

A Common Polymorphism in the Complement Factor H Gene Is Associated With Increased Risk of Myocardial Infarction

The Rotterdam Study

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OBJECTIVES	This study was designed to investigate the association between a common polymorphism (Tyr402His, rs1061170) in the complement factor H (CFH) gene and risk of coronary heart disease.
BACKGROUND	The evidence that inflammation is an important mechanism in atherogenesis is growing. C-reactive protein (CRP), complement factors, and complement regulatory factors have all been linked to coronary heart disease. The CFH gene is an important regulator of the alternative complement cascade. We investigated its association with coronary heart disease.
METHODS	The study was embedded in the Rotterdam Study, a prospective population-based study among men and women aged 55 years and over. A total of 5,520 participants without history of coronary heart disease was genotyped for the Tyr402His polymorphism of the CFH gene. Cox proportional hazards analysis was used to determine risk of myocardial infarction for Tyr402His genotypes.
RESULTS	Mean age among participants was 69.5 years (SD 9.1 years). The overall frequency of the His allele was 36%; genotype frequencies were 41%, 45%, and 14% for TyrTyr, TyrHis, and HisHis, respectively. During a mean follow-up period of 8.4 years, 226 myocardial infarctions occurred. After adjustment for age, gender, established cardiovascular risk factors, and CRP level, HisHis homozygotes had a hazard ratio of 1.77 (95% confidence interval 1.23 to 2.55) for myocardial infarction. Total cholesterol level, diabetes mellitus, and smoking modified the effect. The Tyr402His polymorphism was not associated with established cardiovascular risk factors or CRP level.
CONCLUSIONS	Our data suggest that the CFH gene determines susceptibility to myocardial infarction. This finding underscores the importance of the alternative complement system in cardiovascular disease. (J Am Coll Cardiol 2006;47:1568–75) © 2006 by the American College of Cardiology Foundation

Inflammation has been shown to play an important role in cardiovascular disease (1). Both complement factors and complement regulatory factors have been linked to atherosclerosis (2). Complement inhibitor factor H (CFH) is a plasma protein that is essential in the regulation of the alternative complement pathway. Recently, several studies have found that the Tyr402His (rs1061170) polymorphism in the CFH gene is strongly associated with age-related macular degeneration, with relative risks of 2.5 to 7.4 for homozygotes (3–7).

Complement inhibitor factor H has been suggested to play a part in complement inhibition in atherosclerotic lesions (8), and atherosclerosis has been implicated in the development of age-related macular degeneration (9).

Therefore, the association between the CFH gene polymorphism and age-related macular degeneration may at least in part be mediated by atherosclerosis. Research in coronary artery specimens suggests that interaction of CFH with proteoglycans may be the mechanism by which complement activation in the superficial layer of the coronary intima is controlled (8). Consequently, we hypothesize that CFH may play a protective role in the development of coronary heart disease.

Complement inhibitor factor H is encoded by a single gene (HF1) on human chromosome 1q32. Several polymorphisms have been identified in the CFH gene, but their potential influence on the levels of expression or on the function of CFH is uncertain (10). The Tyr402His polymorphism, representing a tyrosine-histidine change at amino acid 402, is particularly interesting because this change is located within the cluster of positively charged amino acids implicated in the binding of heparin and C-reactive protein (CRP) (11). Binding to these factors augments the ability of CFH to down-regulate the effect of complement. The substitution of a positively charged his-

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Abbreviations and Acronyms

- CABG = coronary artery bypass grafting
- CFH = complement inhibitor factor H
- CI = confidence interval
- CRP = C-reactive protein
- GP = general practitioner
- HDL = high-density lipoprotein
- PTCA = percutaneous transluminal coronary angioplasty

tidine for a non-charged hydrophobic tyrosine in position 402 may alter the binding properties and have functional implications (10). These changes may alter CFH's ability to suppress excess complement activation, ultimately leading to complement-related damage to arterial walls and vessel injury (4).

We set out to investigate the association between the CFH gene Tyr402His polymorphism and the risk of myocardial infarction in the Rotterdam Study, a population-based cohort study in men and women age 55 years and over.

