Genetic and biochemical risks factors in coronary artery disease
Boekholdt, S.M.

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Variants of the toll-like receptor 4 modify the efficacy of statin therapy and the risk of cardiovascular events

S. Matthijs Boekholdt, Willem R.P. Agema, Ron J.G. Zwinderman, Ernst E. van der Wall, Pieter H. Reitsma, J. Wouter Jukema

Departments of Cardiology, Vascular Medicine, and Clinical Epidemiology and Biostatistics, and Laboratory for Experimental Internal Medicine, Academic Medical Center, Amsterdam, Department of Cardiology, Leiden University Medical Center, Leiden, Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands

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Abstract

Background
Atherosclerosis is increasingly considered to be a chronic inflammatory process. We examined whether genetic variants of the toll-like receptor 4 (TLR4), which are correlated with impaired innate immunity and with progression of carotid atherosclerosis, are also associated with coronary atherosclerosis and predict the risk of cardiovascular events.

Methods and results
Two polymorphisms of the TLR4 gene (Asp299Gly and Thr399Ile) were determined in 655 men with angiographically documented coronary atherosclerosis. All patients participated in a prospective cholesterol-lowering trial evaluating the effect on coronary artery disease (CAD), and were randomly assigned to either pravastatin or placebo for two years. There were no significant differences between genetically defined subgroups with respect to baseline risk factors, treatment, or in-trial changes of lipid, lipoprotein, or angiographic measurements. Genotype was not associated with progression of atherosclerosis. In the pravastatin group, 299Gly carriers had a lower risk of cardiovascular events during follow-up, than non-carriers (2.0% versus 11.5%, p=0.045). Among non-carriers, pravastatin reduced the risk of cardiovascular events from 18.1% to 11.5% (p=0.03), while among 299Gly carriers this risk was strikingly reduced from 29.6% to 2.0% (p=0.0002, p=0.025 for interaction).

Conclusion
Among symptomatic men with documented CAD, the TLR4 Asp299Gly polymorphism was associated with the risk of cardiovascular events. This variant also modified the efficacy of pravastatin in preventing cardiovascular events, such that carriers of the variant allele had significantly more benefit from pravastatin treatment.
Background
Atherosclerosis is increasingly considered to be a chronic inflammatory or even infectious disease. In a longitudinal study, subjects with chronic infections had a significantly higher risk of developing carotid atherosclerosis than subjects without chronic infections, and the antibody response to multiple micro-organisms is an independent risk factor for the presence and severity of coronary artery disease (CAD). Chlamydia pneumoniae has been receiving particularly much attention as a potential risk factor in different stages of atherogenesis. C. pneumoniae has been detected in atherosclerotic lesions, and the distribution of C. pneumoniae co-localizes with atherosclerosis within an individual. Gram-negative micro-organisms activate the immune system via lipopolysaccharide (LPS) which, in combination with CD14, serves as ligand to the toll-like receptor 4 (TLR4).

The presence of LPS in the circulation is not confined to sepsis, but also occurs in healthy subjects, and is associated with advanced progression of early atherosclerosis. TLR4 is expressed on cardiomyocytes, macrophages, endothelial and smooth muscle cells, and importantly, activation of this receptor results in the release of anti-microbial peptides and cytokines that initiate innate and adaptive immunity. Recently, Arbour and colleagues discovered two single nucleotide polymorphisms in the TLR4 gene, that result in amino acid substitutions in the extracellular domain of the receptor, with functional consequences. These variants, Asp299Gly and Thr399Ile, lead to a blunted immunological response to inhaled LPS, and to lower levels of proinflammatory cytokines, acute-phase reactants, and soluble adhesion molecules. Lastly and most strikingly, they seem associated with reduced extent and progression of carotid atherosclerosis as quantified by B-mode ultrasound.

Inflammation may not only play a role in the progression of early atherosclerosis, but may also be important in advanced atherosclerosis by determining the stability of atherosclerotic plaques and their proneness to rupture. Compared with lesions causing stable angina, the plaques of patients with acute coronary syndromes contain considerably more inflammatory cells. In particular, the immediate vicinity of the site of plaque rupture is invariably infiltrated by an inflammatory process. Furthermore, C-reactive protein (CRP), a marker of inflammation, has been identified as an independent predictor of mortality and cardiovascular events in patients with stable and unstable angina, high-risk individuals and apparently healthy individuals. Statin therapy is most effective in patients with elevated CRP levels, which underscores the hypothesis that statins, beside lipid lowering effects, have plaque stabilizing and anti-inflammatory effects.

