Journal of the American College of Cardiology Vol. 51, No. 4, 2008

Published by Elsevier Inc.
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2007.05.057

Association of the Trp719Arg Polymorphism in Kinesin-Like Protein 6 With Myocardial Infarction and Coronary Heart Disease in 2 Prospective Trials

The CARE and WOSCOPS Trials

Olga A. Iakoubova, MD, PhD,* Carmen H. Tong, BS,* Charles M. Rowland, MS,* Todd G. Kirchgessner, PhD,† Bradford A. Young, PhD,* Andre R. Arellano, BS,* Dov Shiffman, PhD,* Marc S. Sabatine, MD, MPH,‡ Hannia Campos, PhD,§ Christopher J. Packard, DSc,‖ Marc A. Pfeffer, MD, PhD,‡ Thomas J. White, PhD,* Eugene Braunwald, MD, FACC,‡ James Shepherd, PhD,‖ James J. Devlin, PhD,* Frank M. Sacks, MD‡§

Alameda, California; Princeton, New Jersey; Boston, Massachusetts; and Glasgow, United Kingdom

Objectives We asked whether 35 genetic polymorphisms, previously found to be associated with cardiovascular disease, were associated with myocardial infarction (MI) in the CARE (Cholesterol and Recurrent Events) trial and with coronary heart disease (CHD) in the WOSCOPS (West of Scotland Coronary Prevention Study) trial and whether the risk associated with these polymorphisms could be reduced by pravastatin treatment.

Background Identification of genetic polymorphisms associated with CHD may improve assessment of CHD risk and understanding of disease pathophysiology.

Methods We tested the association between genotype and recurrent MI in the CARE study and between genotype and primary CHD in the WOSCOPS trial using regression models that adjusted for conventional risk factors: Cox proportional hazards models for the CARE study and conditional logistic regression models for a nested case-control study of the WOSCOPS trial.

Results We found that Trp719Arg (rs20455) in *KIF6* was associated with coronary events. *KIF6* encodes kinesin-like protein 6, a member of the molecular motor superfamily. In placebo-treated patients, carriers of the *KIF6* 719Arg allele (59.4% of the CARE trial cohort) had a hazard ratio of 1.50 (95% confidence interval [CI] 1.05 to 2.15) in the CARE trial and an odds ratio of 1.55 (95% CI 1.14 to 2.09) in the WOSCOPS trial. Among carriers, the absolute risk reduction by pravastatin was 4.89% (95% CI 1.81% to 7.97%) in the CARE trial and 5.49% (95% CI 3.52% to 7.46%) in the WOSCOPS trial.

Conclusions In both the CARE and the WOSCOPS trials, carriers of the *KIF6* 719Arg allele had an increased risk of coronary events, and pravastatin treatment substantially reduced that risk. (J Am Coll Cardiol 2008;51:435–43) © 2008 by the American College of Cardiology Foundation

Current algorithms used for assessing risk of coronary heart disease (CHD) events are based on established clinical risk factors, yet these algorithms fail to predict many CHD events (1,2). Because genetics influences predisposition to CHD, evaluating genetic polymorphisms that are associated with risk of CHD may help to improve CHD risk assessment (3,4). Studies that investigate the association between CHD and genetic polymorphisms may test polymorphisms in candidate genes, which provide biologic plausibility to any observed association. An alternative approach is to test polymorphisms in an unbiased collection of genes (5).

See page 456

As the technology for genotyping polymorphisms has improved, the number of polymorphisms reported to be

From *Celera, Inc., Alameda, California; †Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, New Jersey; ‡Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; §Harvard School of Public Health, Boston, Massachusetts; and the ‖University of Glasgow and Royal Infirmary, Glasgow, United Kingdom. Supported by research grants from Bristol-Myers Squibb Pharmaceutical Research Institute (to Drs. Sacks, Braunwald, Shepherd, and Packard). Genotyping and statistical analysis funded by Celera.

Manuscript received May 8, 2007; accepted May 23, 2007.

JACC Vol. 51, No. 4, 2008
January 29, 2008:435–43

Abbreviations and Acronyms

CHD = coronary heart disease

CI = confidence interval

DNA = deoxyribonucleic acid

HDL-C = high-density lipoprotein cholesterol

HR = hazard ratio

LDL-C = low-density lipoprotein cholesterol

MI = myocardial infarction

OR = odds ratio

SNP = single nucleotide polymorphism

associated with CHD has increased; however, many of these associations are not confirmed in subsequent studies (6). Confirmation of associations between genetic polymorphisms and CHD can be difficult to achieve for a variety of reasons. The typically modest risk for CHD associated with each genetic polymorphism can lead to false-positive associations in initial studies or to false-negative associations in confirmation studies. Additionally, the case-control design of an initial study can affect subsequent confirmation, because unanticipated biases in the selection of subjects for case-control studies can lead to spurious associations. Thus, polymorphisms reported to be associated with CHD should be further investigated in multiple studies, including prospective studies, which may avoid some of the inherent bias of case-control studies.

We selected 35 polymorphisms, which had been associated with cardiovascular disease in previous studies, for further investigation. Ten of these polymorphisms are in 9 candidate genes and have been reported to be associated with cardiovascular disease or intermediate phenotypes in 2 studies. Four of these 10 polymorphisms are in genes related to hemostasis: rs1126643 in *ITGA2* (integrin alpha 2) has been associated with nonfatal myocardial infarction (MI) in younger patients (7,8); rs1866389 in *THBS4* (thrombospondin 4) with premature MI (9,10); rs1799768 in *SERPINE1* (plasminogen activator inhibitor-1) with MI and with high plasma plasminogen activator inhibitor-1 activity (11,12); and rs1799963 in *F2* (coagulation factor II) with MI in young women (13,14). Two of the 10 polymorphisms are in genes related to hypertension: a 278-basepair insertion–deletion polymorphism in *ACE* (angiotensin I-converting enzyme) has been associated with MI (15,16); and rs5186 in *AGTR1* (angiotensin II receptor type 1), with cardiovascular risk independent of blood pressure (17,18). The remaining 4 of these 10 polymorphisms are in genes involved in vascular biology: rs1041981 in *LTA* (lymphotoxin alpha) has been associated with MI (19,20); rs3025058 in *MMP3* (matrix metallopeptidase-3) with progression of coronary atherosclerosis (21,22); rs429358, the ε4 polymorphism in *APOE* (apolipoprotein E), with CHD (23,24); and rs405509 in the *APOE* promoter with increased risk of MI (25) and coronary artery disease (23). The other 25 of the 35 polymorphisms had been previously found to be associated with MI in a case-control study that tested putative functional single nucleotide polymorphisms (SNPs) present in an unbiased collection of noncandidate genes (26).

