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A Kinesin Family Member 6 Variant Is Associated With Coronary Heart Disease in the Women's Health Study

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Objectives: We asked if carriers of the 719Arg allele of kinesin family member 6 (*KIF6*) have increased risk of coronary heart disease (CHD) in a cohort of initially healthy Caucasian American women.

Background: The 719Arg allele of *KIF6* (rs20455) has been reported to be associated with increased risk of CHD in a large population-based prospective study, ARIC (Atherosclerosis Risk in Communities), and in the placebo arms of 2 statin trials, CARE (Cholesterol and Recurrent Events) and WOSCOPS (West of Scotland Coronary Prevention Study). However, this *KIF6* variant was not specifically investigated in the female subgroup in the ARIC study, and the CARE and WOSCOPS trials included only a small number of female patients.

Methods: Genotypes of the rs20455 single nucleotide polymorphism (SNP) were determined among 25,283 initially healthy Caucasian women, age 45 years and older, participating in the WHS (Women's Health Study) who were prospectively followed over a 12-year period for incident cardiovascular events. The risk associated with the 719Arg allele of *KIF6* was estimated using Cox proportional hazards models that adjusted for age and traditional risk factors.

Results: During follow-up, 953 women suffered a first-ever CHD event (myocardial infarction, coronary revascularization, or cardiovascular death) or first-ever ischemic stroke. Compared with noncarriers, carriers of the 719Arg allele had an increased risk of CHD (hazard ratio [HR] = 1.24 [95% confidence interval (CI) 1.04 to 1.46, p = 0.013]) and myocardial infarction (HR = 1.34 [95% CI 1.02 to 1.75, p = 0.034]) but not ischemic stroke.

Conclusions: Confirming and extending previous reports, carriers of the 719Arg allele of *KIF6* have 34% higher risk of myocardial infarction and 24% higher risk of CHD compared with noncarriers among 25,283 women from the WHS. (J Am Coll Cardiol 2008;51:444–8) © 2008 by the American College of Cardiology Foundation

Coronary heart disease (CHD) is known to be affected by genetic factors (1). For example, it has recently been observed that the 719Arg allele of the kinesin family member 6 (*KIF6*) gene is associated with increased risk of incident CHD events in ARIC (Atherosclerosis Risk in Communities), a large population-based prospective study of several American communities (2). Carriers of this 719Arg allele of *KIF6* were also found to have increased risk of incident CHD in the placebo arms of 2 statin trials (3): CARE (Cholesterol and Recurrent Events) (secondary prevention of CHD) and WOSCOPS (West of Scotland Coronary Prevention Study) (primary prevention of CHD). However, as with any reported genetic association, replication in additional populations is important to validate these initial observations (4) and to explore whether the association can be generalized to other settings.

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Kinesins are a large family of proteins involved in intracellular transport. Kinesins share a conserved motor domain that interacts with microtubules and a nonconserved tail domain that interacts with a specific cargo either directly or through an adaptor protein (5). Kinesins have been shown to mediate axonal transport in neurons (6), and some kinesins have been implicated in the pathogenesis of neuronal diseases, for example, a variant *KIF2* was reported to be associated with schizophrenia (7), *KLC1* with Alzheimer's disease (6), and *KIF5A* with spastic paraplegia (8). *KIF6* is expressed in many tissues and cell types, including

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vascular cells (9). However, the role of kinesins in CHD and, more specifically, the roles of *KIF6* and the Trp719Arg variant in CHD are not understood.

Two of the studies that provided early evidence for the association between the *KIF6* 719Arg allele and increased risk of CHD included mainly male patients. One study, CARE, included mostly male patients, and the other study, WOSCOPS, did not include any women. Thus, we sought to confirm the association between this kinesin polymorphism and vascular risk in a large prospective cohort of women.