We hypothesized that the TLR4 Asp299Gly and the Thr399Ile polymorphisms would be associated, firstly with progression of coronary atherosclerosis as documented by quantitative coronary angiography, and secondly with the risk of cardiovascular events by affecting plaque stability.
Finally, we hypothesized that carriers of the variant allele would respond differently to statin therapy than non-carriers. We tested these hypotheses in a group of patients with symptomatic CAD who were included in the REGRESS study.

**Methods**

**Study Design**

The REGRESS study and design have been described previously. Briefly, REGRESS was a randomized, placebo-controlled multicenter study designed to assess the effect of two years of treatment with pravastatin 40 mg on progression and regression of angiographically documented coronary atherosclerosis in 885 male patients with a normal to moderately raised serum cholesterol, i.e. between 4 and 8 mmol/L (155 to 310 mg/dL), and triglycerides <4.0 mmol/L (354 mg/dL). Patients were randomized to receive pravastatin 40 mg once daily or matching placebo. Patients and physicians were blinded to the randomization throughout the study. A number of substudies were performed in addition to the main angiographic study, including specialized lipid and lipoprotein and genetic studies.

**Clinical outcome measures**

Coronary angiograms were analyzed quantitatively by the Cardiovascular Measurement System (CMS, MEDIS Medical Imaging Systems, Nuenen, the Netherlands). The quantitative coronary arteriographic procedures have been described in detail previously. Primary end points were (a) the change in average minimal obstruction diameter (MOD) per patient and (b) the change in average mean segment diameter (MSD) per patient. Change in MOD mainly reflects focal progression-regression of atherosclerosis, and change in MSD mainly reflects diffuse progression-regression of atherosclerosis. If a segment or lesion was adequately visualized in two (preferably orthogonal) projections and free of significant foreshortening in both views, the average values of the parameters in both projections were calculated. To calculate average MOD and MSD per patient, the MOD and MSD of all qualifying segments or obstructions were added and divided by the number of contributing segments or obstructions. The following clinical events (according to prespecified criteria) were analyzed during the study and identified before unblinding: myocardial infarction (fatal or nonfatal); coronary heart disease death (other than known fatal myocardial infarction); non-scheduled PTCA or CABG; stroke and transient ischemic attack, and death (all other causes).

**Biochemical and DNA analyses**

Total cholesterol, high-density lipoprotein cholesterol (HDL), and triglycerides were measured on fasting blood samples at the Lipid Reference Laboratory, as published previously. Low-density lipoprotein cholesterol (LDL) was calculated according to the Friedewald formula. Genomic DNA was extracted according to a standard protocol. PCR amplification was
Table 1. Baseline characteristics by toll-like receptor 4 genotype

<table>
<thead>
<tr>
<th></th>
<th>299Asp/Asp</th>
<th>299Gly-positive</th>
<th>399Ile-positive</th>
<th>399Ile-negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>577</td>
<td>69</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>56 ± 8</td>
<td>56 ± 8</td>
<td>57 ± 9</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26 ± 3</td>
<td>26 ± 3</td>
<td>25 ± 2</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>135 ± 18</td>
<td>136 ± 20</td>
<td>130 ± 12</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>82 ± 10</td>
<td>80 ± 10</td>
<td>78 ± 8</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Current or former smoker</td>
<td>509 (88)</td>
<td>58 (84)</td>
<td>7 (78)</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Current smoker</td>
<td>166 (29)</td>
<td>14 (20)</td>
<td>2 (22)</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.0 ± 0.9</td>
<td>6.0 ± 0.7</td>
<td>5.9 ± 0.9</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>4.3 ± 0.8</td>
<td>4.3 ± 0.6</td>
<td>4.3 ± 0.9</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.67 (0.49-4.03)</td>
<td>1.70 (0.46-3.92)</td>
<td>1.10 (0.63-2.32)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>279 (48)</td>
<td>28 (41)</td>
<td>5 (56)</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>70 ± 12</td>
<td>71 ± 14</td>
<td>63 ± 15</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Mean segment diameter</td>
<td>2.80 ± 0.48</td>
<td>2.87 ± 0.41</td>
<td>2.66 ± 0.63</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Minimal obstruction diameter</td>
<td>1.89 ± 0.55</td>
<td>1.91 ± 0.39</td>
<td>1.91 ± 0.51</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Stenosis</td>
<td>35 ± 13</td>
<td>33 ± 8</td>
<td>33 ± 8</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1 vessel</td>
<td>249 (43)</td>
<td>32 (46)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2 vessels</td>
<td>195 (34)</td>
<td>22 (32)</td>
<td>7 (78)</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>- 3 vessels</td>
<td>131 (23)</td>
<td>15 (22)</td>
<td>2 (22)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as mean (±SD) or as number (%). The statistical analysis for triglyceride levels was performed on log-transformed values, but untransformed median (minimum-maximum) values are given in the table.