In the present study, we asked if these 35 polymorphisms were associated with MI in the placebo arm of the CARE (Cholesterol and Recurrent Events) study and with CHD in the placebo arm of the WOSCOPS (West of Scotland Coronary Prevention Study) trial and if the risk associated with these polymorphisms could be reduced by pravastatin treatment.

Methods

Study design. We conducted genetic association studies in populations derived from 2 prospective trials that assessed the effect of pravastatin in the prevention of MI and CHD: the CARE and WOSCOPS trials. The inclusion criteria for the CARE study, a secondary prevention trial, and the WOSCOPS, a primary prevention trial, have been described elsewhere (27–29).

The CARE study included 3,847 Caucasian patients, and the present study comprised 2,913 Caucasian patients who provided deidentified deoxyribonucleic acid (DNA) for genetic studies. We used a composite end point of confirmed fatal or nonfatal MI to focus on the genetics of MI (nonfatal MI constituted 86.7% of the events in this composite end point). The DNA was collected approximately 1.5 years after the start of the CARE trial. Therefore, the proportion of patients with fatal MI was higher in those who did not provide DNA than in those who did provide DNA; however, there was no significant difference for the end point of the present genetic study (fatal or nonfatal MI) between those who provided DNA and those who did not (30). The CARE trial genetic cohort was restricted to Caucasian patients, because the number of non-Caucasian patients (7.3% of the CARE trial population) did not provide sufficient power for a separate analysis.

The present genetic study of the WOSCOPS trial was derived from a previously described prospective nested case-control study, which included all of the 580 on-trial CHD events from the WOSCOPS cohort as case subjects and 1,160 control subjects matched to case subjects by age and smoking (29). The present genetic study of the WOSCOPS trial included all patients in the nested case-control study for whom sufficient DNA was available for genotype analysis: 481 case and 1,086 control subjects. Because the control subjects had been matched to case subjects for age and current smoking status, the present genetic study used the same CHD end point as that reported in the prospective nested case-control study (death from coronary heart disease, nonfatal MI, or revascularization procedures). The baseline characteristics for patients included in these genetic studies of CARE and WOSCOPS are presented in Table 1. All patients enrolled in CARE and WOSCOPS provided written informed consent. The CARE study was approved by the institutional review boards of the Brigham and Women's Hospital and all participating centers. The WOSCOPS study was approved by the ethics committees of the University of Glasgow and all participating health boards.

Table 1 Baseline Characteristics of Patients Genotyped for 35 Polymorphisms

Characteristic	CARE			WOSCOPS		
	On-Trial MI	No On-Trial MI	p Value	Case	Control	p Value
Patients, n	244	2,471		481	1,080	
Men, n (%)	211 (86.5)	2,138 (86.5)	0.98	481 (100)	1,080 (100)	*
Age, mean (SD), yrs	58.8 (9.8)	58.5 (9.3)	0.66	56.4 (5.1)	56.2 (5.2)	*
BMI, mean (SD), kg/m^2	28.0 (4.8)	27.4 (4.2)	0.083	26.0 (3.2)	25.6 (3.2)	0.036
Smoking			<0.001			*
Current	65 (26.6)	383 (15.5)		260 (54.1)	587 (54.5)	
Former	133 (54.5)	1,568 (63.5)		141 (29.3)	306 (28.4)	
Never	46 (18.9)	520 (21.0)		80 (16.6)	185 (17.2)	
History of diabetes	50 (20.5)	294 (11.9)	<0.001	10 (2.1)	13 (1.2)	0.19
History of hypertension	113 (46.3)	1,013 (41.0)	0.11	108 (22.5)	170 (15.8)	0.002
Low-density lipoprotein cholesterol, mg/dl						
Mean (SD)	141.3 (14.7)	138.9 (14.6)	0.01	194.0 (17.6)	191.5 (17.1)	0.008
Median (range)	141.0 (115.0–175.0)	138.0 (100.5–179.5)		191.4 (156.6–250.4)	188.5 (164.3–248.5)	
High-density lipoprotein cholesterol, mg/dl						
Mean (SD)	38.5 (9.1)	38.8 (8.8)	0.70	41.5 (8.7)	44.2 (9.6)	<0.001
Median (range)	36.8 (23.0–68.0)	37.0 (20.0–89.0)		40.0 (21.2–70.3)	43.2 (23.1–127.8)	
Total cholesterol, mg/dl						
Mean (SD)	211.5 (16.7)	209.0 (17.3)	0.03	273.6 (23.6)	271.5 (22.2)	0.09
Median (range)	214.5 (170.5–239.5)	209.5 (159.0–246.5)		271.7 (229.1–364.5)	268.8 (224.3–369.3)	
Triglycerides, mg/dl						
Mean (SD)	157.9 (54.9)	157.0 (60.6)	0.82	174.6 (74.0)	163.4 (68.1)	0.005
Median (range)	147.8 (54.0–337.0)	146.5 (43.0–349.5)		161.6 (53.1–482.7)	146.1 (44.3–491.6)	

*Case and control subjects were matched for age (in 2-year age groups) and smoking (current vs. noncurrent); all were men.
BMI = body mass index; CARE = Cholesterol And Recurrent Events study; MI = myocardial infarction; WOSCOPS = West of Scotland Coronary Prevention Study.

