Polymorphism in KIF6 Gene and Benefit From Statins After Acute Coronary Syndromes

Results From the PROVE IT-TIMI 22 Study

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Objectives

We explored whether the benefit of intensive versus moderate statin therapy would be greater in carriers of KIF6 719Arg than in noncarriers.

Background

The 719Arg variant of Trp719Arg (rs20455), a polymorphism in kinesin-like protein 6, is associated with greater risk of coronary events and greater benefit from pravastatin versus placebo.

Methods

We genotyped 1,778 acute coronary syndrome patients within the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction 22) trial and investigated different intensities of statin therapy in carriers of 719Arg and in noncarriers using Cox proportional hazards models that adjusted for traditional risk factors.

Results

Benefit from intensive, compared with moderate, statin therapy was significantly greater in the 59% of the cohort who were carriers (hazard ratio [HR] 0.59, 95% confidence interval [CI] 0.45 to 0.77) than in those who were noncarriers (HR 0.94, 95% CI 0.70 to 1.27; p = 0.018 for interaction between 719Arg carrier status and treatment). Absolute risk reduction was 10.0% in carriers versus 0.8% in noncarriers. The benefit of intensive therapy in carriers was significant as early as day 30 of therapy. Carriers and noncarriers did not differ in on-treatment low-density lipoprotein cholesterol, triglyceride, or C-reactive protein (CRP) levels.

Conclusions

Carriers of 719Arg receive significantly greater benefit from intensive statin therapy than do noncarriers, a superior benefit that appears to be due to a mechanism distinct from lipid or CRP lowering. Functional studies of the KIF6 kinesin are warranted, given the consistent association of Trp719Arg with risk of coronary events and statin benefit. (J Am Coll Cardiol 2008;51:449–55) © 2008 by the American College of Cardiology Foundation

Differential risk of disease and differential response to treatment due to the genetic diversity of patient populations has been reported for a number of diseases and treatments (1–3). If the genetic variants responsible for these differences were known, such variants could facilitate personalized medicine by identifying those at elevated risk of disease, those likely to benefit from a particular therapy, or those less likely to benefit (4,5).

To that end, we have previously reported that the Trp719Arg polymorphism in kinesin-like protein 6 (encoded by KIF6) is associated with a greater risk of coronary events in the placebo arms of the CARE (Cholesterol and Recurrent Events) and WOSCOPS (West of Scotland Coronary Prevention Study) trials. The KIF6 kinesin is a member of a family of molecular motors involved in intracellular transport of protein complexes, membrane organelles, and messenger ribonucleic acid along microtubules (6,7). Carriers of the 719Arg allele of the KIF6 kinesin had a 50% increased risk of events compared with noncarriers (8). The 719Arg allele has also been associated with risk of coronary heart disease (CHD) in the ARIC (Atherosclerosis Risk in Communities) study, a large prospective, population-based study of over 15,000 middle-aged Americans with long-term follow-up (9), and in the WHS
which has been described previously (14). Briefly, the trial study was derived from the PROVE IT-TIMI 22 trial, The study population for this genetic study comprised 4,162 patients who had been hospitalized for Myocardial Infarction 22) trial (14). In this trial, intensive therapy with high-dose atorvastatin (80 mg/day), compared with standard-dose pravastatin (40 mg/day), resulted in greater protection against death or cardiovascular events than did moderate therapy with standard-dose pravastatin (40 mg/day, the same dose used in the CARE and WOSCOPS trials) in patients with a recent acute coronary syndrome.

Methods

Study population. The study population for this genetic study was derived from the PROVE IT-TIMI 22 trial, which has been described previously (14). Briefly, the trial comprised 4,162 patients who had been hospitalized for an acute coronary syndrome within 10 days preceding enrollment and who were enrolled while in stable condition and after any planned revascularization. Patients were enrolled at 349 centers in 8 countries between November 2000 and December 2001 and randomized to high-dose atorvastatin (80 mg/day) or standard-dose pravastatin (40 mg/day) and to gatifloxacin or placebo using a double-blind, 2 × 2 factorial design. The 2,061 patients who provided institutional review board–approved informed consent for genetic analysis were eligible for inclusion in this genetic study (all of these patients were enrolled in North America). We excluded 32 of these patients because of inadequate quantity or quality of deoxyribonucleic acid and 39 owing to missing values for one or more of the traditional risk factors that were required for multivariate analysis. Because only 10.3% of the patients were nonwhite, which did not provide sufficient power for a separate analysis of ethnic groups, we included only the white patients in this genetic analysis. After these exclusions, 1,778 patients remained in the genetic cohort of the PROVE IT-TIMI 22 trial.