performed on 1 µl DNA in 10 µl ReddyMix™ (ABgene, Epsom, UK) according to methods previously described. Researchers and laboratory personnel had no access to identifiable information, and could identify samples by number only.

Statistical analysis

Within each of the treatment groups, the assumption of Hardy-Weinberg equilibrium was tested by means of gene counting and chi-square analysis. Patients were classified according to genotype combination (normal TLR4; 299Gly-carriership in the presence of 399Ile-carriership; or 299Gly-carriership in the absence of 399Ile-carriership). These groups were compared with respect to relevant baseline characteristics, lipid and lipoprotein concentrations, in-trial changes of lipid and lipoprotein concentrations, and angiographic parameters. Differences in baseline parameters were analyzed with the independent samples t-test, one-way analysis of variance or the Pearson’s chi-square test where appropriate.
Table 2. In-trial changes of lipids, lipoproteins and angiographic measurements by toll-like receptor 4 genotype

|                      | Placebo |                  | Pravastatin |                  |       |  |       |  |       |  |       |  |
|----------------------|---------|------------------|-------------|------------------|-------|  |-------|  |-------|  |-------|  |
|                      | 299Asp  | 299Gly-positive  | 299Gly-positive | 399Ile-positive | 399Ile-negative | 299Asp | 299Gly-positive | 299Gly-positive | 399Ile-positive | 399Ile-negative | P* | P† |
| Subjects             | 299     | 24               | 3           |                  |       |  | 278   | 45    | 6     |       |  |     |
| Lipids and lipoproteins |        |                  |             |                  |       |  |       |       |       |  |     |
| TC, mmol/l           | 0.13 ± 0.64 | 0.24 ± 0.93     | 0.63 ± 0.37 | 0.3              |       | -1.15 ± 0.75 | -1.24 ± 0.63 | -1.60 ± 1.05 | 0.1 | 0.06 |
| LDL-c, mmol/l        | 0.04 ± 0.58 | 0.10 ± 0.69     | 0.49 ± 0.20 | 0.4              |       | -1.19 ± 0.69 | -1.22 ± 0.58 | -1.82 ± 0.78 | 0.1 | 0.1  |
| HDL-c, mmol/l        | 0.04 ± 0.13 | 0.04 ± 0.13     | 0.12 ± 0.04 | 0.7              |       | 0.11 ± 0.16  | 0.12 ± 0.14  | -0.04 ± 0.10 | 0.1 | 0.2  |
| Triglycerides, mmol/l| 0.14 ± 0.62 | 0.21 ± 0.78     | 0.23 ± 0.64 | 0.8              |       | -0.14 ± 0.66 | -0.30 ± 0.69 | -0.23 ± 0.94 | 0.5 | 0.5  |
| Angiography           |          |                  |             |                  |       |  |       |       |       |  |     |
| MSD, mm              | -0.09 ± 0.20 | -0.09 ± 0.18    | 0.03 ± 0.17 | 0.8              |       | -0.07 ± 0.19 | -0.04 ± 0.19 | 0.02 ± 0.02 | 0.5 | 1    |
| MOD, mm              | -0.10 ± 0.21 | -0.09 ± 0.19    | 0.05 ± 0.20 | 0.6              |       | -0.09 ± 0.36 | -0.10 ± 0.36 | 0.02 ± 0.11 | 0.9 | 0.9  |

