age, sex, and body mass index were not collected. Furthermore, the presence of other traditional risk factors was defined in various ways, and the timing of enrollment of cases hampered the accurate measurement of these risk factors in some studies. For example, several studies enrolled cases weeks to months (and sometimes years) after their initial CAD event, making it difficult to reliably discern whether certain medications being taken by a participant at the time of enrollment were prescribed to treat risk factors present before their first ever clinical manifestation of CAD versus for secondary prevention reasons or to treat CAD symptoms (e.g., beta-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and statins). No attempt was made to standardize risk factor definitions across all studies.

Statistical analysis. We first calculated the crude ORs and 95% CIs based on the 2×2 table of *KIF6* Trp719Arg allele-by-trait counts for each case-control study. In case-control studies with >1 race/ethnic group, we calculated an OR for each race/ethnic group. We then compared the distribution of the raw genotype counts in each case-control stratum with Armitage's trend test (30). Next, we calculated ORs and 95% CIs adjusted for age at onset of CAD and sex using standard unconditional logistic regression. Because of the difficulties in ascertaining risk factors other than age and sex, we did not adjust ORs for any additional CAD risk factors. Log additive and dominant ORs were calculated because both modes of inheritance were reported to date. The 719Trp allele served as the reference allele given that the 719Arg allele was identified as the high-risk allele in previous publications. Analyses were repeated after excluding case subjects with an age at onset of CAD of 50 years and older for men and 60 years and older for women. Controls for this subgroup analysis were also restricted to those in the same sex-specific age range as cases at the time of enrollment. The GeneSTAR family study analyses were performed using generalized linear latent and mixed models with the logistic link and family membership as the random effect to account for family structure, whereas the Irish Family Study used likelihood-based association statistics incorporated in the UNPHASED software package (31) to account for family structure. In the latter family study, only the crude ORs for the additive model using all subjects could be calculated.

Last, we combined crude and adjusted ORs, 95% CIs, and p values using a Mantel-Haenszel model in which the groups were allowed to have different population frequencies for genotypes but were assumed to have common relative risks (32). We weighed the results from each study by the standard error of the effect derived from the p value of the OR (see the Online Appendix for details). Using this meta-analytic approach, we calculated the overall combined ORs, 95% CIs, and p values separately for all case control studies of Europeans and all case-control studies of African Americans. The meta-analyses were repeated for the subgroup of cases in each study with early-onset disease and their respective controls. For each meta-analysis, we also performed 2 standard heterogeneity tests (Cochran's Q and Higgins' I^2) to assess the appropriateness of a fixed-effects model over a random-effects model (33).

From previous work, we know that 1 of the 2 admixed race/ethnic strata in the ADVANCE study, the admixed non-Hispanics, has significant differences in the degree of admixture between cases and controls (14). Specifically, cases in this stratum are, on average, significantly more European (and less African American) than controls. Therefore, we included a covariate in the multivariate regression model indicating the proportion of white ancestry for each individual estimated by the program STRUCTURE (34,35) in this stratum. The other admixed stratum in the ADVANCE study, the admixed Hispanics, did not show differences in degree of admixture between cases and controls. Therefore, no additional covariates were added to the regression model for this stratum.

Some Icelandic affected individuals and controls are related, both within and between groups, causing the chi-square test statistic to have a mean >1 and a median larger than 0.6752. The inflation factor, lambda (λ), was estimated at 1.21 using a method of genomic control (36) by calculating the average of the observed chi-square statistics for the genome-wide SNP set, which accounts for relatedness and for potential population stratification. The 95% CIs and p values presented for deCODE are based on adjusting the chi-square statistics by dividing it by this inflation factor. The inflation factor was also found to be high in the GerMIFS I study ($\lambda \sim 1.27$); therefore, p values and 95% CIs for this study were also adjusted in the same manner. For all other studies with genome-wide data, a genomic control correction was not applied to the data because the inflation factor was found to be very low ($\lambda \leq 1.05$).

We performed sensitivity analyses by repeating all analyses after excluding non-MI cases in the subset of case-control studies that included both MI and non-MI cases of CAD. These sensitivity analyses were performed only in Europeans.