End point and laboratory values. The end point for this genetic study was the same as the primary end point of the original PROVE IT-TIMI 22 study (i.e., death from any cause or major cardiovascular events), which included myocardial infarction, documented unstable angina requiring hospitalization, revascularization with either percutaneous coronary intervention or coronary artery bypass grafting (if these procedures were performed at least 30 days after randomization), and stroke. All laboratory measurements were made in a core facility (14). Low-density lipoprotein cholesterol (LDL-C) and triglyceride levels were measured at baseline and at the following scheduled visits: 30 days, 4 months, 8 months, 16 months, and the end of follow-up; follow-up lasted 18 to 36 months (mean 24 months). C-reactive protein (CRP) levels were measured at baseline and at the following scheduled visits: 30 days, 4 months, and the end of follow-up. KIF6 Trp719Arg genotypes were determined using an allele-specific real-time polymerase chain reaction genotyping assay at a core facility (15). The Trp719Arg genotypes determined with this assay have been shown to be >99.8% concordant with genotypes determined using a different genotyping technology (16). The primer sequences are available upon request.

Statistical analyses. All reported p values are 2-sided. Differences between baseline characteristics were assessed by the Wilcoxon rank-sum test (continuous variables) or by Fisher exact test (discrete variables). We assessed deviation from Hardy-Weinberg equilibrium using an exact test (17). A chi-square test was used to test for differences in genotype frequencies across studies. Cox proportional hazards models were used to assess the effect of intensive therapy, compared with moderate therapy, on incident events in KIF6 719Arg carriers and noncarriers. To estimate hazard ratios (HRs) adjusted for the effects of other risk factors and study design variables, baseline covariates were included in the Cox models and were coded either continuously (age, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglyceride, and CRP levels) or dichotomously (gender, smoking [current vs. never or past], hypertension [defined as history of hypertension, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg], diabetes, and treatment with gatifloxacin). After estimating the benefit of 80 mg atorvastatin, compared with 40 mg pravastatin, separately in carriers and noncarriers, the p value for interaction between KIF6 719Arg carrier status and statin treatment was determined from likelihood ratio tests that evaluated potential heterogeneity of the HRs. The Kaplan-Meier method was used to estimate the cumulative incidence of death or major cardiovascular events, and differences were assessed with the log-rank test at 2 years of follow-up. Absolute risk reduction was estimated from the differences...
in the cumulative incidence of events at 2 years of follow-up. SAS version 9 software (SAS Institute, Cary, North Carolina) was used for all regression models and for generating Kaplan-Meier estimates.

**Results**

**Baseline characteristics and allele frequencies.** The baseline characteristics for traditional risk factors of this genetic cohort of the PROVE IT-TIMI 22 trial according to KIF6 Trp719Arg carrier status are presented in Table 1. The prevalence of risk factors for CHD was similar in carriers and noncarriers of the 719Arg variant, with the exception that current smoking was less frequent among 719Arg carriers (p = 0.042). The treatment groups in this genetic cohort also were well balanced with regard to baseline traditional risk factors (p > 0.29; data not presented). Carriers of the 719Arg variant constituted 58.9% of the PROVE IT-TIMI 22 trial genetic cohort. The genotype frequencies were 13.5%, 45.4%, and 41.1% for Arg/Arg, Arg/Trp, and Trp/Trp, respectively, and the distribution of these genotypes did not deviate from Hardy-Weinberg expectations (p = 0.51). These genotype frequencies were not significantly different (p = 0.32) than the frequencies reported for the CARE trial cohort and the control group in a nested case-control study of the WOSCOPS trial (8).

**Outcome of therapy and KIF6 Trp719Arg.** Carriers and noncarriers of KIF6 719Arg received significantly different benefit from high-dose atorvastatin therapy compared with standard-dose pravastatin therapy. Specifically, in carriers of the 719Arg allele, high-dose atorvastatin therapy, compared with standard-dose pravastatin therapy, reduced the risk of death or major cardiovascular events by 41% (adjusted HR 0.59, 95% confidence interval [CI] 0.45 to 0.77; p < 0.001) (Table 2). The HRs were similar in Arg/Arg homozygotes and in Arg/Trp heterozygotes, which is consistent with a dominant genetic model. In contrast, high-dose atorvastatin, compared with standard-dose pravastatin, was of no significant benefit in noncarriers (adjusted HR 0.94, 95% CI 0.70 to 1.27; p = 0.70) (Table 2). This difference in benefit between carriers and noncarriers was significant (p = 0.018 for interaction between KIF6 719Arg carrier status and treatment) (Table 2).