Abbreviations and Acronyms

CHD = coronary heart disease

HDL-C = high-density lipoprotein cholesterol

HWE = Hardy-Weinberg equilibrium

LDL-C = low-density lipoprotein cholesterol

Lp(a) = lipoprotein(a)

MI = myocardial infarction

Methods

Study design. The primary study population was derived from participants in the WHS (Women's Health Study), a randomized, double-blind, placebo controlled 2 × 2 factorial design trial of aspirin and vitamin E in the prevention of cardiovascular disease conducted among initially healthy women age at least 45 years at enrollment, who were followed over a mean 12-year period for incident vascular events (10). At enrollment, participants provided baseline clinical and demographic information; 28,345 WHS participants also provided adequate blood samples for both plasma and genetic analysis. Participants were followed for the occurrence of first-ever myocardial infarction (MI), ischemic stroke, coronary revascularization procedures, or cardiovascular-related death. The CHD end point is a composite of MI, coronary revascularization, or cardiovascular death. The methods of cohort assembly, follow-up, and end-point validation have been described elsewhere (10). All participants provided written informed consent, and the study protocol was approved by the Institutional Review Board of Brigham and Women's Hospital (Boston, Massachusetts).

Genotypes for rs20455 (*KIF6* Trp719Arg) in WHS participants were determined by a previously described procedure that combined polymerase chain reaction amplification of target sequences from 3 ng of genomic deoxyribonucleic acid with subsequent allele-specific oligonucleotide ligation as described (2). The ligation products of the 2 alleles were separated by hybridization to product-specific oligonucleotides, each coupled to spectrally distinct Luminex 100 xMAP microspheres (Luminex, Austin, Texas). The captured ligation products were fluorescently labeled with streptavidin R-phycoerythrin (Prozyme, San Leandro, California), sorted on the basis of microsphere spectral properties, and detected by a Luminex 100 instrument. The fraction of samples with successful genotype determination was 97.9%. A total of 25,283 Caucasian women with available deoxyribonucleic acid samples also had full ascertainment for rs20455 genotype, age, and incident cardiovascular events.

Statistical methods. Deviations from Hardy-Weinberg equilibrium (HWE) were evaluated using a log-likelihood ratio test. Clinical covariate differences between carriers and noncarriers were assessed by the Wilcoxon rank sum test for continuous variables and by a chi-square test of proportions for categorical characteristics. Association between the 719Arg allele and the risk of incident disease was tested with Cox proportional hazards models adjusted for age or traditional risk factors (age, blood pressure, history of diabetes, smoking status, familial history of MI, low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]) using a pre-specified dominant inheritance model. Analyses were additionally adjusted for randomized treatment assignment within the WHS either to aspirin, vitamin E, or placebo. Potential interaction between traditional risk factors and 719Arg carrier status was tested in separate Cox models that included the interaction (cross-product) term between the risk factor and carrier status; these models also included main effect terms for carrier status and age. For the Caucasian participants of WHS, there was at least 80% power to detect a significant ($p \leq 0.05$) association between carriers of the rs20455 risk allele (719Arg) of *KIF6* and incident cardiovascular disease for hazard ratios of 1.25 or higher for CHD, and 1.42 or higher (for MI or stroke).

Results

The frequency of the 719Arg allele of rs20455 in the Caucasian participants of WHS was 0.36, which is similar to that reported for other Caucasian populations (0.36 in ARIC; 0.36 in the CARE cohort; 0.34 in the control group of the WOSCOPS nested case-control study). The genotype distribution of rs20455 in the Caucasian WHS participants deviated slightly from HWE expectations ($p = 0.047$), principally because the heterozygous group contained 146 more individuals (1.2% more) than expected. This deviation is unlikely to be due to genotyping error, since the genotype concordance in 628 duplicate samples in WHS was >99.5%. Additionally, the genotype distribution did not deviate from HWE expectations in ARIC, where the genotypes for this SNP were determined using the same technology used in WHS (2).

Baseline clinical characteristics, including traditional risk factors for cardiovascular disease (age, blood pressure, history of diabetes, familial history of MI, smoking status, LDL-C, and HDL-C) did not differ between carriers of the 719Arg allele and noncarriers (Table 1).

