ORIGINAL INVESTIGATION

Genetic variants in the *KIF6* region and coronary event reduction from statin therapy

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Abstract A single nucleotide polymorphism (SNP) in *KIF6*, a member of the *KIF9* family of kinesins, is associated with differential coronary event reduction from statin therapy in four randomized controlled trials; this SNP (rs20455) is also associated with the risk for coronary heart disease (CHD) in multiple prospective studies. We investigated whether other common SNPs in the *KIF6* region were associated with event reduction from statin therapy. Of the 170 SNPs in the *KIF6* region investigated in the Cholesterol and Recurrent Events trial (CARE), 28 were associated with differential event reduction from statin therapy ($P_{interaction} < 0.1$ in Caucasians, adjusted for age and sex) and were further investigated in the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI22) and West of Scotland Coronary Prevention Study (WOSCOPS).

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These analyses revealed that two SNPs (rs9462535 and rs9471077), in addition to rs20455, were associated with event reduction from statin therapy ($P_{interaction} < 0.1$ in each of the three studies). The relative risk reduction ranged from 37 to 50% ($P < 0.01$) in carriers of the minor alleles of these SNPs and from −4 to 13% ($P > 0.4$) in non-carriers. These three SNPs are in high linkage disequilibrium with one another ($r^2 > 0.84$). Functional studies of these variants may help to understand the role of KIF6 in the pathogenesis of CHD and differential response to statin therapy.

Introduction

The risk for coronary heart disease (CHD) varies between individuals and can be ameliorated by lifestyle changes and medications such as statins. Although statins have been shown to be highly effective in reducing CHD events in randomized controlled trials, individual response to statin therapy varies (reviewed in Mangravite et al. 2006; Schmitz and Langmann 2006), a variability thought to be partly due to genetic factors.

Recently, a common single nucleotide polymorphism (SNP) in *KIF6*, a member of the *KIF9* family of kinesins, has been reported to be associated with CHD risk and differential reduction of coronary events from statin therapy (Bare et al. 2007; Iakoubova et al. 2008a, b, 2009; Shiffman et al. 2008a, b); this SNP (rs20455) encodes the missense polymorphism KIF6 Trp719Arg. In a meta-analysis of seven prospective studies, carriers of the 719Arg variant had over 20% increased risk of CHD, compared with non-carriers ($P = 1.02 \times 10^{-6}$) (Li et al. 2010). This increased risk was independent of traditional risk factors including age, blood pressure, diabetes, smoking, low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein

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cholesterol (HDL-C). However, an association between the *KIF6* Trp719Arg polymorphism and CHD was not observed in two case–control studies (Stewart et al. 2009; Wellcome Trust Case Control Consortium 2007), possibly due to statin use.

The 719Arg variant was also associated with greater reduction of coronary events from both pravastatin (a hydrophilic statin) and atorvastatin (a lipophilic statin) therapy. In retrospective genetic analyses of four prospective clinical trials, the Cholesterol and Recurrent Events trial (CARE) (Sacks et al. 1996), the West of Scotland Coronary Prevention Study (WOSCOPS) (Shepherd et al. 1995), Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI22) (Cannon et al. 2004), and the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), carriers of the 719Arg variant (~60% of people of European ancestry) had substantial and significant reduction of CHD events from statin therapy, whereas non-carriers did not (Iakoubova et al. 2008a, b, 2009).