Genetic analysis. Genotypes were determined using multiplexed polymerase chain reaction-based amplification of genomic DNA followed by multiplexed allele detection using oligonucleotide ligation as described by Iannone et al. (31) (Online Appendix). Primer sequences are available upon request. This multiplex panel was initially intended to test 10 polymorphisms in 9 candidate genes (Table 2) that others had previously reported to be associated with cardiovascular disease in 2 studies. We investigated the 278-basepair insertion–deletion polymorphism in *ACE* using an assay for an SNP (rs4344) reported to be in strong linkage disequilibrium with this insertion–deletion polymorphism (32). Because the multiplex technology could accommodate 35 SNP assays in a single panel, we included 25 assays for putative functional SNPs that had been found to be associated with disease in primary or subgroup analyses of a single case-control study of MI, study 1 in Shiffman et al. (26). Most of these 25 SNPs were not in typical cardiovascular disease candidate genes.

To test for association between CHD and other SNPs that may be in linkage disequilibrium with the *KIF6* Trp719Arg SNP, we selected 27 tagging SNPs, including Trp719Arg, in the regions flanking the Trp719Arg SNP using pairwise tagging in Tagger (33) as implemented in Haploview (34) (Online Appendix).

Statistical analysis. All reported p values are 2-sided. Differences between baseline characteristics of patients were assessed by *t* tests (continuous variables) or by chi-square tests (discrete variables). We assessed deviation from Hardy-Weinberg expectations using an exact test in the CARE trial cohort and in the control patients of the WOSCOPS trial (35). The power to detect significant associations with disease in the CARE and WOSCOPS trials for each of the 35 genetic polymorphisms was estimated by simulation (Online Appendix) and is shown in Table 1 of the Online Appendix.

Cox proportional hazards models in the CARE trial and conditional logistic regression models in the WOSCOPS trial were used to assess the association of genotype with incident disease in the placebo arms (Wald tests) and were also used to assess the effect of pravastatin compared with placebo in subgroups defined by genotypes. Likelihood ratio tests were used to evaluate potential interactions between genotype and each conventional risk factor in separate regression models that included an interaction term between the risk factor and genotype.

Absolute risk reduction was estimated in the CARE trial at 5 years of follow-up using Kaplan-Meier estimates of MI-free survival of subgroups defined by *KIF6* genotype. Absolute risk and absolute risk reduction in the WOSCOPS trial was projected for the first 4.9 years of follow-up using estimates of the genotype frequencies, an estimate that assumed that the outcome and treatment arm-specific genotype frequencies of subjects included in the nested case-control study were equal to the corresponding treatment arm-specific genotype frequencies in the WOSCOPS trial cohort. The SAS version 9 software (SAS Institute, Cary, North Carolina) was used for all regression

Table 2 Association of 35 Polymorphisms With MI in CARE and CHD in WOSCOPS

Symbol	Name	SNP (Alias)*	Minor Allele Frequency†		p Value‡		p Value§
			CARE	WOSCOPS	CARE	WOSCOPS	Combined
THBS4‖	Thrombospondin 4	rs1866389 (1186G/C)	0.22	0.22	0.034	0.825	0.1273
ACE‖	Angiotensin I-converting enzyme	rs4344 (I/D 278bp)	0.45	0.48	0.938	0.738	0.9469
ITGA2‖	Integrin alpha 2	rs1126643 (C807T)	0.40	0.40	0.986	0.166	0.4605
APOE‖	Apolipoprotein E	rs429358 (3932T/C)	0.14	0.19	0.943	0.337	0.6825
AGTR1‖	Angiotensin II receptor type 1	rs5186 (A1166C)	0.30	0.30	0.285	0.474	0.4056
LTA‖	Lymphotoxin alpha	rs1041981 (804C/A)	0.33	0.37	0.072	0.937	0.2496
APOE‖	Apolipoprotein E	rs405509 (−219T)	0.49	0.46	0.234	0.897	0.5376
MMP3‖	Matrix metallopeptidase- 3	rs3025058 (−1171 6A/5A)	0.50	0.49	0.862	0.940	0.9806
SERPINE1‖	Serpin peptidase inhibitor clade E member 1	rs1799768 (4G/5G)	0.47	0.445	0.692	0.929	0.9267
F2‖	Coagulation factor II	rs1799963 (20210G/A)	0.02	0.01	0.852	0.394	0.7022
ROS1	Proto-oncogene c-ros-1 protein	rs529038	0.26	0.26	0.437	0.249	0.3507
BRE	TNFRSF1A modulator	rs1506536	0.49	0.50	0.463	0.833	0.7530
GALNTL4	GalNAc-transferase	rs901550	0.26	0.24	0.231	0.144	0.1463
AKAP13	A kinase (PRKA) anchor protein 13	rs7162168	0.36	0.36	0.773	0.998	0.9717
BDNF	Brain-derived neurotropic factor	rs6265	0.18	0.20	0.599	0.383	0.5671
CR2	Complement component receptor 2	rs17615	0.32	0.31	0.481	0.417	0.5227
CALCOCO2	Nuclear domain 10 protein	rs1422645	0.29	0.31	0.044	0.660	0.1328
IL12A	Interleukin 12A	rs2243131	0.16	0.18	0.722	0.415	0.6607
ALDH4A1	Aldehyde dehydrogenase 4A1	rs2230709	0.15	0.14	0.231	0.785	0.4914
LILRA4	Leukocyte immunoglobulin-like receptor subfamily A member 4	rs2241384	0.16	0.17	0.023	0.713	0.0849
WDR55	WD repeat domain 55	rs2286394	0.23	0.21	0.331	0.973	0.6868
PPP4R1L	Protein phosphatase 4, regulatory subunit 1-like	rs614507	0.27	0.27	0.279	0.532	0.4316
GIPR	Gastric inhibitory polypeptide receptor	rs1800437	0.19	0.17	0.476	0.692	0.6948
KIF6	Kinesin family member 6	rs20455	0.36	0.34	0.029	0.004	0.0012
MTRR	Methionine synthase reductase	rs1801394	0.46	0.44	0.223	0.249	0.2162
ACAA1	Acetyl-coenzyme A acyltransferase 1	rs156265	0.15	0.15	0.905	0.576	0.8609
LRRC25	Leucine-rich repeat–containing 25	rs6512265	0.34	0.34	0.398	0.872	0.7140
R3HDM1	R3H domain-containing 1	rs961360	0.18	0.09	0.624	0.309	0.5104
ACTR1B	Centractin beta	rs3474	0.28	0.28	0.830	0.434	0.7284
LOC646871	Hypothetical LOC646871	rs3736919	0.46	0.47	0.284	0.287	0.2855
ARL5C	ADP-ribosylation factor–like 5C	rs593772	0.10	0.09	0.747	0.002	0.0093
LTK	Leukocyte tyrosine kinase	rs35932273	0.03	0.03	0.925	0.177	0.4609
KCNQ4	Potassium voltage-gated channel	rs34287852	0.25	0.26	0.449	0.344	0.4430
LGALS14	Lectin, galactoside-binding, soluble, 14	rs35541195	0.13	0.11	0.615	0.243	0.4336
IQCC	IQ motif-containing C	rs12032332	0.06	0.06	0.939	0.777	0.9697