During follow-up, 953 of the Caucasian women with ascertained rs20455 genotype suffered a first-ever CHD event (MI, coronary revascularization, or cardiovascular death) or first-ever ischemic stroke. The cumulative incidence of CHD and MI events was higher among carriers of the *KIF6* 719Arg allele than in noncarriers (Fig. 1). The cumulative incidence of CHD events was 2.94% in carriers

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Table 1 Baseline Characteristics of WHS Caucasian *KIF6* 719Arg Noncarriers and Carriers

	Noncarriers	Carriers	p Value
Clinical characteristics (U)			
Age (yrs)	52 (48.0–59.0)	53.0 (48.0–59.0)	0.49
BMI (kg/m^2)	24.8 (22.4–28.3)	24.9 (22.5–28.3)	0.34
History of hypertension	2,534 (24.8)	3,702 (24.6)	0.61
Framingham systolic/diastolic blood pressure categories (mm Hg)			0.31
<120/<75	3,406 (33.7)	5,031 (33.8)	
120/75 to 129/84	3,242 (32.1)	4,859 (32.6)	
130/85 to 139/89	1,889 (18.7)	2,803 (18.8)	
140/90 to 159/94	1,321 (13.1)	1,901 (12.8)	
≥160/≥95	240 (2.4)	299 (2.0)	
Family history of MI	1,212 (13.2)	1,754 (12.9)	0.54
Current smoking	1,207 (11.8)	1,718 (11.4)	0.30
History of diabetes	239 (2.3)	405 (2.7)	0.097
HRT	4,401 (43.2)	6,609 (43.9)	0.28
Menopause	5,529 (54.3)	8,246 (54.8)	0.47
Lipid biomarkers (U)			
Total cholesterol (mg/dl)	208 (184–236)	208 (184–235)	0.44
LDL cholesterol (mg/dl)	121.5 (100.6–144.6)	121.3 (100.5–144.4)	0.56
ApoB (mg/dl)	100.2 (84.1–121.8)	100.0 (83.8–121.1)	0.28
HDL cholesterol (mg/dl)	51.7 (43.1–62.1)	51.8 (43.2–62.3)	0.64
ApoA (mg/dl)	149.0 (132.1–167.8)	148.9 (132.6–167.8)	0.53
Triglycerides (mg/dl)	119 (84–176)	118.0 (84.0–175.0)	0.50
Lp(a) (mg/dl)	10.8 (4.4–33.0)	10.1 (4.2–31.0)	0.002
Inflammation biomarkers (U)			
CRP (mg/l)	2.0 (0.8–4.3)	2.0 (0.8–4.4)	0.36
ICAM (ng/ml)	341.9 (302.0–396.0)	343.9 (301.7–394.0)	0.59
Fibrinogen (mg/l)	349.9 (306.8–402.7)	349.8 (307.2–400.5)	1.00
Other biomarkers (U)			
Creatinine (mg/dl)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.50
Homocysteine (μmol/dl)	10.5 (8.7–13.0)	10.5 (8.7–12.9)	0.41
HbA1c (%)	5.0 (4.8–5.2)	5.0 (4.8–5.2)	0.11

Values are number of participants (%) or median (interquartile range).

ApoA = apolipoprotein A; ApoB = apolipoprotein B; BMI = body mass index; CRP = C-reactive protein; HbA1c = hemoglobin A1c; HDL = high-density lipoprotein; HRT = hormone replacement therapy; ICAM = intracellular adhesion molecule; LDL = low-density lipoprotein; Lp(a) = lipoprotein(a); MI = myocardial infarction; WHS = Women's Health Study.

and 2.51% in noncarriers (log-rank p = 0.043), and the cumulative incidence of MI events was 1.23% in carriers and 0.94% in noncarriers (log-rank p = 0.032). For carriers of the 719Arg allele compared with noncarriers, the age-adjusted hazard ratio (HR) was 1.18 for CHD (95% confidence interval [CI] 1.01 to 1.37, p = 0.037) and 1.32 for MI (95% CI 1.03 to 1.69, p = 0.028) (Table 2). These HRs were somewhat increased by adjustment for traditional risk factors, aspirin treatment, and vitamin E treatment: for CHD the fully adjusted HR was 1.24 (95% CI 1.04 to 1.46, p = 0.013) and for MI the fully adjusted HR was 1.34 (95% CI 1.02 to 1.75, p = 0.034). We investigated potential interaction between *KIF6* carrier status and each of the following risk factors: age (>60 years), diabetes, or smoking status; however, we did not observe any significant interactions (p > 0.09). The risk for ischemic stroke did not differ between carriers of the 719Arg allele and noncarriers (age-adjusted HR = 1.08; 95% CI 0.85 to 1.37, p = 0.52). However, the 95% confidence intervals suggest that 719Arg homozygotes may be