Here we have conducted a fine mapping study to determine (1) whether other SNPs or haplotypes in the *KIF6* region account for the association of rs20455 (Trp719Arg) with differential coronary event reduction from statin therapy and (2) whether other variants in the *KIF6* region are independently associated with differential reduction of events from statin therapy.
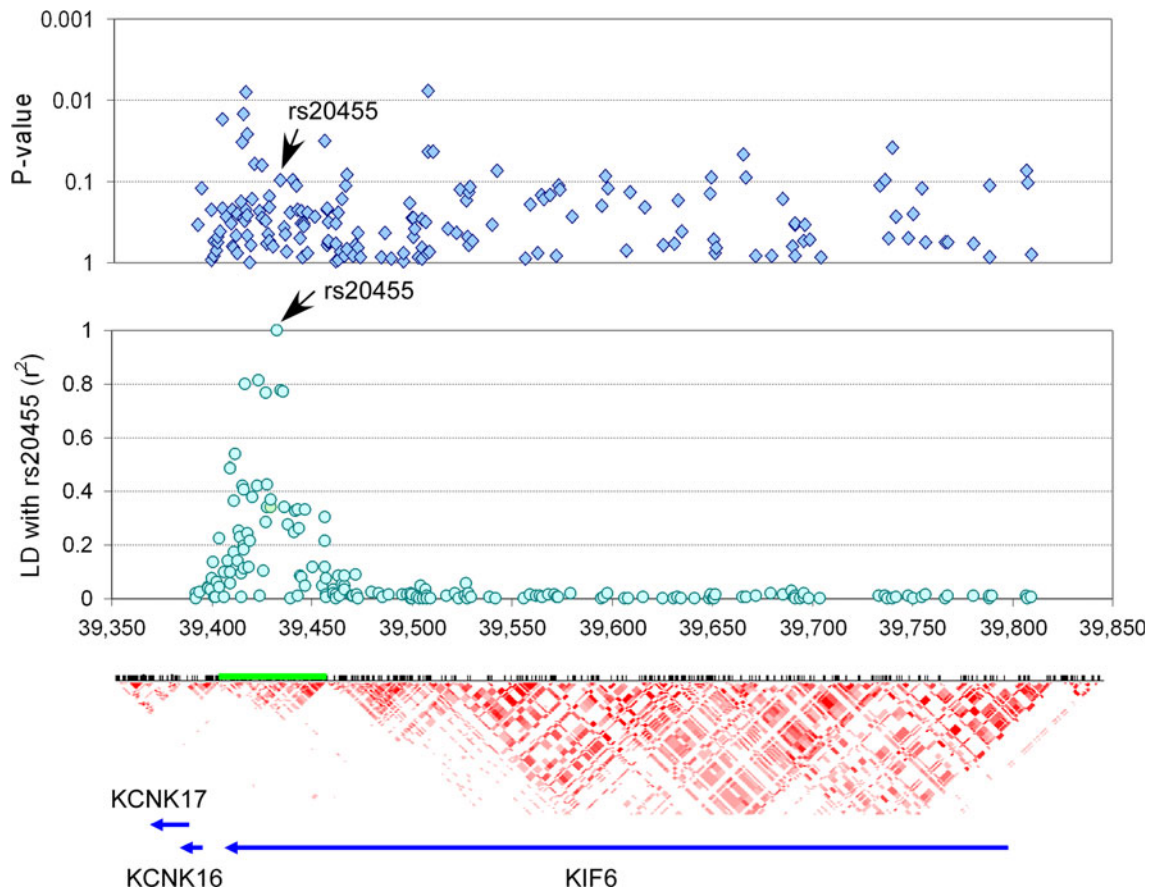
Methods

Study population

The populations in this genetic study were derived from three prospective double blind, randomized clinical trials that evaluated the effect of statin therapy on coronary events: CARE, PROVE IT-TIMI22, and WOSCOPS. CARE evaluated the effect of 40 mg/day pravastatin, compared with placebo, on the reduction of fatal CHD and nonfatal myocardial infarction (MI) in men and women with a prior MI and LDL-C levels between 115 and 174 mg/dL (average age of 59 years old); WOSCOPS evaluated the effect of 40 mg/day pravastatin, compared with placebo, on the reduction of fatal and nonfatal CHD in men with LDL-C levels between 174 and 232 mg/dL (age ranged from 45 to 64 years old) and no history of MI; PROVE IT-TIMI 22 evaluated the effect of intensive statin therapy (80 mg/day atorvastatin), compared with moderate statin therapy (40 mg/day pravastatin), on fatal and nonfatal cardiovascular events in men and women after acute coronary syndromes (average age of 58 years old). The average follow-up period was 5, 2 and 4.9 years for the CARE,

PROVE IT-TIMI 22 and WOSCOPS trials, respectively. The current genetic analyses were performed in those patients whose DNA was available: 2,913 in CARE, 1,817 in PROVE IT-TIMI22, and 1,570 in WOSCOPS; note that for WOSCOPS we obtained access to a previously described nested case–control study that included all cases, who had CHD events and age- and smoking status-matched controls who did not have events during the WOSCOPS trial (Packard et al. 2000). This study was limited to Caucasian patients to avoid complications arising from potential differences in linkage disequilibrium (LD) structure in different populations and because each trial enrolled only about 10% or fewer non-Caucasian patients. Characteristics of the patients included in this genetic study can be found in Supplementary Table 1.