Results

Table 1 summarizes the ascertainment scheme and the inclusion criteria for phenotype, age, sex, and race stratified by study and case-control status. The distribution and frequency of all traditional risk factors of CAD stratified by study and case-control status can be found in Table 1S in the Online Appendix. Of the 19 case-control studies, 12

enrolled only cases with MI and 14 also restricted enrollment or genotyping to early-onset disease (when considering the traditional age cutoffs of 65 years and younger for women and 55 years and younger for men at the time of first onset of disease). However, the largest study (deCODE) enrolled both MI and non-MI cases of CAD and included CAD cases with any age at onset. The proportion of cases that were women patients varied from study to study and was influenced by the ascertainment scheme and matching (either one to one or frequency matching). The overall weighted average age at onset of CAD was 55.7 years.

Table 2 summarizes the genotype counts stratified by study, case control status, and race/ethnic group as well as the adjusted OR, 95% CI, and p values for the association between *KIF6* rs20455 SNP and CAD. Crude ORs were not materially different from their respective ORs adjusted for age and sex. Therefore, only ORs adjusted for age and sex are presented. Among Europeans, only one out of the 19 studies (deCODE) demonstrated a nominally significant association between the *KIF6* polymorphism and CAD but in the opposite direction of the published literature (the 719Arg allele inversely associated with risk: log additive OR of 0.93, 95% CI: 0.88 to 0.99, log dominant OR of 0.91, 95% CI: 0.85 to 0.99). The meta-analysis produced a point estimate of the log additive OR near unity with very tight confidence intervals (log additive OR of 0.98, 95% CI: 0.95 to 1.02, log dominant OR of 0.97, 95% CI: 0.93 to 1.01). We also found no significant association between the *KIF6* rs20455 SNP and CAD among South Asian participants in the INTERHEART study (log additive OR of 1.02, 95% CI: 0.91 to 1.14, log dominant OR of 1.04, 95% CI: 0.87 to 1.24), African American participants in the ADVANCE, CATHGEN, and GeneSTAR studies (fixed-effects meta-analysis log additive OR of 0.91, 95% CI: 0.73 to 1.13, log dominant OR of 0.81, 95% CI: 0.42 to 1.55), and a smaller number of Hispanic, East Asian, and admixed individuals participating in the ADVANCE study (see Table 2 for details).

Table 3 shows genotype counts and association analyses restricted to the subgroup of case subjects with very early onset disease (at younger than 50 years for men and at younger than 60 years for women) and controls within the same age range at the time of enrollment. Among this subgroup, we also observed no increase in the risk of CAD in either the European carriers of the 719Arg allele compared with noncarriers (fixed-effects meta-analysis log-additive OR: 0.99, 95% CI: 0.94 to 1.04; log-dominant OR: 1.01, 95% CI: 0.95 to 1.08) or the non-European subjects (see Table 3 for details).

Last, our sensitivity analyses in Europeans restricting cases to those defined on the basis of an MI in *all* 19 case-control studies also revealed no increased risk of CAD in European carriers of the 719Arg allele compared with noncarriers, both for the overall analysis (fixed-effects meta-analysis log-additive OR: 0.99, 95% CI: 0.96 to 1.03; log-dominant OR: 0.98, 95% CI: 0.94 to 1.02) and the subgroup analysis of early-onset MI cases (fixed-effects meta-analysis log-additive OR: 1.03, 95% CI: 0.98 to 1.09; log-dominant OR: 1.03, 95% CI: 0.97 to 1.11). Additional details for these analyses are provided in Tables 2S and 3S in the Online Appendix.

None of the Q_tests revealed heterogeneity in our meta-analyses (lowest p value = 0.252), and all meta-analyses in Europeans had an I^2 value of 0%, suggesting that the variability in effect sizes in Europeans was due entirely to sampling error within the studies (see Table 4S in the Online Appendix). Thus, performing random-effects model meta-analyses was not necessary in Europeans because the results would be identical to the fixed-effects model. Only the log-dominant model for CAD overall among African Americans demonstrated an I^2 value >0%. For this stratum, the random-effects model (DerSimonian and Laird method) revealed an OR that was similar to that of the fixed-effects model but with wider CIs (Table 4S in the Online Appendix).

Discussion

The principal finding of this study was the uniform lack of elevated risk of clinical CAD among carriers of the *KIF6* 719Arg allele compared with noncarriers in 19 case-control studies performed around the world. Our meta-analysis involving a very large number of subjects with European ancestry (17,000 cases and 39,369 controls) suggests that the risk of CAD among European carriers of the 719Arg allele is unlikely to be increased by >2% compared with noncarriers.