METHODS

Study population. The present study is part of the Rotterdam Study, a population-based cohort study aimed at assessing the occurrence of and risk factors for chronic diseases in the elderly. Objectives and methods of the Rotterdam Study have been described in detail elsewhere

(12). The medical ethics committee of Erasmus Medical Center, Rotterdam, approved the study. Participants gave written informed consent and permission to retrieve information from treating physicians.

Figure 1 shows a flow chart describing the study population. The Rotterdam Study cohort includes 7,983 men and women aged 55 years and over (78% of the eligible population), living in a well-defined suburb of the city of Rotterdam, the Netherlands. Baseline data were collected from 1990 until 1993. A trained interviewer visited all subjects at home and collected information using a computerized questionnaire. Additionally, established cardiovascular risk factors were measured at the research center in 7,129 participants. Deoxyribonucleic acid (DNA) was available for 6,571 subjects. Genotyping was successful in 6,345 participants. After excluding participants with a history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass grafting (CABG) at baseline, 5,520 subjects were left for analysis.

Genotyping. The participants were genotyped for the Tyr402His (1277T > C) polymorphism of the CFH gene. This polymorphism has been described under identification number rs1061170 (13).

Deoxyribonucleic acid was extracted with proteinase K and sodium dodecyl sulfate digestion at 37°C overnight and purified with phenol-chloroform extractions. The extracted DNA was then precipitated with NaCl at 4 mol/l and 2 vol of cold absolute ethanol. DNA was solubilized in double-

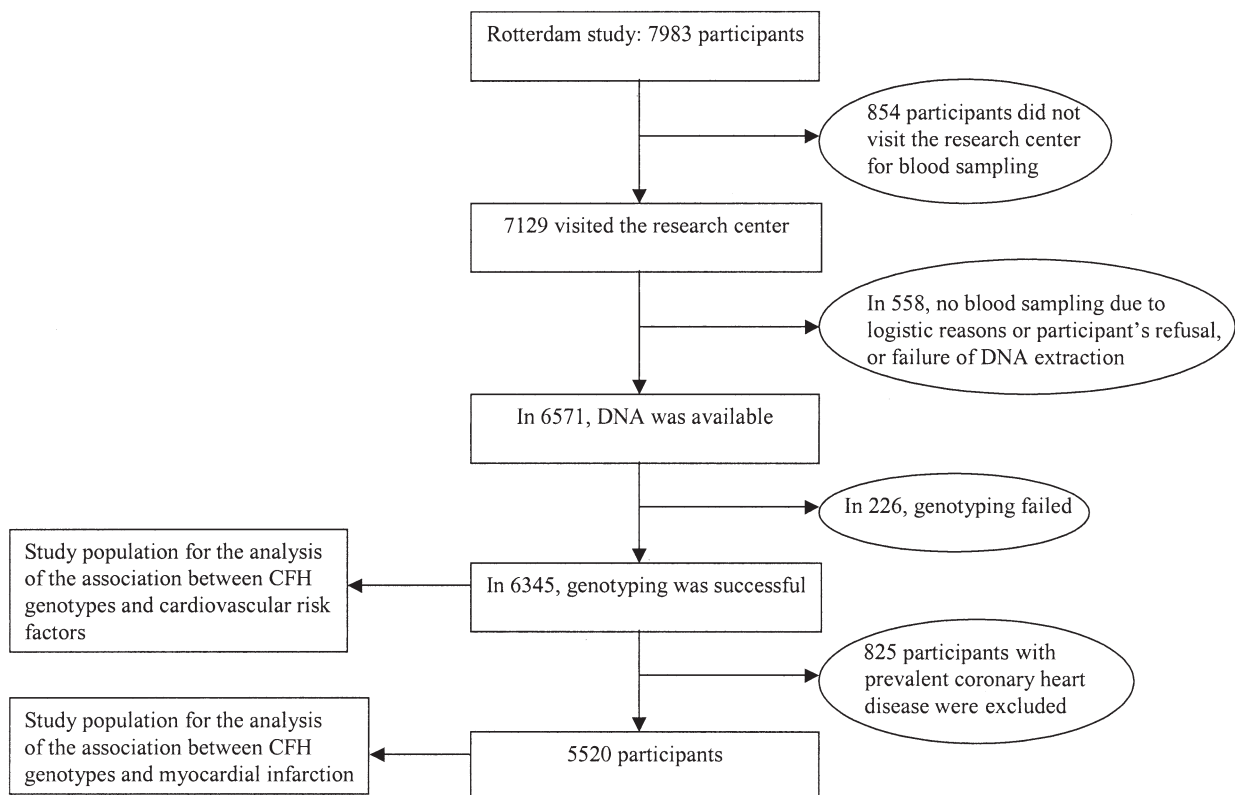


Figure 1. Flow chart describing study population. CFH = complement inhibitor factor H; DNA = deoxyribonucleic acid.