The endpoints were either the primary endpoint of the original clinical trials (for CARE and PROVE IT-TIMI22) or that of the previously reported nested case–control study of WOSCOPS (Packard et al. 2000). For CARE, the endpoint was fatal coronary event or nonfatal MI (Shiffman et al. 2010); for PROVE IT-TIMI22, the endpoint was death from any cause or major cardiovascular events including MI, revascularization, unstable angina requiring hospitalization and stroke (Cannon et al. 2004); and for WOSCOPS, the endpoint was CHD death, nonfatal MI, and revascularization (Shepherd et al. 1995). Genotypes were determined in a core facility by either allele-specific real-time PCR (Germer et al. 2000) or multiplexed PCR of genomic DNA followed by multiplexed allele detection (Iannone et al. 2000). Genotyping was carried out in plates with randomly arrayed samples, and genotype calls were made without the knowledge of the sample event status.

SNP selection

The interrogated region on chromosome 6 spanned from 39,392,028 to 39,809,728 bp, corresponding to the positions of rs1535500 and rs215497. This region includes the entire *KIF6* gene plus 8.6 kbp upstream and 18.8 kbp downstream of the *KIF6* gene (Fig. 1). A total of 178 SNPs in this *KIF6* region were genotyped in CARE, including rs20455 and 26 other SNPs whose association with risk of CHD had been investigated previously (Iakoubova et al. 2008b). Of the 178 SNPs included in this study, the genotype distributions of five SNPs did not meet Hardy–Weinberg expectations ($P < 0.001$) and three SNPs had an allele frequency less than 2%; the remaining 170 SNPs were analyzed for association with differential coronary event reduction from statin therapy. All 450 SNPs in the *KIF6* region in the HapMap dataset (de Bakker et al. 2005) with an allele frequency greater than 2% were either included in

Fig. 1 Linkage disequilibrium and association with statin therapy in CARE. Gene and SNP locations and SNP to SNP LD in the HapMap dataset (in D′ ranging from 1 in *red* to 0 in *white*) in the *KIF6* region are shown in the *lower part* of the figure. The LD block containing rs20455 is indicated with a *green bar*. LD between rs20455 and other genotyped SNPs in CARE is shown in the *middle part* of the figure. Nucleotide position on the chromosome is indicated below the *x* axis in kilobases. Association between SNP and event reduction in CARE (dominant model $P_{\text{interaction}}$) is presented in the *top panel*

these 170 SNPs or in LD ($r^2 > 0.8$) with one of the 170 SNPs.

One objective of the current study was to determine the presence of a SNP in the *KIF6* region that could explain the association of *KIF6* Trp719Arg SNP with differential reduction of coronary events from statin. Therefore, to avoid missing such a hypothetical 'causative' SNP that, by chance, had a weak association in one of the studies we set a relaxed threshold ($P_{\text{interaction}} < 0.1$) for advancing SNPs from one study to the next. Therefore, the flow of the study was as following: SNPs were first tested in CARE, and those that were associated with differential reduction of events from statin therapy ($P_{\text{interaction}} < 0.1$) were then analyzed in PROVE IT-TIMI22. SNPs that were associated with statin response in PROVE IT-TIMI22 ($P_{\text{interaction}} < 0.1$ and hazard ratio in the same direction as in CARE) were further analyzed in WOSCOPS. We adopted this sequence of analysis partly because only a limited amount of DNA was available from patients in the WOSCOPS study.

Statistical analyses

Genotype distribution in the individual studies was tested for deviation from Hardy–Weinberg equilibrium (HWE) by an exact test.

For each SNP, likelihood ratio tests comparing Cox (CARE and PROVE IT-TIMI22) or logistic regression models (WOSCOPS) with and without an interaction term between genotype and statin therapy were used to evaluate heterogeneity (measured by $P_{\text{interaction}}$) of event reduction from statin therapy. Additive, dominant, and recessive models for each SNP were coded using the major allele (defined in CARE) as the reference allele so that the direction of estimated parameters would be consistently measured across all three studies.

The LD measure r^2 was calculated from unphased data with the LDMAX program in the GOLD package (Abecasis and Cookson 2000). Haplotype frequencies were estimated from the genotype data and, using the posterior probabilities of possible haplotype pairs for each subject,

tested for association with heterogeneity of event reduction from statin therapy using logistic regression as implemented in the R package haplo.stats (Lake et al. 2003; Schaid et al. 2002). A sliding window was used to select haplotypes consisting of three or five adjacent SNPs.