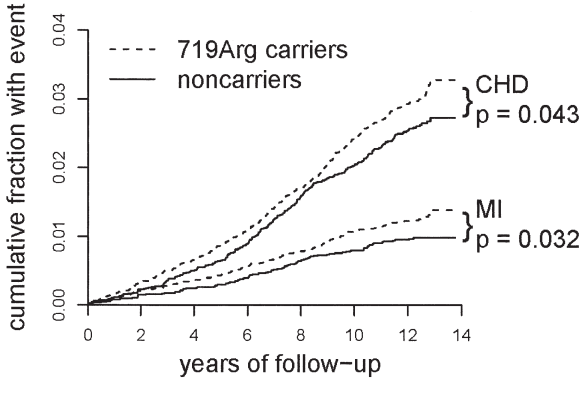

Figure 1 Risk of CHD or MI

Comparison of Kaplan-Meier estimates of the cumulative incidence of coronary heart disease (CHD) events and myocardial infarction (MI) events among carriers of the *KIF6* 719Arg allele and noncarriers. Log-rank p values are for mean follow-up time of 12 years.

Table 2 Association of *KIF6* Trp719Arg With CHD, MI, and Ischemic Stroke

Genotype	n	Events	Adjusted for Age		Fully Adjusted*	
			HR (95% CI)†	p Value	HR (95% CI)†	p Value
CHD						
ArgArg	3,249	95	1.18 (0.93–1.50)	0.16	1.25 (0.96–1.61)	0.092
ArgTrp	11,831	349	1.14 (0.97–1.35)	0.11	1.23 (1.03–1.47)	0.020
ArgArg + ArgTrp	15,080	444	1.18 (1.01–1.37)	0.037	1.24 (1.04–1.46)	0.013
TrpTrp	10,203	256	Reference		Reference	
MI						
ArgArg	3,249	37	1.21 (0.83–1.77)	0.32	1.15 (0.75–1.77)	0.53
ArgTrp	11,831	149	1.35 (1.04–1.74)	0.023	1.39 (1.05–1.83)	0.022
ArgArg + ArgTrp	15,080	186	1.32 (1.03–1.69)	0.028	1.34 (1.02–1.75)	0.034
TrpTrp	10,203	96	Reference		Reference	
Stroke						
ArgArg	3,249	48	1.35 (0.96–1.90)	0.080	1.42 (0.98–2.06)	0.061
ArgTrp	11,831	130	1.01 (0.78–1.30)	0.96	1.99 (0.75–1.31)	0.93
ArgArg + ArgTrp	15,080	178	1.08 (0.85–1.37)	0.52	1.11 (0.86–1.42)	0.42
TrpTrp	10,203	112	Reference		Reference	

*Adjusted for age, blood pressure, history of diabetes, smoking status, familial history of myocardial infarction, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, aspirin treatment, and vitamin E treatment; †hazard ratio and 2-sided p values estimated using Cox proportional hazards models.

CHD = coronary heart disease; CI = confidence interval; HR = hazard ratio.

at elevated risk of ischemic stroke: for 719Arg homozygotes compared with 719Trp homozygotes, the fully adjusted HR was 1.42 (95% CI 0.98 to 2.06, p = 0.061).