distilled water and stored at -20°C until used for DNA amplification. Genotypes were determined in 2 ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Foster City, California). Primer and probe sequences were optimized by using the SNP assay-by-design service of Applied Biosystems (14). Reactions were performed with the Taqman Prism 7900HT 384 wells format.

Assessment of covariates. The information obtained during the interview included current health status, medical history (including history of PTCA and CABG), drug use, and smoking status. At the research center, height and weight were measured, and the body mass index was calculated (weight [kg]/height [m^2]). Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. Diabetes mellitus was defined as the use of blood glucose lowering medication or a random or post-load serum glucose level ≥ 11.1 mmol/l (15). C-reactive protein was measured in serum, kept frozen at -20°C , using a nephelometric method (Image, Beckman Coulter, Fullerton, California). A 12-lead resting electrocardiogram (ECG) was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz, and stored digitally. All ECGs were processed by the Modular ECG Analysis System (MEANS [16]) to obtain ECG measurements and interpretations. Myocardial infarction found on ECG was based on criteria partly derived from the Minnesota code (17). A history of myocardial infarction was considered present in case of a self-report of myocardial infarction confirmed by ECG or additional clinical information, or the presence of an ECG characteristic of prior myocardial infarction.

Follow-up procedure. Follow-up started at the baseline examination and lasted until January 1, 2002, for the present study. Information on fatal and non-fatal myocardial infarctions for the participants enlisted with the general practitioners (GPs) working in the study district (85% of the cohort) was obtained from these GPs. Computerized records were sent to the Rotterdam Study data center regularly. Subsequently, research assistants gathered information about these events at the GP offices. All medical records of the participants under the care of GPs outside the study area (15% of the cohort) were checked annually for possible events. Letters and, in case of hospitalization, discharge reports from medical specialists were obtained. With respect to the vital status of participants, information was also obtained regularly from the municipal health authorities in Rotterdam. After notification, cause and circumstances of death were established by questionnaire from the GPs.

Subsequently, two research physicians independently coded all reported events according to the International Classification of Diseases-10th edition (ICD-10) (18). In case of disagreement, consensus was reached. Finally, a

medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events.

In identifying incident myocardial infarctions (ICD-10 code I21), all available information, which included ECG, cardiac enzyme levels, and the clinical judgment of the treating specialist, was used. Persons with incident silent myocardial infarctions were not identified.

Data analysis. Hardy-Weinberg equilibrium of the Tyr402His polymorphism was tested using a chi-square test. Differences in established cardiovascular risk factors for the three genotypes were examined by using analysis of covariance, adjusting for age and gender. For age, this analysis was only adjusted for gender, and for gender, this analysis was only adjusted for age. C-reactive protein serum levels were log-transformed because of their skewed distribution.

Cox proportional hazards analysis was used to determine the relative risks of myocardial infarction associated with Tyr402His genotypes. The proportional hazards assumption was tested by drawing log minus log plots of the survival function. In model 1, we adjusted for age and gender; in model 2, we adjusted for age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, smoking, and diabetes mellitus; and in model 3, we adjusted for the covariates in model 2 and additionally for CRP. Age- and gender-adjusted survival curves were drawn showing event-free survival until the occurrence of myocardial infarction. These curves were evaluated at the mean of the covariates (age and gender) in the 5,520 subjects used. For age, this was 69.1 years; for gender, this was 62% women.

First, analyses were performed in all participants. Thereafter, to examine effect modification, subgroup analyses were performed in strata of age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, smoking, diabetes mellitus, and CRP level. For continuous variables, participants were divided into subgroups according to the median values of the variables. Similarly, models 1, 2, and 3, as described above, were used for these subgroup analyses. Interaction terms were tested for the Tyr402His polymorphism and each of the covariates we stratified on, adjusting for age and gender. For this purpose, the polymorphism was entered into the model as a continuous variable with three values, namely homozygotes for the common allele (value 0), heterozygotes (value 1), and homozygotes for the rare allele (value 2).