Our study has 2 strengths in addition to the very high power conferred by studying a large number of European subjects. First, we studied a large number of early-onset CAD case subjects (men younger than 50 years of age and women younger than 60 years of age) but observed no association with *KIF6* Trp719Arg and CAD despite the expectation that susceptibility alleles would be more prevalent in this subgroup (37). In this subgroup with early-onset disease, we were able to rule out an increase in risk of ≥8% in Europeans. Second, this is the only study to date that examines several non-European racial/ethnic groups. In each of the non-European case-control strata, we also found no significant associations between the *KIF6* Trp719Arg polymorphism and CAD. However, the point estimates of the ORs for these non-European subgroups have substantially wider CIs than those derived in Europeans due to relatively small sample sizes (particularly in our Hispanic, East Asians, admixed Hispanic, and admixed non-Hispanics groups). Thus, further study of all these non-European race/ethnic groups is needed to rule out more modest effects on risk.

Study limitations. Our study has 3 important limitations related to the case-control design. The first limitation is the potential selection bias by studying only nonfatal cases of CAD. If the 719Arg allele increases the risk of incident fatal CAD more than the risk of incident nonfatal CAD, the exclusion of incident fatal CAD cases could conceivably bias the OR toward the null (i.e., OR: 1). However, the difference in relative risk between these 2 subgroups of cases

Table 2 Kinesin-Like Protein-6 Trp719Arg Polymorphism (rs20455) Allele Frequencies, Genotype Counts, and Odds Ratios Adjusted for Age and Sex in 19 Case-Control Studies of CAD, Stratified by Race/Ethnic Group

	Cases (CAD)					Controls					OR (95% CI) and p Values			
	n	Allele Freq 719Arg	719Trp/ 719Trp	719Arg/ 719Trp	719Arg/ 719Arg	n	Allele Freq 719Arg	719Trp/ 719Trp	719Arg/ 719Trp	719Arg/ 719Arg	Log-Additive Mode of Inheritance	p Value	Log-Dominant Mode of Inheritance	p Value
Europeans														
ADVANCE	275	0.345	119	122	34	311	0.378	122	143	46	0.87 (0.69–1.10)	0.249	0.84 (0.60–1.16)	0.289
AMI Gene	793	0.369	311	379	103	1,121	0.381	430	528	163	0.89 (0.75–1.04)	0.142	0.90 (0.71–1.13)	0.349
CATHGEN	1,298	0.361	545	570	183	730	0.355	297	347	86	1.02 (0.89–1.18)	0.749	0.96 (0.79–1.17)	0.674
deCODE*	4,313	0.300	2,131	1,779	403	24,952	0.312	11,813	10,689	2,450	0.93 (0.88–0.99)	0.018	0.91 (0.85–0.99)	0.020