Data are given as in-trial change, calculated as value at follow-up minus value at baseline. Data are shown as mean (±SD). A larger reduction in mean segment diameter and minimal obstruction diameter indicates more progression of atherosclerosis. * P-value for analysis of covariance of the changes between the three genetic groups, with baseline values as the covariate. † P-value for interaction between genotype and treatment by analysis of covariance, with baseline values as covariate. MSD refers to mean segment diameter, MNOD refers to minimal obstruction diameter.
Since triglyceride concentrations had a skewed distribution, the statistical analyses were based on log-transformed data. Changes in lipid concentrations, lipoprotein concentrations, and angiographic measurements during the trial were compared with one-way analysis of covariance, with baseline values as covariates. The interaction between genotype and treatment (placebo or pravastatin) was tested with the interaction test of two-way analysis of covariance, with genotype and treatment as fixed factors and baseline values as covariates. Differences in the rate of events were illustrated with Kaplan-Meier curves, and compared using a logrank test. We further used the Cox regression model to test for an interaction between the TLR4 genotype and treatment (pravastatin or placebo).

Results

Frequency of the TLR4 Asp299Gly and Thr399Ile polymorphisms
Of the 885 patients enrolled in REGRESS, DNA was available from 670 individuals. Of these, 655 could be genotyped for both the Asp299Gly and the Thr399Ile polymorphisms. These 655 individuals did not differ significantly in any baseline parameter from the original 885 patients. In the entire cohort, 78 individuals carried the 299Gly variant allele, and 69 carried the 399Ile variant allele. The three common genotype combinations (normal TLR4; 299Gly-carriership in the presence of 399Ile-carriership; and 299Gly-carriership in the absence of 399Ile-carriership) were found at frequencies of 0.88, 0.11 and 0.014, respectively. These frequencies did not differ significantly between the two treatment groups (data not shown). For the placebo group, the pravastatin group, and the total cohort, the observed allele frequencies were in Hardy-Weinberg equilibrium.

Baseline characteristics
When patients were classified according to TLR-4 genotype combination, there were no statistically significant differences with respect to CAD risk factors, or treatment (table 1). The individuals with isolated 299Gly-carriership had more severe CAD than individuals with other genotypes, as evidenced by a higher proportion of individuals with two-vessel disease. However, there were only 9 individuals with this genotype, so the data must be interpreted with caution. In both groups, approximately half of the patients were randomly assigned to pravastatin treatment.

In-trial changes of lipid concentrations and angiographic parameters
The TLR4 genotype did not affect in-trial changes of total cholesterol, LDL cholesterol, triglycerides, or HDL cholesterol (table 2). Pravastatin affected total cholesterol, LDL, HDL, and triglycerides to a similar extent in all genetic subgroups. Changes in angiographic measurements were also not significantly affected by the Asp299Gly polymorphism, with similar effects of pravastatin treatment in the genetic subgroups.
Risk of cardiovascular events
Carriership of the 299Gly allele did not significantly affect the risk of cardiovascular events in the entire cohort, when compared with non-carriers (11.5% versus 14.9%, p=0.58) (table3). However, in the pravastatin group carriers of the variant 299Gly-allele had a significantly lower risk of cardiovascular events than non-carriers (2.0% versus 11.5%, p=0.045). Pravastatin reduced the risk of cardiovascular events in the entire cohort by 50% (19.0% versus 10.0%, p=0.0007). Strikingly, among non-carriers, the risk of cardiovascular events was reduced from 18.1% to 11.5% (p=0.03), while among 299Gly carriers the risk was reduced significantly more from 29.6% to 2.0% (p=0.0002)(figure 1). Testing for interaction between the Asp299Gly genotype and treatment group revealed that the efficacy of pravastatin in reducing the time to first cardiovascular event was significantly different between the genetic groups (p=0.025). Subdivision of the group of 299Gly-carriers according to carriership of the 399Ile variant allele resulted in genotype groups that were too small to detect significant differences and interactions. The least common genotype combination (299Gly-carriership in the absence of 399Ile-carriership) occurred only 9 times (3 on placebo, 6 on pravastatin). Although the prevalence of cardiovascular events appeared similar in the 399-positive and 399-negative group, no solid conclusions can be made.