Values for cardiovascular covariates were missing in $<4\%$ of participants, except for CRP, which was missing in 7%. Missing values were handled by single imputation using the expectation-maximization algorithm based on age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, smoking, diabetes mellitus, and CRP.

All analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Cary, North Carolina).

Table 1. Baseline Characteristics of the Total Population

	Total (n = 6,345)
Age (yrs)	69.5 ± 9.1
Women (%)	60.0
Body mass index (kg/m ²)	26.3 ± 3.7
Systolic blood pressure (mm Hg)	139 ± 22
Diastolic blood pressure (mm Hg)	74 ± 11
Total cholesterol (mmol/l)	6.6 ± 1.2
HDL cholesterol (mmol/l)	1.3 ± 0.4
Diabetes mellitus (%)	10.4
Smokers (%)	
Never	35.5
Current	22.7
Former	41.8
History of hypertension (%)	34.3
C-reactive protein (mg/l)*	1.87 (0.90–3.67)

Categorical variables are expressed as percentage. Values of continuous variables are expressed as mean ± standard deviation for total. *Median and interquartile range because of skewed distribution.

HDL = high-density lipoprotein.

RESULTS

Genotype distributions were in Hardy-Weinberg equilibrium. The overall frequency of the His allele was 36%; genotype frequencies were 41%, 45%, and 14% for TyrTyr, TyrHis, and HisHis, respectively. Table 1 shows baseline characteristics of all participants in which genotyping was successful. None of the studied characteristics, namely age, gender, body mass index, systolic and diastolic blood pressure, hypertension, total and HDL cholesterol, diabetes mellitus, smoking, and CRP, were associated with Tyr402His genotype.

During a mean of 8.4 (SD 2.7) years of follow-up, 226 cases of myocardial infarction occurred among participants who had no history of myocardial infarction, CABG, or PTCA at baseline. Follow-up information on myocardial infarction was complete for 5,237 of 5,520 participants used for the analysis (94.9%). The potential number of person-years that could have been contributed by these 5,520 participants until myocardial infarction, death, or the end of

the follow-up period was 46,516.7. We were able to observe 46,173.5 person-years (99.3%) until the date that a participant was last known to be alive, myocardial infarction, death, or the end of the follow-up period.

In Table 2, hazard ratios for myocardial infarction are displayed according to Tyr402His genotype. In reference to wild type, heterozygotes had a 14% to 16% increased risk of myocardial infarction, but this did not reach statistical significance. After adjustment for age and gender (model 1), HisHis homozygotes had a hazard ratio of 1.72 (95% confidence interval [CI] 1.20 to 2.47) for developing myocardial infarction. After additional adjustment for established cardiovascular risk factors (model 2), the hazard ratio slightly increased to 1.77 (95% CI 1.23 to 2.54). Further adjustment for CRP (model 3) did not change the estimate. When we repeated the analysis without excluding participants with coronary heart disease at baseline, the results did not change materially.

The hazard ratios in men and women are also displayed in Table 2. After full adjustment (model 3), male HisHis homozygotes had a hazard ratio of 1.95 (95% CI 1.21 to 3.13) for developing myocardial infarction, whereas for female HisHis homozygotes this was 1.54 (95% CI 0.87 to 2.73). The latter value may not have reached statistical significance due to lack of power in women.

Event-free survival until the occurrence of myocardial infarction is displayed in Figure 2. Homozygotes for the rare His allele had a significantly lower event-free survival.

Figure 3 displays the effects of potential modifiers of the association between Tyr402His and myocardial infarction in an age- and gender-adjusted subgroup analysis. Overall, risk of myocardial infarction was increased for HisHis homozygotes in all subgroups. The risk of myocardial infarction was considerably higher in participants with total cholesterol levels above the median versus those with levels below the median (p for interaction 0.05), in subjects with diabetes versus subjects without diabetes (p for interaction