FINRISK	167	0.374	64	81	22	172	0.355	73	76	23	1.09 (0.80–1.49)	0.596	1.18 (0.76–1.82)	0.458
GeneSTAR	285	0.368	106	148	31	1,579	0.365	626	752	201	0.99 (0.82–1.21)	0.939	1.09 (0.83–1.43)	0.543
GerMIFS I†	722	0.367	293	328	101	1,643	0.368	662	753	228	0.97 (0.84–1.13)	0.742	0.97 (0.88–1.08)	0.661
GerMIFS II	1,126	0.367	447	529	150	1,277	0.359	522	593	162	1.01 (0.89–1.14)	0.893	0.99 (0.91–1.08)	0.770
HARPS	505	0.404	187	228	90	559	0.381	216	260	83	1.10 (0.92–1.31)	0.279	1.07 (0.83–1.37)	0.602
INTERHEART	789	0.362	335	337	117	859	0.354	354	402	103	1.03 (0.90–1.19)	0.671	0.95 (0.78–1.15)	0.573
IFS*‡	482	0.344	203	226	53	622	0.346	261	292	69	1.03 (0.81–1.30)	0.835	—	—
MDC	86	0.372	35	38	13	99	0.394	33	54	12	0.91 (0.60–1.39)	0.667	0.73 (0.40–1.33)	0.305
MedStar	875	0.349	370	399	106	447	0.364	174	221	52	0.99 (0.80–1.22)	0.919	0.91 (0.68–1.22)	0.527
MGH PCAD	204	0.353	89	86	29	260	0.348	114	111	35	1.04 (0.82–1.31)	0.878	1.03 (0.69–1.52)	0.904
MAHI	807	0.367	322	377	108	637	0.359	256	304	77	1.04 (0.89–1.21)	0.647	1.01 (0.82–1.25)	0.919
PennCATH	933	0.379	359	441	133	468	0.358	194	213	61	1.04 (0.86–1.26)	0.666	1.03 (0.79–1.33)	0.850
REGICOR	312	0.348	134	139	39	317	0.339	141	137	39	1.02 (0.78–1.34)	0.747	1.06 (0.77–1.45)	0.716
VHS	1,106	0.378	437	501	168	383	0.372	145	191	47	1.03 (0.87–1.22)	0.757	0.93 (0.73–1.19)	0.568
WTCCC CAD	1,922	0.356	792	890	240	2,933	0.355	1,242	1,299	392	1.02 (0.93–1.12)	0.624	1.09 (0.96–1.24)	0.189
Total meta-analysis	17,000					39,369					0.98 (0.95–1.02)	0.349	0.97 (0.93–1.01)	0.137
Non-Europeans														
ADVANCE admixed Hispanic	34	0.412	12	16	6	22	0.455	7	10	5	0.77 (0.34–1.67)	0.507	0.73 (0.21–2.36)	0.607
ADVANCE admixed non-Hispanic	74	0.419	27	32	15	37	0.622	10	8	19	0.83 (0.44–1.57)	0.555	1.44 (0.49–4.41)	0.506
ADVANCE African Americans	49	0.745	4	17	28	87	0.816	5	22	60	0.70 (0.39–1.23)	0.207	0.70 (0.18–3.00)	0.619
ADVANCE East Asians	45	0.356	19	20	6	35	0.486	9	18	8	0.60 (0.30–1.13)	0.114	0.48 (0.18–1.25)	0.135
ADVANCE Hispanic	28	0.375	11	13	4	22	0.364	8	12	2	1.07 (0.45–2.58)	0.876	0.88 (0.27–2.82)	0.831
CathGEN African Americans	277	0.762	16	100	161	240	0.783	9	86	145	0.89 (0.66–1.21)	0.465	0.58 (0.25–1.37)	0.213
GeneSTAR African Americans	93	0.796	2	34	57	1,073	0.786	53	353	667	1.05 (0.72–1.51)	0.813	2.35 (0.56–9.83)	0.241
INTERHEART (South Asians)	1,092	0.451	351	498	243	1,187	0.449	389	531	267	1.02 (0.91–1.14)	0.791	1.04 (0.87–1.24)	0.669
African Americans total meta-analysis	419					1,400					0.91 (0.73–1.13)	0.380	0.81 (0.42–1.55)	0.520