Results

To fine map the *KIF6* region, we analyzed 170 SNPs to determine whether reduction of fatal CHD or confirmed nonfatal MI events from statin therapy in CARE differed between genotype-defined subgroups as assessed by the interaction between genotype and treatment group ($P_{interaction}$, see "Methods"). We found that 44 SNPs met the $P_{interaction}$ threshold of <0.1 (Supplementary Tables 2, 3 and 4). Of the 17 SNPs associated with differential reduction of events from statin therapy in the recessive model, 16 SNPs were excluded from further analysis, because for each of these SNPs, fewer than 4% of the patients were minor homozygotes and there were very few events (<10) among the minor homozygotes. The remaining 28 SNPs (including rs20455) associated with differential reduction of events from statin therapy included 12 SNPs that were in LD with rs20455 and

clustered in a 53 kbp region (Fig. 1, indicated by a green bar above the LD map).

We then analyzed these 28 SNPs in the PROVE IT-TIMI22 cohort using the genetic model for each SNP that was most significant in the CARE analysis. Six SNPs were associated with differential event reduction from statin therapy ($P_{interaction}$ < 0.1) in PROVE IT-TIMI22, and event reduction from statin therapy was greatest in the same genotype for all six SNPs: 2 in the additive model and 4 in the dominant model (Supplementary Tables 5 and 6). Further analyses of these six SNPs in the WOSCOPS study showed that three SNPs (rs20455, rs9462535 and rs9471077) were differentially associated with coronary event reduction by statin use ($P_{interaction}$ < 0.1, dominant model; Table 1; Fig. 2). These three SNPs were in high LD with each other (r^2 = 0.84–0.98) (Supplementary Table 7).

We next carried out haplotype analyses in CARE in the 53 kbp region of high LD surrounding rs20455 (green bar in Fig. 1) using a sliding window approach (Schaid et al. 2002). Results of haplotypes comprising three SNPs along with the results of the most significant individual SNP in a given haplotype are shown in Supplemental Fig. 1. In CARE, only the haplotypes based on rs45460596, rs9394585 and rs9357303 were more significantly associated with event reduction than the best of the three SNPs