Discussion
We examined whether the Asp299Gly and Thr399Ile polymorphisms of the TLR4 gene influenced the progression of coronary atherosclerosis and the risk of cardiovascular events in a large cohort of men with symptomatic CAD. Our results revealed an important interaction between these genetic variants and pravastatin treatment on the risk of cardiovascular events. In particular, the efficacy of pravastatin treatment in preventing cardiovascular events was significantly higher in 299Gly carriers than in non-carriers. This observation extends on previous reports in which we attempted to identify genetic factors that affect the clinical presentation of the patients and their response to pravastatin therapy.25-27

Frequency of the TLR4 Asp299Gly and Thr399Ile polymorphisms
Genotyping of study participants revealed that the allele frequency of the 299Gly variant was 5.9% in the REGRESS cohort. In the Bruneck study, the allele frequency was 3.5%,12 and in three other populations they were reported to be 6.6%, 7.9%, and 3.3%.12 These allele frequencies are all within the expected range. In addition, the frequencies of the three common genotype combinations (normal TLR4; 299Gly-carriership in the presence of 399Ile-carriership; and 299Gly-carriership in the absence of 399Ile-carriership) were similar to those described in previous reports. Thus, the genotype combinations that were associated with decreased progression of carotid atherosclerosis (299Gly-carriership with and without 399Ile-carriership), do not occur at a substantially lower frequency in the REGRESS
Table 3. Incidence of cardiovascular events by toll-like receptor 4 genotype and treatment

<table>
<thead>
<tr>
<th></th>
<th>299AspAsp</th>
<th>299Gly-positive</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>18.1 (54/299)</td>
<td>29.6 (8/27)</td>
<td>19.0 (62/326)</td>
<td>0.10*</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>11.5 (32/278)</td>
<td>2.0 (1/51)</td>
<td>10.0 (33/329)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Total</td>
<td>14.9 (86/577)</td>
<td>11.5 (9/78)</td>
<td></td>
<td>0.6*</td>
</tr>
<tr>
<td>P</td>
<td>0.03†</td>
<td>0.0002†</td>
<td>0.0007†</td>
<td>0.025**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>399Ile-positive</th>
<th>399Ile-negative</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>18.1 (54/299)</td>
<td>29.1 (7/24)</td>
<td>19.0 (62/326)</td>
<td>0.3*</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>11.5 (32/278)</td>
<td>2.2 (1/45)</td>
<td>10.0 (33/329)</td>
<td>0.1*</td>
</tr>
<tr>
<td>Total</td>
<td>14.9 (86/577)</td>
<td>11.5 (8/69)</td>
<td></td>
<td>0.6*</td>
</tr>
<tr>
<td>P</td>
<td>0.03†</td>
<td>0.0007†</td>
<td>0.2†</td>
<td>0.1**</td>
</tr>
</tbody>
</table>

Data are given as incidence of cardiovascular events during 2-year follow-up in % (n/N). * P-value for logrank test between the genetic groups. † P-value for logrank test between the treatment groups. ** P-value for interaction between genotype and treatment by Cox regression model.

cohort of men with symptomatic CAD, than in random population samples.

Risk of cardiovascular events
Among individuals randomized to placebo, carriers of the 299Gly-allele were at a non-significantly higher risk of cardiovascular events, compared to non-carriers (hazard ratio 1.84, 95%CI 0.8-3.87). In contrast, among those randomized to pravastatin treatment, 299Gly carriers were at a non-significantly lower risk of events (hazard ratio 0.16, 95%CI 0.02-1.20, p for interaction=0.025). In the Bruneck study, 299Gly carriers were at a non-significantly lower risk for cardiovascular disease (hazard ratio 0.16, 95%CI 0.02-1.24, p=0.08) but data on use of statin therapy are not reported. It has been suggested that the Asp299Gly-mediated loss of TLR4 function is in turn influenced by the polymorphism at residue 399. These reports indicate that 299Gly-carriership in the presence of 399Ile-carriership results in intermediate TLR4 function, while isolated 299Gly-carriership yields the worst TLR4 functionality. Whether or not the interaction between TLR4 genotype and pravastatin treatment is also affected by this Thr399Ile polymorphism, cannot be concluded from the present study due to the limited number of individuals with isolated 299Gly-carriership.

Mechanism
The systemic inflammatory response to low-grade infectious stimuli plays a role in the progression of atherosclerosis. The TLR4 Asp299Gly and Thr399Ile polymorphisms blunt this response, thereby reducing generalized arterial wall inflammation and subsequent generalized atherosclerosis. This process is slow and well compensated for by
The graphs represent the cumulative survival free of cardiovascular events calculated by Cox regression, for non-carriers (299AspAsp) treated with placebo, carriers (299Gly-positive) treated with placebo, non-carriers treated with pravastatin, and carriers treated with pravastatin.

outward arterial remodelling which salvages lumen size. This seems an important reason why we did not observe any association between these polymorphisms and changes in MSD and MOD, two parameters of arterial lumen size. In addition, the time span of 2 years may have been too short to detect a small difference.