*Adjusted for relatedness. †p Values adjusted by genomic control method as $\lambda = 1.27$ ‡Approach to analysis unable to produce dominant model odds ratios (OR).
CI = confidence interval; other abbreviations as in Table 1.

Table 3 Kinesin-Like Protein-6 Trp719Arg Polymorphism (rs20455) Allele Frequencies, Genotype Counts, and Odds Ratios Adjusted for Age and Sex in 19 Case-Control Studies of CAD, Stratified by Race/Ethnic Group and Restricted to Early-Onset Disease (Age at Onset of CAD Younger Than 50 Years of Age for Men and Younger Than 60 Years of Age for Women) and Similarly Aged Controls

	Cases (CAD)					Controls					OR (95% CI) and p Values			
	n	Allele Freq 719Arg	719Trp/ 719Trp	719Arg/ 719Trp	719Arg/ 719Arg	n	Allele Freq 719Arg	719Trp/ 719Trp	719Arg/ 719Trp	719Arg/ 719Arg	Log-Additive Mode of Inheritance	p Value	Log-Dominant Mode of Inheritance	p Value
Europeans														
ADVANCE	275	0.345	119	122	34	311	0.378	122	143	46	0.87 (0.69–1.10)	0.249	0.84 (0.60–1.16)	0.289
AMI Gene	296	0.380	117	133	46	193	0.415	68	90	35	0.98 (0.74–1.30)	0.875	1.02 (0.67–1.54)	0.929
CATHGEN	585	0.366	233	276	76	295	0.359	121	136	38	0.99 (0.78–1.28)	0.990	0.98 (0.70–1.37)	0.900
deCODE*	750	0.292	375	312	63	12,548	0.312	5,955	5,366	1,227	0.91 (0.72–1.16)	0.450	0.95 (0.69–1.29)	0.730
FINRISK	167	0.374	64	81	22	172	0.355	73	76	23	1.09 (0.80–1.49)	0.596	1.18 (0.76–1.82)	0.458
GeneSTAR	201	0.358	79	100	22	1,579	0.365	626	752	201	0.95 (0.76–1.18)	0.627	0.99 (0.73–1.35)	0.951
GerMIFS I†	412	0.367	168	186	58	387	0.377	152	178	57	0.99 (0.80–1.23)	0.949	0.99 (0.87–1.16)	0.999
GerMIFS II	524	0.365	210	245	69	709	0.363	286	331	92	0.97 (0.81–1.16)	0.760	0.97 (0.86–1.10)	0.611
HARPS	505	0.404	187	228	90	559	0.381	216	260	83	1.10 (0.92–1.31)	0.279	1.07 (0.83–1.37)	0.602
INTERHEART	195	0.364	85	78	32	216	0.343	92	90	24	1.09 (0.82–1.44)	0.559	0.96 (0.65–1.42)	0.837
IFS*‡	371	0.342	154	180	37	309	0.353	129	142	38	0.86 (0.63–1.16)	0.315		
MDC	86	0.372	35	38	13	99	0.394	33	54	12	0.91 (0.60–1.39)	0.667	0.73 (0.40–1.33)	0.305
MedStar	601	0.351	255	270	76	151	0.358	61	72	18	0.98 (0.74–1.29)	0.878	0.91 (0.62–1.33)	0.612
MGH PCAD	204	0.353	89	86	29	260	0.348	114	111	35	1.04 (0.82–1.31)	0.878	1.03 (0.69–1.52)	0.904
MAHI	227	0.392	81	114	32	190	0.363	77	88	25	1.14 (0.85–1.51)	0.386	1.12 (0.80–1.79)	0.374
PennCATH	354	0.383	134	169	51	128	0.359	53	58	17	1.11 (0.82–1.49)	0.508	1.16 (0.73–1.83)	0.539
REGICOR	312	0.348	134	139	39	317	0.339	141	137	39	1.02 (0.78–1.34)	0.747	1.06 (0.77–1.45)	0.716
VHS	197	0.409	71	91	35	113	0.420	33	65	15	0.88 (0.56–1.38)	0.570	0.72 (0.38–1.37)	0.320
WTCCC CAD	1,085	0.359	444	503	138	2,612	0.355	1101	1167	344	1.04 (0.94–1.16)	0.444	1.10 (0.95–1.27	0.216
Total meta-analysis	7,347					21,148					0.99 (0.94–1.04)	0.729	1.01 (0.95–1.08)	0.831
Non-Europeans														
ADVANCE admixed Hispanic	34	0.412	12	16	6	22	0.455	7	10	5	0.77 (0.34–1.67)	0.507	0.73 (0.21–2.36)	0.607
ADVANCE admixed non-Hispanic	74	0.419	27	32	15	37	0.622	10	8	19	0.83 (0.44–1.57)	0.555	1.44 (0.49–4.41)	0.506
ADVANCE African Americans	49	0.745	4	17	28	87	0.816	5	22	60	0.70 (0.39–1.23)	0.207	0.70 (0.18–3.00)	0.619
ADVANCE East Asians	45	0.356	19	20	6	35	0.486	9	18	8	0.60 (0.30–1.13)	0.114	0.48 (0.18–1.25)	0.135
ADVANCE Hispanic	28	0.375	11	13	4	22	0.364	8	12	2	1.07 (0.45–2.58)	0.876	0.88 (0.27–2.82)	0.831
CathGEN African Americans	157	0.755	7	63	87	128	0.781	5	46	77	1.02 (0.64–1.61)	0.950	0.89 (0.23–3.46)	0.860
GeneSTAR African Americans	70	0.807	2	23	45	1,073	0.786	53	353	667	1.15 (0.75–1.76)	0.532	1.79 (0.43–7.50)	0.427
INTERHEART South Asians	515	0.458	163	232	120	633	0.453	203	286	144	1.03 (0.88–1.21)	0.701	1.04 (0.81–1.34)	0.735
African Americans total meta-analysis	276					1,288					1.06 (0.76–1.49)	0.721	1.05 (0.87–1.26)	0.609

Controls are restricted to men younger than 50 years of age and women younger than 60 years of age at the time of blood draw to match the sex-specific age range of cases. *Adjusted for relatedness. †p Values adjusted by genomic control method as $\lambda = 1.27$. ‡Approach to analysis unable to produce dominant model OR.