*All of the gene symbols, gene names, and rs numbers are from National Center for Biotechnology build 36 unless noted otherwise. †Based on the cohort for the CARE trial and the control group for the WOSCOPS trial. ‡Association between genotype and disease in Cox proportional hazards model (CARE) or conditional logistic regression model (WOSCOPS); 2-degrees-of-freedom Wald test. §Fisher combined p value. ‖Polymorphisms previously reported to be associated with cardiovascular disease in 2 studies.

CHD = coronary heart disease; SNP = single nucleotide polymorphism; other abbreviations as in Table 1.

models and for generating Kaplan-Meier estimates of survival. We used the Fisher method (36) of combining p values to assess the evidence for association from the combined CARE and WOSCOPS trials. In this method, under the null hypothesis of no association, the statistic $S = -2 (\ln [p1] + \ln [p2])$ and has a chi-square distribution with 4 degrees of freedom, where p1 and p2 are the p values from 2 degrees of freedom genotypic tests of association in the CARE and WOSCOPS trials, respectively. We then applied a Bonferroni correction to the combined p value of each SNP to adjust for the multiple hypothesis tests performed.

Estimates of absolute risk reduction and differences in absolute risk reduction were evaluated for statistical significance in the CARE trial by assuming the difference in the estimate divided by its standard error approximates a standard normal distribution under the null hypothesis and by Monte Carlo simulation (100,000 iterations) in the WOSCOPS trial.

Results

Association with coronary events. In this genetic study of CARE and WOSCOPS, we investigated 35 genetic poly-

Table 3 Assoociation of *KIF6* Trp719Arg with MI and CHD in the Placebo Arms of the CARE and WOSCOPS Trials

Study	Genotype	On-Trial MI*	Total*	Unadjusted HR	Unadjusted 95% CI	Unadjusted p Value	Adjusted† HR	Adjusted† 95% CI	Adjusted† p Value
CARE	Arg/Arg	16	155	1.33	0.75–2.35	0.33	1.33	0.75–2.36	0.33
	Arg/Trp	82	636	1.63	1.13–2.35	0.009	1.54	1.07–2.23	0.02
	Arg/Arg + Arg/Trp	98	791	1.57	1.10–2.25	0.01	1.50	1.05–2.15	0.03
	Trp/Trp	44	542	1.00	ref		1.00	ref	
				Matched‡			Adjusted§		
		Case*	Control*	OR	95% CI	p Value	OR	95% CI	p Value
WOSCOPS	Arg/Arg	35	59	1.49	0.92–2.40	0.10	1.48	0.91–2.41	0.11
	Arg/Trp	137	204	1.61	1.18–2.21	0.003	1.56	1.14–2.15	0.006
	Arg/Arg + Arg/Trp	172	263	1.59	1.18–2.14	0.003	1.55	1.14–2.09	0.005
	Trp/Trp	104	256	1.00	ref		1.00	ref	

*Number of patients. †Adjusted for gender, age (continuous), current versus noncurrent smoking, history of hypertension, history of diabetes, body mass index (continuous), baseline low-density lipoprotein cholesterol level (LDL-C; continuous), and baseline high-density lipoprotein cholesterol level (HDL-C; continuous). ‡Case and control subjects were matched for age (in 2-year age groups) and smoking (current versus noncurrent); all were men. §Matched for age and smoking and adjusted for history of hypertension, history of diabetes, body mass index (continuous), baseline LDL-C level (continuous), and baseline HDL-C level (continuous).

CI = confidence interval; HR = hazard ratio; OR = odds ratio; ref = reference group; other abbreviations as in Tables 1 and 2.

morphisms (Table 2). The genotype distributions for these polymorphisms were all in accord with Hardy-Weinberg expectations ($p > 0.05$) after Bonferroni correction for testing 35 polymorphisms.

Four SNPs (in *KIF6, THBS4, CALCOCO2,* and *LILRA4*) were associated with MI in the placebo arm of the CARE trial and 2 SNPs (in *KIF6* and *ARL5C*) were associated with CHD in the placebo arm of the WOSCOPS trial (Table 2). The SNPs in *KIF6* ($p = 0.001$) and *ARL5C* ($p = 0.009$) remained significantly associated with disease after combining the evidence for association from both the CARE and the WOSCOPS trials; however, only the *KIF6* association remained significant ($p = 0.04$) after applying a Bonferroni multiple testing correction to the combined p values of the 35 SNPs tested in the present study.