Table 1 Association of the three SNPs in the *KIF6* region with differential reduction of coronary events from statin therapy in CARE, PROVE IT-TIMI 22 and WOSCOPS studies
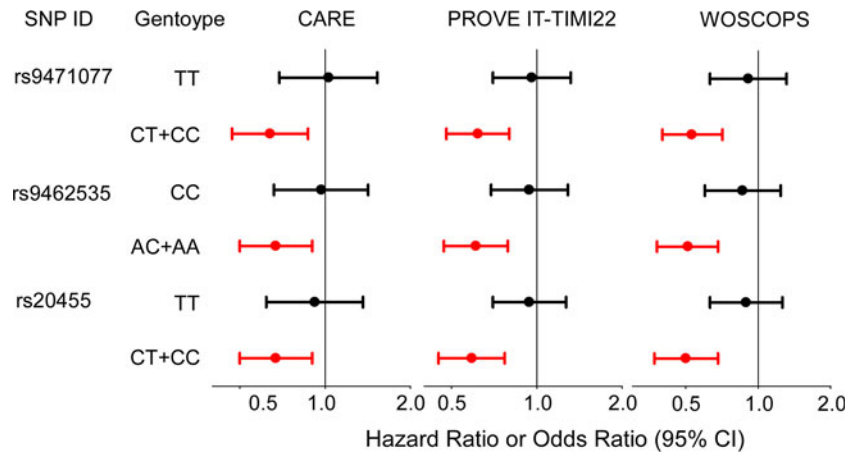
SNP type	Study	Genotype	Drug* Events	Total	Comparator* Events	Total	Model 1** HR or OR (95% CI)	$P_{response}$	$P_{interaction}$	Model 2*** HR or OR (95% CI)	$P_{response}$	$P_{interaction}$
rs20455 Trp719Arg	CARE	CT + CC	76	810	111	791	0.65 (0.49–0.88)	0.0045	0.0975	0.67 (0.50–0.90)	0.0074	0.1708
		TT	52	554	51	542	0.98 (0.67–1.45)	0.9241		0.93 (0.63–1.38)	0.6749	
	PROVE IT-TIMI22	CT + CC	90	533	142	532	0.60 (0.46–0.78)	0.0001	0.0206	0.62 (0.48–0.80)	0.0003	0.0292
		TT	87	375	91	375	0.94 (0.70–1.26)	0.6802		0.96 (0.70–1.32)	0.8226	
	WOSCOPS	CT + CC	108	330	172	263	0.50 (0.37–0.67)	<0.0001	0.0063	0.50 (0.37–0.68)	<0.0001	0.0116
		TT	81	213	104	256	0.94 (0.66–1.32)	0.7055		0.89 (0.63–1.26)	0.5126	
rs9462535 Intronic	CARE	AC + AA	78	873	110	832	0.66 (0.50–0.89)	0.0056	0.0634	0.67 (0.50–0.90)	0.0084	0.1171
		CC	55	535	53	538	1.04 (0.71–1.52)	0.8333		0.99(0.67–1.44)	0.9383	
	PROVE IT-TIMI22	AC + AA	97	568	149	558	0.61 (0.47–0.79)	0.0001	0.0258	0.61 (0.47–0.79)	0.0002	0.0273
		CC	80	340	85	349	0.95 (0.70–1.30)	0.7603		0.95 (0.69–1.29)	0.7327	
	WOSCOPS	AC + CC	113	335	184	279	0.51 (0.39–0.68)	<0.0001	0.0144	0.51 (0.38–0.68)	<0.0001	0.0188
		CC	75	197	96	228	0.90 (0.63–1.29)	0.5805		0.87 (0.60–1.25)	0.4538	
rs9471077 Intronic	CARE	CT + CC	70	813	106	789	0.63 (0.47–0.85)	0.0026	0.0258	0.64 (0.47–0.87)	0.0042	0.0517
		TT	52	488	47	488	1.10 (0.74–1.64)	0.6309		1.04 (0.70–1.55)	0.8401	
	PROVE IT-TIMI22	CT + CC	99	569	149	560	0.62 (0.48–0.80)	0.0002	0.0320	0.59 (0.45–0.77)	0.0001	0.0816
		TT	78	335	83	344	0.96 (0.70–1.31)	0.7984		0.94 (0.70–1.28)	0.7081	
	WOSCOPS	CT + CC	113	340	175	284	0.54 (0.41–0.72)	<0.0001	0.0146	0.54 (0.40–0.72)	<0.0001	0.0178
		TT	76	196	94	231	0.95 (0.67–1.36)	0.7914		0.92 (0.64–1.32)	0.6365	

* Drug was Pravastatin in CARE and WOSCPS and Atorvastatin in PROVE IT-TIMI22; Comparator was placebo in CARE and WOSCOPS and pravastatin in PROVE IT-TIMI22

** Model 1 for CARE and PROVE IT-TIMI22 is adjusted for age and sex; WOSCOPS is unadjusted

*** Model 2 is adjusted for age, smoking, hypertension, diabetes, high-density cholestrol (HDL-C) level, and non-HDL-C level; additional adjustments include sex and body mas index for CARE, sex and treatment for gatifloxacin for PROVE IT-TIMI22

Fig. 2 Effect of statin therapy on coronary events stratified by the genotype of three SNPs in CARE, PROVE IT-TIMI22 and WOSCOPS studies. The hazard ratios or odds ratios were calculated for Model 2 in Table 1

Table 2 Association of the three SNPs in the *KIF6* region and coronary events in the placebo groups of CARE and WOSCOPS

SNP	Genotype	Study	Model 1*		Model 2**	
			HR or OR (95% CI)	P	HR or OR (95% CI)	P
rs20455	CT + CC versus TT	CARE	1.54 (1.10–2.14)	0.0112	1.48 (1.06–2.06)	0.0215
		WOSCOPS	1.61 (1.19–2.17)	0.0017	1.55 (1.15–2.10)	0.0045
rs9462535	AC + AA versus CC	CARE	1.38 (1.00–1.92)	0.0530	1.32 (0.95–1.84)	0.0944
		WOSCOPS	1.57 (1.16–2.12)	0.0036	1.53 (1.12–2.08)	0.0069
rs9471077	CT + CC versus TT	CARE	1.44 (1.02–2.03)	0.0376	1.38 (0.98–1.95)	0.0678
		WOSCOPS	1.51 (1.12–2.05)	0.0077	1.46 (1.07–1.99)	0.0160

* Model 1 for CARE is adjusted for age and sex; WOSCOPS is unadjusted

** Model 2 is adjusted for age, smoking, hypertension, diabetes, body mass index, high density cholesterol (HDL-C) level, and non-HDL-C level; CARE is also adjusted for sex

alone (dominant $P_{interaction}$ = 0.0088 vs. 0.097); however, in PROVE IT-TIMI22 these haplotypes were not associated with event reduction (dominant haplotype $P_{interaction}$ = 0.55). Testing of haplotypes consisting of five adjacent SNPs did not reveal any haplotype that was more significant than the corresponding individual SNPs (data not shown).