Blunted inflammatory response leads to ineffective removal of infectious
agents, which may lead to persistence or even progression of the inflammatory trigger. Persistent triggering of the innate immune system may be especially harmful in an atherosclerotic plaque where abundant TLR4 is present to initiate an inflammatory response. It has been hypothesized that such an inflammatory response activates resident cells and macrophages in the atherosclerotic plaque. Systemic LPS administration yielded more proinflammatory cytokine gene expression in the aorta of rabbits with diet-induced atheroma, than in rabbits without atheroma. In addition, the extent of cytokine production was related to the burden of atheroma. Thus, at some point in the natural course of atherosclerosis, the beneficial effect of a blunted immunological response in 299Gly carriers may be outweighed by persistent inflammatory triggering due to ineffective removal of the pro-inflammatory agent. This balance depends on the amount of TLR4 present, and thus on the severity of atherosclerosis. In the REGRESS cohort of men with advanced atherosclerosis, this enhanced inflammatory response may have led to plaque inflammation and instability.

In summary, our observations would be explained by a model in which the extent of plaque inflammation, and thus the risk of plaque rupture, is determined by three factors: (I) the amount of inflammatory trigger that is capable of activating TLR4. This factor may be affected, in turn, by the efficacy of TLR4 in its removal. (II) The amount of TLR4 present in the vessel wall, and (III) the efficacy of TLR4 in mounting a local inflammatory response. This model would explain the striking reduction of cardiovascular events observed in 299Gly carriers using statin therapy, compared with those who did not. Statins are known to reduce LDL cholesterol, and thus oxidized LDL, which is a potent up-regulator of TLR4. Thus, in 299Gly carriers using statin therapy, a persistent inflammatory triggering due to ineffective removal may have been negated by a reduction of TLR4 and a genotype-dependent inefficient initiation of local inflammation.

Limitations
There are several potential limitations to the present study. First, the events defined in REGRESS include a number of different clinical entities, i.e. non-scheduled percutaneous or surgical revascularization, non-fatal myocardial infarction, and fatal myocardial infarction. Nevertheless, the large majority were events in which atherosclerotic plaque rupture and subsequent thrombotic occlusion are the underlying pathophysiological processes. The fact that these plaque rupture-related events led to a range of clinical outcomes may have depended on numerous other factors, and does not negate our findings. Second, the conclusions obviously apply only to symptomatic men with documented CAD. However, this population is representative for the majority of male CAD patients in the Netherlands. Finally, the frequency of isolated 299Gly-carriership (i.e. in the absence of 399Ile-carriership) was low in our cohort, which is consistent with previous
These reports have suggested that the genotype at residue 399 influences the effect of the Asp299Gly polymorphism; 299Gly-carriership in the presence of 399Ile-carriership predicts decreased TLR4 function, but 299Gly-carriership in the absence of 399Ile-carriership yields a TLR4 protein that functions even worse. We did perform analyses to evaluate whether the interaction between Asp299Gly genotype and pravastatin treatment was affected by the Thr399Ile polymorphism as well. However, the results do not allow any solid conclusions due to the low frequency of this genotype combination. An even larger cohort of patients, or one with a higher prevalence of this genotype combination, will be required to address this issue.

Conclusion
We observed that, among symptomatic men with documented CAD, the TLR4 Asp299Gly polymorphism was associated with the risk of cardiovascular events. This genetic variant also predicted the efficacy of pravastatin in preventing cardiovascular events such that carriers of the variant allele had substantially more benefit from pravastatin treatment. A substantial amount of experimental evidence exists regarding the role of inflammation in determining the risk of plaque rupture-related events. However, clinical data are limited. The relevance of our observation lies in the fact that it shows genetic variance in the innate immune system to be associated with the occurrence of plaque rupture-related clinical events. In addition, to our knowledge, this is the first observation of an interaction between genotype and statin treatment in reducing the risk of clinical cardiovascular events without an effect on lipid parameters.

Acknowledgments
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