The *KIF6* SNP (rs20455) is a Trp719Arg polymorphism in the gene encoding kinesin-like protein 6, a member of the superfamily of molecular motors that are involved in intracellular transport (37). This gene was not an a priori candidate gene for CHD; the association between the *KIF6* Trp719Arg polymorphism and CHD was identified in an investigation of polymorphisms in an unbiased collection of genes. In the placebo arm of the CARE trial, carriers of the *KIF6* 719Arg allele had a hazard ratio (HR) for recurrent MI of 1.50 (95% confidence interval [CI] 1.05 to 2.15) (Table 3) in a model adjusted for age, gender, smoking, history of hypertension, history of diabetes, body mass index, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). This HR for the 719Arg allele was similar to that of some of the conventional risk factors tested in the CARE trial (Fig. 1). In the placebo arm of the WOSCOPS trial, we found that carriers of the *KIF6* 719Arg allele had an odds ratio (OR) for CHD of 1.55 (95% CI 1.14 to 2.09) (Table 3) in a model adjusted for history of hypertension, history of diabetes, body mass index, LDL-C, and HDL-C (case and control subjects were matched for age and smoking status).

The genotype frequencies for *KIF6* Trp719Arg were 40.6%, 46.5%, and 12.8%, for Trp/Trp, Arg/Trp, and Arg/Arg, respectively, in the CARE trial cohort. In the WOSCOPS trial control group, the frequencies were 44.2%, 43.6%, and 12.2%, respectively. The *KIF6* Trp719Arg SNP was not associated with any covariates used for adjustment ($p > 0.13$), nor did we observe any consistent interaction between genotype and the covariates used for adjustment in the CARE and WOSCOPS trials (data not presented).

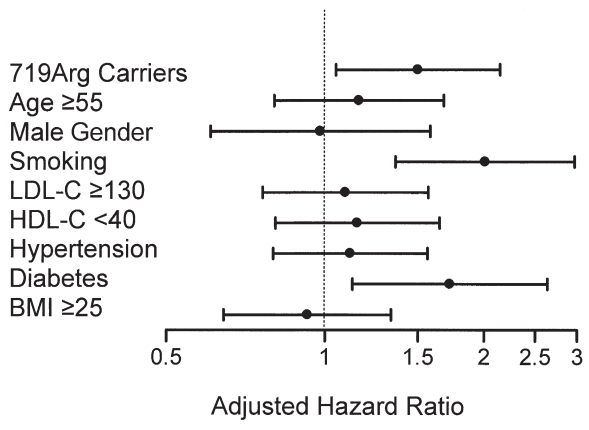

Figure 1 Adjusted Hazard Ratios for Myocardial Infarction in the CARE Study

Comparison of the *KIF6* 719Arg risk allele and conventional risk factors in the CARE study. Hazard ratios were adjusted for age, gender, smoking, baseline low-density lipoprotein cholesterol (LDL-C) level, baseline high-density lipoprotein cholesterol (HDL-C) level, history of diabetes, history of hypertension, and body mass index (BMI). The 719Trp homozygote was the reference group for 719Arg carriers. The 95% confidence intervals are shown.

Table 4 Association of the *THBS4* Ala387Pro SNP With MI and CHD in the Placebo Arms of the CARE and WOSCOPS Trials

		Unadjusted			Adjusted*		
Study	Genotype	HR	95% CI	p Value	HR	95% CI	p Value
CARE	Pro/Pro	2.12	1.20–3.74	0.01	2.07	1.16–3.67	0.01
	Pro/Ala	1.19	0.83–1.70	0.35	1.24	0.86–1.77	0.25
	Ala/Ala	1.00	ref		1.00	ref	
		Matched†			Adjusted‡		
		OR	95% CI	p Value	OR	95% CI	p Value
WOSCOPS	Pro/Pro	1.11	0.56–2.22	0.76	1.19	0.59–2.41	0.62
	Pro/Ala	1.10	0.80–1.52	0.56	1.09	0.78–1.51	0.62
	Ala/Ala	1.00	ref		1.00	ref	

*Adjusted for gender, age (continuous), current versus noncurrent smoking (except in the WOSCOPS trial), history of hypertension, history of diabetes, body mass index (continuous), baseline LDL-C level (continuous), and baseline HDL-C level (continuous). †Case and control subjects were matched for age (in 2-year age groups) and smoking (current versus noncurrent); all were men. ‡Matched for age and smoking and adjusted for history of hypertension, history of diabetes, body mass index (continuous), baseline LDL-C level (continuous), and baseline HDL-C level (continuous).
Abbreviations as in Tables 1 to 3.

The Trp719Arg SNP is located on human chromosome 6 at position 39,433,056 and corresponds to the first nucleotide of codon 719 in the full-length transcript NM_145027 (National Center for Biotechnology Information SNP database, build 36.1). To explore associations between disease and SNPs that might be in linkage disequilibrium with *KIF6* Trp719Arg, we genotyped 26 other SNPs in the regions flanking the Trp719Arg SNP. We found that no SNP or haplotype in the *KIF6* region was more significantly associated with both recurrent MI in the CARE trial and with CHD in the WOSCOPS trial than the *KIF6* Trp719Arg SNP alone (Online Appendix).

We have previously reported that the 94Asn allele of the gene *FCAR* on chromosome 19 was associated with MI in the CARE trial and with CHD in the WOSCOPS trial (30). The risk associated with the *KIF6* SNP seems to be independent of that associated with the *FCAR* SNP, because the risk estimates for carriers of the *KIF6* risk allele were essentially unchanged after adjustment for *FCAR* Asp92Asn and traditional risk factors: In the CARE trial the HR for MI was 1.52 (95% CI 1.06 to 2.17); in the WOSCOPS trial the OR for CHD was 1.55 (95% CI 1.14 to 2.10). There was no indication of an interaction between *KIF6* Trp719Arg and *FCAR* Asp92Asn ($p = 0.48$ in the CARE trial; $p = 0.98$ in the WOSCOPS trial).