We also carried out a haplotype analysis in CARE of the three SNPs (rs9471077, rs9462535, and rs20455) that were associated with event reduction in all three studies. Two common (>5%) haplotypes were observed, which corresponded to *KIF6* 710Arg carriers and non-carriers (data not shown). Due to the high LD between these three SNPs, we were unable to differentiate the effects of one SNP from another despite exploring both pairwise adjustment and stepwise regression analyses. For example, there were only six events each in placebo and statin-treated subjects among the combinations where the genotypes are discordant (data not shown).

Since rs20455 had also been reported to be associated with CHD, we analyzed association of the two other SNPs (rs9462535 and rs9471077) that were consistently associated with event reduction in CARE and WOSCOPS. We found that they were also associated with CHD (Table 2).

Discussion

This study was designed to investigate whether (1) the previously reported association of rs20455 (Trp719Arg) with coronary event reduction from statin therapy could be better explained by another SNP in the *KIF6* region and (2) other SNPs in the *KIF6* region were associated with coronary event reduction from statin therapy independently of rs20455. We found that two additional SNPs in strong LD with rs20455 were consistently associated ($P_{interaction}$ < 0.1) with differential reduction of clinical events from statin therapy in the CARE, WOSCOPS, and PROVE IT-TIMI22 studies. These two SNPs are located in introns, while rs20455 is a missense polymorphism in the *KIF6* gene.

Previously, we have tested 27 SNPs, including rs20455, in a 95.5 kbp portion of the *KIF6* region (from position 39,347,330 to position 39,442,863 on chromosome 6) for association with CHD in the placebo arms of the CARE and WOSCOPS studies (Iakoubova et al. 2008b). This study expands the interrogated region and focuses on differential event reduction from statin therapy, an effect of particular clinical interest. The three SNPs that we found to be associated with differential response to statin therapy are highly correlated and thus indistinguishable from each

other in this association. However, the possibility that the 719Trp versus 719Arg variation has functional consequences is supported by the BLOSUM62 score of −3 for this variation. The BLOSUM62 score is the log odds for amino acid substitutions in conserved protein blocks (Henikoff and Henikoff 1992), and a score of −3 indicates a less likely substitution. As the Trp719Arg substitution is near the putative cargo-binding tail domain of the kinesin, it would be interesting to determine whether the amino acid change affects the binding of cargo molecules by the KIF6 protein.

One limitation of our study is that we were unable to define the causal variant with certainty. Strategies to identify causal variants include performing genetic association studies in a different population that might have a distinct LD structure for these tightly linked SNPs from the initial studies. The CARE and PROVE IT-TIMI22 cohorts had small numbers of non-Caucasian participants; therefore, the power to conduct an association study in the non-Caucasian population is limited. Another limitation is that PROVE IT-TIMI22 may not be comparable to CARE and WOSCOPS—the former tested intensive statin therapy compared with moderate statin therapy, while the latter tested 40 mg/day pravastatin compared with placebo. Therefore, by including PROVE IT-TIMI22, we may have missed the SNPs that are capable of predicting event reduction response to pravastatin compared with placebo but not intensive statin therapy compared with moderate statin therapy. Such SNPs may be independent from rs20455, which predicts differential event reduction from both 40 mg/day pravastatin compared with placebo and intensive statin therapy compared with moderate statin therapy. In addition, PROVE IT-TIMI22 primary endpoint included a small fraction (5.2% of primary events) of death from presumably non-cardiovascular events, while the CARE endpoint and the WOSCOPS case definition did not include non-cardiovascular death.

In conclusion, we have identified three highly linked SNPs in the *KIF6* region that predict differential reduction of coronary events from statin therapy. Functional characterization of these variants is warranted and could help to explain the role of *KIF6* in the pathogenesis of CHD and the variable event reduction in response to statin therapy.

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