In terms of absolute event rates, among carriers of the KIF6 719Arg variant the Kaplan-Meier estimates of the cumulative incidence of death or major cardiovascular events at 2 years was 26.0% in the standard-dose pravastatin group and 16.1% in the high-dose atorvastatin group (log-rank p < 0.001) (Fig. 1A), an absolute risk reduction of 10.0% (95% CI 4.9% to 15.0%) favoring the intensive-therapy regimen. However, among noncarriers, the cumulative in-

**Table 1 Baseline Characteristics of the Patients According to KIF6 Trp719Arg Genotypes and 719Arg Carrier Status**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ArgArg Genotype (n = 240)</th>
<th>Arg/Trp Genotype (n = 808)</th>
<th>Arg Variant Noncarriers, Trp/Trp Genotype (n = 730)</th>
<th>Arg Variant Carriers, Arg/Arg + Arg/Trp Genotype (n = 1,048)</th>
<th>p Value†</th>
<th>p Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>58.1 ± 10.9</td>
<td>57.3 ± 11.0</td>
<td>57.7 ± 11.0</td>
<td>57.5 ± 11.0</td>
<td>0.53</td>
<td>0.54</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>188 (78.3)</td>
<td>636 (79.0)</td>
<td>564 (77.3)</td>
<td>826 (78.8)</td>
<td>0.72</td>
<td>0.45</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.4 ± 6.2</td>
<td>30.1 ± 5.7</td>
<td>30.1 ± 5.8</td>
<td>30.1 ± 5.9</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td>0.09</td>
<td>0.042</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>87 (36.3)</td>
<td>291 (36.0)</td>
<td>306 (41.9)</td>
<td>378 (36.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>251 (34.4)</td>
<td>307 (38.0)</td>
<td>251 (34.4)</td>
<td>389 (37.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>173 (23.7)</td>
<td>210 (26.0)</td>
<td>173 (23.7)</td>
<td>281 (26.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>41 (17.1)</td>
<td>150 (18.6)</td>
<td>113 (15.5)</td>
<td>191 (18.2)</td>
<td>0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>Hypertension, n (%)*</td>
<td>138 (57.5)</td>
<td>452 (55.9)</td>
<td>418 (57.3)</td>
<td>590 (56.3)</td>
<td>0.84</td>
<td>0.70</td>
</tr>
<tr>
<td>Prior statin therapy, n (%)</td>
<td>60 (25.0)</td>
<td>209 (25.9)</td>
<td>183 (25.1)</td>
<td>269 (25.7)</td>
<td>0.93</td>
<td>0.78</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>187</td>
<td>181</td>
<td>184</td>
<td>183</td>
<td>0.65</td>
<td>0.80</td>
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<tr>
<td>Median</td>
<td>163–208</td>
<td>162–207</td>
<td>161–208</td>
<td>162–207</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mg/dl</td>
<td>108</td>
<td>106</td>
<td>108</td>
<td>107</td>
<td>0.79</td>
<td>0.67</td>
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<tr>
<td>Median</td>
<td>89–129</td>
<td>89–130</td>
<td>89–129</td>
<td>89–130</td>
<td></td>
<td></td>
</tr>
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<td>High-density lipoprotein cholesterol, mg/dl</td>
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<td>38</td>
<td>38</td>
<td>0.82</td>
<td>0.74</td>
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<tr>
<td>Median</td>
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<td>33–45</td>
<td>33–45</td>
<td>33–45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>160</td>
<td>163</td>
<td>164</td>
<td>162</td>
<td>0.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Hypertension was defined as the history of hypertension, systolic blood pressure > 140 mm Hg, or diastolic blood pressure > 90 mm Hg. †Associations between baseline characteristics and KIF6 Trp719Arg genotypes were tested with the Kruskal-Wallis test for continuous variables and the chi-square test for discrete variables. ‡Associations between baseline characteristics and KIF6 719Arg carrier status were tested with the Wilcoxon rank sum test for continuous variables and Fisher exact test for discrete variables.