None of the 10 polymorphisms previously reported to be associated with cardiovascular disease in multiple studies was associated with CHD in the placebo arm of the WOSCOPS trial, and only the Ala387Pro SNP in *THBS4* (encoding thrombospondin 4) was associated with recurrent MI in the placebo arm of the CARE trial (Table 4). Compared with *THBS4* Ala387 homozygotes, homozygotes for 387Pro had a hazard ratio for MI of 2.07 (95% CI 1.16 to 3.67) (Table 4). For 5 of these 10 polymorphisms (in *THBS4*, *ITGA2*, *MMP3*, *SERPINE1*, and *F2*), the power to detect association was greater than 80% in both the CARE and the WOSCOPS trials. For the polymorphism in *LTA* the power was 62% in the CARE trial and 72% in the WOSCOPS trial. The power to detect association was <60% in both studies for the other 4 SNPs. The power to detect association with disease for all the polymorphisms studied is shown in Table 1 of the Online Appendix.

***KIF6* Trp719Arg and the effect of pravastatin on MI and CHD.** Because the *KIF6* Trp719Arg SNP was associated with both recurrent MI in the CARE trial and CHD in the WOSCOPS trial, we asked whether carriers of the *KIF6* 719Arg risk allele benefited from pravastatin treatment. In the CARE trial, pravastatin treatment reduced the relative risk of MI by 37% among carriers of the 719Arg risk allele (adjusted HR 0.63, 95% CI 0.46 to 0.87) (Table 5), and among carriers in the WOSCOPS trial pravastatin treatment resulted in an OR for CHD of 0.50 (95% CI 0.38 to 0.68) (Table 5). Thus, in the present genetic study of the CARE trial absolute risk reduction by pravastatin was 4.89% (95% CI 1.82% to 7.97%) (Table 6) for carriers of the 719Arg risk allele and 1.39% (95% CI −1.94% to 4.72%) for noncarriers; when genotype was not considered in the present study of the CARE trial the absolute risk reduction was 3.47% (95% CI 1.19 to 5.74). In the present genetic study of the WOSCOPS trial, projected absolute risk reduction by pravastatin was 5.49% (95% CI 3.52% to 7.46%) (Table 6) for carriers of the 719Arg risk allele and 0.09% (95% CI −1.97% to 2.14%) for noncarriers; when genotype was not considered in the present genetic study the absolute risk reduction was 3.48% (95% CI 2.51 to 5.36).

Because carriers of the *KIF6* 719Arg allele benefited from pravastatin treatment in both the CARE and the WOSCOPS trials, we asked if pravastatin benefit differed between carriers and noncarriers. We observed a significant interaction between genotype and treatment in the WOSCOPS trial (interaction $p = 0.01$) (Table 5) but not in the CARE trial ($p = 0.39$).

Discussion

We found that the Trp719Arg SNP in *KIF6* was associated with risk of recurrent MI in the CARE trial and odds of CHD in the WOSCOPS trial, and this association with risk remained significant after correcting for multiple test-

Table 5 Effect of Pravastatin on MI and CHD in *KIF6* Trp719Arg Subgroups in the CARE and WOSCOPS Trials

Study	Genotype	Treatment	On-Trial MI*	Total*	Unadjusted HR	Unadjusted 95% CI	Unadjusted p Value	Adjusted† HR	Adjusted† 95% CI	Adjusted† p Value
CARE	Arg/Trp	Pravastatin	46	619	0.56	0.39–0.81	0.002	0.59	0.41–0.85	0.004
		Placebo	82	636	1.00	ref		1.00	ref	
	Arg/Arg + Arg/Trp	Pravastatin	64	810	0.63	0.46–0.86	0.004	0.63	0.46–0.87	0.005
		Placebo	98	791	1.00	ref		1.00	ref	
	Trp/Trp	Pravastatin	39	554	0.86	0.56–1.33	0.50	0.80	0.52–1.24	0.32
		Placebo	44	542	1.00	ref		1.00		
						interaction p = 0.25‖			interaction p = 0.43¶	
						interaction p = 0.25¶			interaction p = 0.39¶	

Study	Genotype	Treatment	Case*	Control*	Matched‡ OR	Matched‡ 95% CI	Matched‡ p Value	Adjusted§ OR	Adjusted§ 95% CI	Adjusted§ p Value
WOSCOPS	Arg/Trp	Pravastatin	80	339	0.46	0.33–0.64	<0.0001	0.45	0.32–0.64	<0.0001
		Placebo	137	341	1.00	ref		1.00	ref	
	Arg/Arg + Arg/Trp	Pravastatin	108	330	0.50	0.38–0.67	<0.0001	0.50	0.38–0.68	<0.0001
		Placebo	172	263	1.00	ref		1.00	ref	
	Trp/Trp	Pravastatin	81	213	0.94	0.67–1.33	0.73	0.91	0.64–1.28	0.58
		Placebo	104	256	1.00	ref		1.00	ref	
						interaction p = 0.014‖			interaction p = 0.021‖	
						interaction p = 0.006¶			interaction p = 0.011¶	

*Number of patients. †Adjusted for gender, age (continuous), current versus non-current smoking (except in the WOSCOPS trial), history of hypertension, history of diabetes, body mass index (continuous), baseline LDL-C level (continuous), and baseline HDL-C level (continuous). ‡Case and control subjects were matched for age (in 2-year age groups) and smoking (current versus noncurrent); all were men. §Matched for age and smoking and adjusted for history of hypertension, history of diabetes, body mass index (continuous), baseline LDL-C level (continuous), and baseline HDL-C level (continuous). ‖Interaction between *KIF6* genotype and treatment (2-degrees-of-freedom analysis of all 3 genotypes, likelihood ratio test). ¶Interaction between *KIF6* carrier status and treatment (likelihood ratio test). Abbreviations as in Tables 1 to 3.

ing. Carriers of the *KIF6* 719Arg risk allele had an adjusted HR of 1.50 for recurrent MI in the CARE trial and an adjusted OR of 1.55 for CHD in the WOSCOPS trial. The *KIF6* 719Arg risk allele has also been recently shown to be associated with CHD in the ARIC (Atherosclerosis Risk in Communities) study (38).