Abbreviations as in Tables 1 and 2.

would have to be quite large to result in a substantially biased OR in our study, given that the majority of incident CAD events are not fatal, especially among subjects with early-onset disease (e.g., in the ARIC surveillance study, 20% to 35% of subjects age 60 years or older and 10% to 20% of subjects younger than the age of 60 years died as a consequence of a complication of their initial presentation of CAD [38]). The second potential limitation is our inability to measure traditional risk factors as robustly as they are measured in prospective studies. We therefore made no attempt to fully adjust ORs for all traditional risk factors of CAD. However, prospective studies published to date suggest that traditional risk factors are not correlated with the *KIF6* Trp719Arg polymorphism, making adjustment unnecessary (1–5). Last, our study did not allow us to explore whether statin use modifies the effect of the 719Arg allele on risk as was done in the WOSCOPS, CARE, and PROVE IT–TIMI 22 trials because reliable information on the use of statins in relation to the incident event was not available for most studies. However, we believe it unlikely that our null results are a consequence of a high prevalence of the use of statins at the time of the event in cases. In fact, we suspect that the overall prevalence of the use of statins in our set of 19 case-control studies is actually lower than the prevalence of use observed at the last follow-up for participants in the ARIC, CHS, and WHS studies (39–41) because a majority of our case-control studies restricted their recruitment and/or genotyping efforts to very early onset cases and young controls. For example, in the ADVANCE study, which focused on very early onset CAD (men younger than 45 years of age, women younger than 55 years of age), access to the electronic pharmacy records confirmed that only a small proportion of cases (~14.4%) and controls (~4.7%) were taking statins during the appropriate time window of exposure.

Is it possible that other less obvious sources of bias are responsible for the differences in associations observed between case-control and cohort studies of *KIF6*? Although we cannot rule out this possibility, we also deem this scenario unlikely, given that such cryptic biases, if they exist, have not influenced associations between SNPs at the 9p21.3 locus and CAD in the same manner (9,10). In fact, many of the case-control studies included in this report have produced ORs of disease for the 9p21.3 locus equivalent to or larger than the HRs derived from cohort studies (9,10,12,14,42–44). These cohort studies include the 2 largest cohort studies to date reporting on the association between *KIF6* and CAD (ARIC and WHS). Thus, we are left with the real possibility that the initial reports falsely suggested an association between variation in *KIF6* and CAD. The reasons for this are unclear but include the possibility of chance findings or inadequate correction for multiple testing (45–47). The lack of biological studies implicating *KIF6* in the pathogenesis of coronary atherosclerosis combined with the lack of consistent evidence of expression of this gene in relevant tissues such as the vasculature (48,49) could also argue for a false-positive association. However, many recent population genetic studies of complex traits have clearly demonstrated that such mechanistic data are not necessary to validate highly significant associations uncovered through the genome-wide association approach to discovery (50).

Our findings question not only the usefulness of the *KIF6* test in identifying subjects at increased risk of incident or recurrent CAD but also its usefulness in identifying subjects most likely to benefit from statins. Although we could not test the latter hypothesis directly, the previously reported interaction between genotype and benefit from statins is largely dependent on the validity of the association among subjects not taking statins, which could not be replicated in this study. We also call attention to the fact that the interaction term p value between genotype and statin use was only marginally significant in the WOSCOPS ($p = 0.021$) and PROVEIT–TIMI 22 ($p = 0.018$) trials and not significant in the CARE trial (adjusted $p = 0.39$) (2,3). Despite these observations, additional high-quality prospective cohort studies of the effect of the *KIF6* variant on CAD risk among users and nonusers of statins are needed before any firm conclusions can be made on the validity of this interaction.

Conclusions

We were unable to confirm an increased risk of CAD in carriers of the *KIF6* 719Arg allele compared with noncarriers in a very large number of European subjects. We also observed no compelling evidence of an association between the *KIF6* Trp719Arg SNP and CAD in multiple other race/ethnic groups and among subjects with early-onset CAD. Our null results are unlikely to be a consequence of selection bias, a high prevalence of use of statins among cases, or other cryptic biases. These findings do not support the clinical utility of testing for the *KIF6* Trp719Arg polymorphism to identify subjects at higher risk of CAD and indirectly question whether genotype information at this locus can reliably identify a population of subjects most likely to benefit from the use of statins.

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Key Words: coronary artery disease ■ KIF6 ■ kinesin-like protein 6 ■ myocardial infarction ■ polymorphism.

APPENDIX

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