BMI = body mass index; IQR = interquartile range.
In this genetic study of the PROVE IT-TIMI 22 study, a clinical trial that enrolled patients with a recent acute coronary syndrome, we found that intensive statin therapy (80 mg atorvastatin per day, the highest approved dose of this potent statin), compared with standard statin therapy (40 mg pravastatin per day), was significantly more efficacious in reducing death and major cardiovascular events in the 59% of the patients who were carriers of the KIF6 719Arg variant than in the remaining 41% of the population who were noncarriers (relative risk reduction 41% vs. 6%, respectively). In the face of equal lipid lowering in carriers and in noncarriers, absolute risk reductions were 10.0% in carriers and 0.8% in noncarriers after 2 years of treatment. Moreover, in carriers of 719Arg, the substantial and significant benefit from intensive therapy, compared with moderate therapy, was evident as early as 30 days after randomization and remained significant throughout the trial: the unadjusted HR at 30 days was 0.18 (95% CI 0.04 to 0.81) (Fig. 2). In contrast to the early benefit seen in carriers, in noncarriers there was no significant benefit of intensive therapy, compared with moderate therapy, at any time point (Fig. 2).

Because LDL-C cholesterol, CRP, and triglyceride levels are risk factors for cardiovascular events and can be reduced by intensive statin therapy (18,19), we explored whether the changes in the levels of these risk factors in response to treatment with either high-dose atorvastatin or standard-dose pravastatin differed between carriers and noncarriers of the KIF6 719Arg allele. We found no evidence in either the high-dose atorvastatin group or in the standard-dose pravastatin group that these risk factors differed between carriers and noncarriers at any time during therapy. Specifically, in both treatment groups, no significant differences in median LDL-C, CRP, or triglyceride levels were found between carriers and noncarriers at baseline or at any scheduled visit during the study (p ≤ 0.3; data not presented).

**KIF6 719Arg and risk of events.** Because a previous genetic study of the CARE and WOSCOPS trials had shown that carriers of the KIF6 719Arg variant were at increased risk of CHD compared with noncarriers in placebo-treated patients and that standard-dose pravastatin treatment ameliorated the increased risk associated with the 719Arg variant (8,9), we investigated the association between 719Arg allele and risk in the PROVE IT-TIMI 22 trial. As observed in the CARE and WOSCOPS trials, we found that in the standard-dose pravastatin–treated patients carriers of the KIF6 719Arg allele were not at increased risk of death or major coronary events compared with noncarriers (adjusted HR 1.12, 95% CI 0.85 to 1.45; p = 0.45). In the high-dose atorvastatin group, carriers were actually at decreased risk (adjusted HR 0.65, 95% CI 0.48 to 0.88; p = 0.005).

**Discussion**

In this genetic study of the PROVE IT-TIMI 22 study, a clinical trial that enrolled patients with a recent acute coronary syndrome, we found that intensive statin therapy (80 mg atorvastatin per day, the highest approved dose of this potent statin), compared with standard statin therapy (40 mg pravastatin per day), was significantly more efficacious in reducing death and major cardiovascular events in the 59% of the patients who were carriers of the KIF6 719Arg variant than in the remaining 41% of the population who were noncarriers (relative risk reduction 41% vs. 6%, respectively). In the face of equal lipid lowering in carriers and in noncarriers, absolute risk reductions were 10.0% in carriers and 0.8% in noncarriers after 2 years of treatment. Thus, only 10 carriers of the KIF6 719Arg variant needed to be treated with intensive statin therapy to prevent 1 event; in contrast, the number needed to treat for noncarriers was 125.

Our findings in the PROVE IT-TIMI 22 trial are consistent with and extend observations made in 2 other distinct clinical trial populations: CARE and WOSCOPS (8). The KIF6 719Arg variant has now been investigated in genetic studies of 3 trials that included over 6,200 patients: 2 studies of 40 mg pravastatin (a lipophilic statin) per day versus placebo (8) and the present study of 80 mg atorvastatin (a hydrophilic statin) per day versus 40 mg pravastatin per day. In all 3 of these genetic studies, the efficacy of the more intensive lipid-lowering therapy in reducing coronary events was greater in carriers (37% to 50% relative risk reductions) than in noncarriers (6% to 20% risk reductions) (Fig. 3).
Among carriers of KIF6 719Arg the superiority of intensive, compared with standard, statin therapy was substantial and significant as early as 30 days after the start of the treatment; however, in noncarriers there was no significant benefit from intensive therapy at any point during the study. This early superiority of intensive therapy in carriers may be due to an early plaque-stabilizing effect of the intensive-treatment regimen, a pleiotropic effect that has been proposed to explain the early benefit from statin therapy that appears not to be due to LDL lowering (20,21). A pleiotropic mechanism would be consistent with our observation that on-treatment median LDL-C levels did not differ between carriers and noncarriers. Additionally, this early benefit from intensive statin therapy in carriers of KIF6 719Arg variant is likely to be distinct from anti-inflammatory mechanisms related to the reduction of CRP levels, because carriers and noncarriers did not differ in median CRP levels at baseline or during the trial.