Although the discovery of genetic polymorphisms that are associated with CHD may aid in the assessment of an individual's risk of disease, a therapy that specifically counteracts the mechanism of action of the deleterious gene variant is unlikely to be immediately available. However, carriers of the deleterious gene variant might benefit from aggressive treatment of modifiable CHD risk factors. And this may be the case for carriers of the *KIF6* risk allele: In both the CARE and the WOSCOPS trials, carriers of the 719Arg allele significantly benefited from pravastatin treatment. Among carriers of the *KIF6* 719Arg allele, pravastatin treatment in the CARE trial resulted in an absolute risk reduction of 4.9% and a relative risk reduction of 37% and in the WOSCOPS

Table 6 Absolute Risk and Absolute Risk Reduction of MI in the CARE Trial and of CHD in the WOSCOPS Trial: Effect of Pravastatin in *KIF6* Trp719Arg Subgroups

Study	Genotype	Treatment	AR (%)	ARR (%)	95% CI	p Value
CARE	All	Pravastatin	7.37	3.47	1.19–5.74	0.003
		Placebo	10.83			
	Arg/Arg + Arg/Trp	Pravastatin	7.60	4.89	1.82–7.97	0.002
		Placebo	12.49			
	Trp/Trp	Pravastatin	7.00	1.39	−1.94–4.72	0.41
		Placebo	8.40			
WOSCOPS	All	Pravastatin	7.06	3.48	2.51–5.36	<0.0001
		Placebo	10.54			
	Arg/Arg + Arg/Trp	Pravastatin	6.89	5.49	3.52–7.46	<0.0001
		Placebo	12.38			
	Trp/Trp	Pravastatin	7.95	0.09	−1.97–2.14	0.99
		Placebo	8.04			

Absolute risk reduction (ARR) was estimated in the CARE trial at 5 years of follow-up using Kaplan–Meier estimates of MI-free survival within subgroups defined by *KIF6* genotype. In the WOSCOPS trial, ARR was projected for the first 4.9 years of follow–up. In the WOSCOPS trial, absolute risk reduction due to pravastatin was significantly greater in carriers of the Arg allele than in noncarriers (p = 0.003), whereas in the CARE trial the greater ARR in carriers than in noncarriers did not reach statistical significance (p = 0.13).
AR = absolute risk; other abbreviations as in Tables 1 to 3.

trial resulted in a projected absolute risk reduction of 5.5% and a relative risk reduction of 50%.

KIF6 encodes a kinesin, a class of motor proteins involved in the intracellular transport along microtubules; the cargos transported include membrane organelles, protein complexes, and mRNAs (37). Kinesins consist of a conserved motor domain that propels the kinesin along microtubules in an ATP dependent manner and a nonconserved tail domain that binds to its cargo (37). The tail domains contain coiled-coil structures that facilitate protein-protein interactions (39), and the *KIF6* tail domain contains an alpha-helical region that is predicted by the COILS program to form a coiled-coil structure (40). The Trp719Arg polymorphism is in this predicted coiled-coil structure; therefore, the Trp719Arg polymorphism, a nonconservative amino acid change that replaces a nonpolar residue with a basic residue, might affect the cargo binding of the kinesin encoded by *KIF6*. Several kinesins have been implicated in the pathogenesis of chronic diseases, such as neurodegenerative diseases, type 2 diabetes, and Alzheimer's disease (41); however, the role of *KIF6* and the Trp719Arg SNP in cardiovascular disease remains to be elucidated.

Of the 10 polymorphisms previously reported to be associated with cardiovascular disease, only the *THBS4* Ala387Pro SNP (9) was associated with recurrent MI in the CARE trial, and the risk allele in the CARE trial was the same as the previously reported risk allele. Although this SNP was not significantly associated with CHD in the WOSCOPS trial, the OR for CHD was higher in carriers of the *THBS4* 387Pro allele than in noncarriers.

Study limitations. One limitation of the present study is that only a small number of women were enrolled in the CARE trial and none were enrolled in the WOSCOPS trial; therefore, the association of *KIF6* Trp719Arg with cardiovascular risk should be investigated in cohorts that are adequately powered for analysis in women. Additional limitations are that the end points and entry criteria of the CARE and WOSCOPS trials differed and that for 4 of the 10 polymorphisms previously reported in multiple studies to be associated with cardiovascular disease (in *ACE*, *APOE*, and *AGTR1*), power to detect association was <60%. Finally, because *KIF6* was not an a priori candidate gene for cardiovascular disease, the role of kinesin-like protein 6 and the Trp719Arg polymorphism in the pathogenesis of cardiovascular disease remains to be determined in future studies.

Of the 35 polymorphisms tested, only the *KIF6* 719Arg allele was associated with both MI in the CARE trial and CHD in the WOSCOPS trial. In both the CARE and the WOSCOPS trials, carriers of the 719Arg allele received significant and substantial absolute risk reduction from pravastatin treatment.

Acknowledgments

The authors express their gratitude to the CARE and WOSCOPS patients and to Drs. John Sninsky and Koustubh Ranade for helpful comments on this manuscript, to Joe Catanese, Sheng-Yung Chang, David Lew, Dr. Diane Leong, and the Celera high throughput group for generating genotyping data, to Drs. Anand Chokkalingam, Lynn Ploughman, and Kit Lau for their input into statistical analysis, and to Dr. David Ross, Dr. Goran Gogic, Joel Bolonick, Migdad Machrus, Daniel Civello, Alla Smolgovsky, David Wolfson, and Kevin Tsai for computational biology support.