Except for lipoprotein(a) [Lp(a)] levels, none of the baseline measurements available for WHS participants (Table 1) was associated with the 719Arg *KIF6* polymorphism carrier status. Carriers of the 719Arg allele of *KIF6* had modestly lower median baseline plasma levels of Lp(a) than noncarriers had (10.1 mg/dl for carriers compared with 10.8 mg/dl for noncarriers; p < 0.01). However, the risk estimates for CHD (age- and Lp(a)-adjusted HR = 1.18; 95% CI 1.01 to 1.38, p = 0.043) and MI (age- and Lp(a)-adjusted HR = 1.30; 95% CI 1.00 to 1.68, p = 0.047) were not appreciably changed by adjustment for baseline Lp(a) levels.

Discussion

We have confirmed the pre-specified hypothesis that Caucasian carriers of the 719Arg allele of *KIF6* had increased risk of CHD and MI in the WHS. This finding extends previously reported associations between this *KIF6* variant and CHD (Table 3). Carriers of the 719Arg allele were previously reported to be at increased risk of CHD in the placebo arms of the CARE and WOSCOPS statin trials (3). These 2 trials were designed to study mostly male populations at high risk for CHD; CARE, a secondary prevention trial, included only individuals (mostly men) who had already experienced an MI: the incidence of MI or CHD death in the placebo arm was 13.2% during 5 years of follow-up (11); WOSCOPS included only men who had high LDL-C levels and other traditional risk factors: the event rate was 15.8 events (MI and CHD death) per 1,000 person-years (12). In contrast, the female participants in the WHS were healthy at baseline and free of prevalent cardiovascular disease, as evidenced by the relatively low event rate: 2.3 CHD events per 1,000 person-years. The 719Arg allele of *KIF6* was also associated with increased risk of CHD in ARIC (2), a prospective study of middle-aged Americans. Thus, carriers of the 719Arg allele of *KIF6* have increased risk of CHD in 4 studies that cover men and women and a spectrum of CHD risk—from high risk in CARE and WOSCOPS to low risk in WHS.

The availability of a panel of lipid and inflammatory biomarkers assessed among the over 25,000 Caucasian WHS participants allowed us to ask whether the effects of 719Arg on CHD could be understood in terms of corre-

Table 3 Association Between *KIF6* 719Arg and Risk of CHD*

Study	Risk Ratio*	95% CI	p Value	Context
ARIC	1.09†	1.00–1.19	0.05	Prospective cohort study of middle-age men and women
CARE	1.50	1.05–2.15	0.03	Placebo arm of a statin secondary prevention study of men (86.5%) and women
WOSCOPS	1.55	1.14–2.09	0.005	Placebo arm of a statin primary prevention study of men
WHS	1.24	1.04–1.46	0.013	Prospective cohort study of healthy women

*Risk ratios are hazard ratios for CARE (Cholesterol and Recurrent Events), WHS (Women's Health Study), and ARIC (Atherosclerosis Risk in Communities), and odds ratio for the nested case-control study of WOSCOPS (West of Scotland Coronary Prevention Study); †In CARE, WOSCOPS, and WHS, the risk ratios are for carriers compared with noncarriers. In ARIC, the risk ratio was based on an additive inheritance model, in which the hazard ratios for carriers of 1 or 2 copies of the 719Arg allele were 1.09 or 1.19, respectively (2).

Abbreviations as in Table 1.

sponding effects on these biomarkers for traditional and emerging risk factors for CHD (13). We found that the only risk factor associated with the *KIF6* 719Arg carrier status was Lp(a) levels. This association is unlikely to explain the association between 719Arg and CHD, since lower plasma levels of Lp(a) were found among carriers of the risk allele of *KIF6* (719Arg) than among noncarriers, whereas higher plasma levels of Lp(a) are typically associated with increased risk of CHD (14). Moreover, the CHD risk estimates of the 719Arg allele did not appreciably change after adjustment for plasma Lp(a) levels. However, the association with Lp(a) could plausibly reflect an effect of the *KIF6* variant on intracellular transport processes involved in the assembly or secretion of Lp(a).

Conclusions

We found that the 719Arg allele of *KIF6* is associated with CHD and MI in WHS, a population of initially healthy Caucasian women with a relatively low CHD event rate. This is the fourth prospective study in which the *KIF6* 719Arg allele has been associated with increased risk of CHD. Replication of this association in independent prospective population studies would be of great interest.

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