The KIF6 719Arg variant was associated with an increased risk of coronary events in the ARIC study, a large population-based cohort of over 15,000 subjects (9), in the WHS, a large population-based cohort of over 25,000 women (10), and in the placebo groups of the WOSCOPS and CARE trials (8). However, statin therapy appears to ameliorate this risk: carriers of the 719Arg variant who were treated with standard-dose pravastatin were not at increased risk compared with noncarriers in the CARE and WOSCOPS trials (8). And similarly, in the PROVE IT-TIMI 22 trial, carriers of 719Arg who were treated with standard-dose pravastatin were not at increased risk compared with noncarriers who were treated with standard-dose pravastatin. A hypothesis that could explain these observations is that, for patients on statin therapy, the risk of coronary events depends on the balance between the 2 effects associated with the 719Arg variant: increased risk of coronary events and enhanced benefit from statin therapy. Thus, among patients on placebo, carriers of 719Arg were at 50% greater risk compared with noncarriers. However, among patients on standard-dose pravastatin, carriers and noncarriers were at similar levels of risk, because the increased risk associated with the 719Arg allele was balanced, or offset, by the increased benefit from statin therapy associated with the 719Arg allele. Finally, among patients on the more potent high-dose atorvastatin regimen, carriers, with their enhanced benefit from statin therapy, were actually at decreased risk of coronary events compared with noncarriers.

In the KIF6 719Arg variant, a basic arginine residue replaces a nonpolar tryptophan residue. Because this amino acid substitution is in a predicted coiled-coil region of the KIF6 protein, the change might affect the cargo binding of the kinesin (22). Several kinesins have been implicated in the pathogenesis of chronic diseases, including neurodegenerative diseases, type 2 diabetes, and Alzheimer's disease (23). We believe that functional studies that explore the mechanism by which the KIF6 kinesin and the 719Arg variant affect cardiovascular disease are now warranted, given the consistency of the association in multiple prospective cohorts. Such studies are particularly important to help explain why the benefit from atorvastatin (80 mg), compared with pravastatin (40 mg) differed between carriers and noncarriers despite similar on-therapy plasma LDL-C and CRP levels.

Study limitations. This genetic study was not a part of the study protocol for the original PROVE IT-TIMI 22 trial and included only the white patients who were predominately middle-aged men; therefore, the effect of the intensive statin therapy in carriers and noncarriers of the KIF6 719Arg variant should be further investigated in additional ethnic groups, women, and older patients after acute coronary syndromes. Although genetic studies of the PROVE IT-TIMI 22, WOSCOPS, and CARE trials (comprising...
about 6,000 patients) showed that carriers of the KIF6 Trp719Arg allele receive significant clinical benefit from atorvastatin (80 mg) compared with pravastatin (40 mg), and from pravastatin (40 mg) compared with placebo (8), whereas noncarriers do not, these observations should be confirmed in other larger studies. In addition, although 2 different statins were studied, our findings may not extend to other statins. It is also not clear whether differential benefit between carriers and noncarriers from intensive statin therapy compared with standard treatment is restricted only to benefit from high-dose atorvastatin compared with standard-dose pravastatin or can be extrapolated to different doses of the same statin. This question could be addressed by conducting a genetic study of KIF6 Trp719Arg polymorphism in clinical trials that compare different doses of the same statin.

Conclusions

Carriers of the 719Arg allele are at increased risk of fatal or nonfatal coronary events when not treated with statins (8–10), and they clearly benefit from statin therapy, whether it be standard-dose pravastatin compared with placebo (8) or high-dose atorvastatin therapy compared with standard-dose pravastatin. In contrast, noncarriers not only were at lower risk than carriers in the ARIC and WHS trials and in the placebo groups of the CARE and WOSCOPS trials, but noncarriers also received less benefit from statin treatment than did carriers in the CARE, WOSCOPS, and PROVE IT-TIMI 22 trials. The absence of significant benefit from intensive statin therapy in noncarriers does not preclude the possibility that a portion of noncarriers do benefit from statin therapy, but it does suggest that noncarriers may be treated with standard statin therapy and also with other lipid-modifying drugs or by strategies that target other risk factors such as hypertension, diabetes, or smok-
The biological explanations for the observed difference in treatment benefit should be provided by functional studies of this kinesin motor protein, studies which could also offer important additional insights into the biology of lipid metabolism and acute coronary syndromes that could, in turn, generate novel targets for therapeutic intervention and lead to better-tailored therapy for patients.

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**REFERENCES**