Reprint requests and correspondence: Dr. Olga A. Iakoubova, Celera, Inc., 1401 Harbor Bay Parkway, Alameda, California 94502. E-mail: olga.iakoubova@celera.com.

REFERENCES

1. Grundy SM. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. J Am Coll Cardiol 2006;47:1093–100.
2. Grundy SM, Cleeman JI, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227–39.
3. Gibbons GH, Liew CC, Goodarzi MO, et al. Genetic markers: progress and potential for cardiovascular disease. Circulation 2004; 109:IV47–58.
4. Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis: part II: clinical implications. Circulation 2004;110:2066–71.
5. Cohen JC. Genetic approaches to coronary heart disease. J Am Coll Cardiol 2006;48:A10–4.
6. Ioannidis JP, Trikalinos TA, Khoury MJ. Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases. Am J Epidemiol 2006;164:609–14.
7. Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807T gene polymorphism with nonfatal myocardial infarction in younger patients. Blood 1999; 93:2449–53.
8. Antoniades C, Tousoulis D, Vasiliadou C, Stefanadi E, Marinou K, Stefanadis C. Genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: effects on the release of sCD40L during the acute phase of premature myocardial infarction. J Am Coll Cardiol 2006;47:1959–66.
9. Topol EJ, McCarthy J, Gabriel S, et al. Single nucleotide polymorphisms in multiple novel thrombospondin genes may be associated with familial premature myocardial infarction. Circulation 2001;104: 2641–4.
10. Wessel J, Topol EJ, Ji M, Meyer J, McCarthy JJ. Replication of the association between the thrombospondin-4 A387P polymorphism and myocardial infarction. Am Heart J 2004;147:905–9.
11. Eriksson P, Kallin B, van 't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A 1995;92:1851–5.
12. Margaglione M, Cappucci G, Colaizzo D, et al. The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. Arterioscler Thromb Vasc Biol 1998;18:152–6.
13. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood 1997;90:1747–50.
14. Doggen CJ, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. Circulation 1998;97:1037–41.
15. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 1996;94:708–12.

16. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992;359:641–4.
17. Jones A, Dhamrait SS, Payne JR, et al. Genetic variants of angiotensin II receptors and cardiovascular risk in hypertension. Hypertension 2003;42:500–6.
18. Canavy I, Henry M, Morange PE, et al. Genetic polymorphisms and coronary artery disease in the south of France. Thromb Haemost 2000;83:212–6.
19. Ozaki K, Ohnishi Y, Iida A, et al. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet 2002;32:650–4.
20. Laxton R, Pearce E, Kyriakou T, Ye S. Association of the lymphotoxin-alpha gene Thr26Asn polymorphism with severity of coronary atherosclerosis. Genes Immun 2005;6:539–41.
21. Ye S, Watts GF, Mandalia S, Humphries SE, Henney AM. Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. Br Heart J 1995;73:209–15.
22. Hirashiki A, Yamada Y, Murase Y, et al. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol 2003;42:1429–37.
23. Ye S, Dunleavey L, Bannister W, et al. Independent effects of the −219 G>T and epsilon 2/epsilon 3/epsilon 4 polymorphisms in the apolipoprotein E gene on coronary artery disease: the Southampton Atherosclerosis Study. Eur J Hum Genet 2003;11:437–43.
24. Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. Lancet 2001;358:115–9.
25. Lambert JC, Brousseau T, Defosse V, et al. Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations—the ECTIM study. Hum Mol Genet 2000;9:57–61.
26. Shiffman D, Ellis SG, Rowland CM, et al. Identification of four gene variants associated with myocardial infarction. Am J Hum Genet 2005;77:596–605.
27. Sacks FM, Pfeffer MA, Moye LA, et al., Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996;335:1001–9.
28. Shepherd J, Cobbe SM, Ford I, et al., West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995; 333:1301–7.
29. Packard CJ, O'Reilly DS, Caslake MJ, et al., West of Scotland Coronary Prevention Study Group. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. N Engl J Med 2000;343:1148–55.
30. Iakoubova OA, Tong CH, Chokkalingam AP, et al. Asp92Asn polymorphism in the myeloid IgA Fc receptor is associated with myocardial infarction in two disparate populations: CARE and WOSCOPS. Arterioscler Thromb Vasc Biol 2006;26:2763–8.
31. Iannone MA, Taylor JD, Chen J, et al. Multiplexed single nucleotide polymorphism genotyping by oligonucleotide ligation and flow cytometry. Cytometry 2000;39:131–40.
32. Zhu X, McKenzie CA, Forrester T, et al. Localization of a small genomic region associated with elevated ACE. Am J Hum Genet 2000;67:1144–53.
33. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005; 37:1217–23.
34. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21: 263–5.
35. Weir BS. Genetic Data Analysis 2: Methods for Discrete Population Genetic Data. 2nd edition. Sunderland: Sinauer Associates, 1996:98.
36. Elston RC. On Fisher's method of combining p-values. Biom J 1991;3:339–345.
37. Miki H, Setou M, Kaneshiro K, Hirokawa N. All kinesin superfamily protein, KIF, genes in mouse and human. Proc Natl Acad Sci U S A 2001;98:7004–11.
38. Morrison AC, Bare LA, Chambless LE, et al. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities Study. Am J Epidemiol 2007;166:28–35.
39. Seiler S, Kirchner J, Horn C, Kallipolitou A, Woehlke G, Schliwa M. Cargo binding and regulatory sites in the tail of fungal conventional kinesin. Nat Cell Biol 2000;2:333–8.
40. Lupas A, Van Dyke M, Stock J. Predicting coiled coils from protein sequences. Science 1991;252:1162–4.
41. Seog DH, Lee DH, Lee SK. Molecular motor proteins of the kinesin superfamily proteins (KIFs): structure, cargo and disease. J Korean Med Sci 2004;19:1–7.

APPENDIX

For supplemental procedure descriptions and a table, please see the online version of this article.