Table 2. Hazard Ratios for Myocardial Infarction According to Tyr402His Genotype

Genotype	Events/Subjects	Hazard Ratio (95% Confidence Interval)		
		Model 1	Model 2	Model 3
All				
TyrTyr	81/2,251	1.00 (reference)	1.00 (reference)	1.00 (reference)
TyrHis	99/2,509	1.14 (0.85–1.53)	1.16 (0.86–1.55)	1.16 (0.86–1.55)
HisHis	46/760	1.72 (1.20–2.47)	1.77 (1.23–2.54)	1.77 (1.23–2.55)
Men				
TyrTyr	46/863	1.00 (reference)	1.00 (reference)	1.00 (reference)
TyrHis	58/924	1.22 (0.83–1.79)	1.25 (0.85–1.84)	1.25 (0.84–1.84)
HisHis	28/293	1.82 (1.16–2.96)	1.94 (1.21–3.12)	1.95 (1.21–3.13)
Women				
TyrTyr	35/1,388	1.00 (reference)	1.00 (reference)	1.00 (reference)
TyrHis	41/1,585	1.03 (0.66–1.62)	1.02 (0.65–1.60)	1.02 (0.65–1.61)
HisHis	18/467	1.54 (0.87–2.71)	1.53 (0.86–2.70)	1.54 (0.87–2.73)

Model 1: adjusted for age and gender; model 2: adjusted for age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, smoking, and diabetes mellitus; model 3: adjusted for age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, smoking, diabetes mellitus, and C-reactive protein.

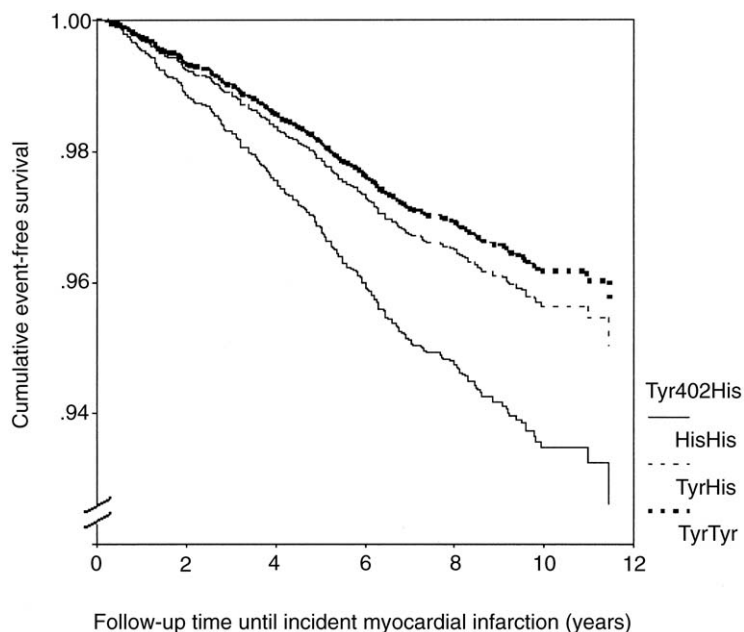


Figure 2. Age- and gender-adjusted event-free survival until incident myocardial infarction.

0.02), and in current smokers versus former and never smokers (p for interaction 0.06). The risk for subjects with above-median CRP levels was somewhat higher, but the interaction did not reach statistical significance (p for interaction 0.31). The remaining age- and gender-adjusted interaction coefficients of the Tyr402His polymorphism and the covariates upon which we stratified were not significant. Subgroup analysis with additional adjustment for age, gender, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, HDL cholesterol, smoking, diabetes mellitus, and CRP did not materially alter the results.

DISCUSSION

In the present study, the CFH gene Tyr402His polymorphism was associated with an increased risk of myocardial infarction in participants without a history of coronary heart disease. Total cholesterol levels, diabetes mellitus, and smoking modified the effect. The CFH gene Tyr402His polymorphism was not associated with established cardiovascular risk factors, or with serum CRP levels. Adjustment for these factors did not essentially alter the risk of myocardial infarction. This study was performed within a large, prospective, population-based cohort, with lengthy follow-up time and a considerable number of incident myocardial infarction cases available for analysis.

Inflammation has been recognized as an important mechanism in coronary heart disease and other manifestations of atherosclerosis (1). The complement system contributes to inflammation in the arterial intima, and thus may exert unfavorable effects on atherosclerosis (2). The complement system contains several plasma and membrane-associated proteins that are organized in three activation pathways: the classical, the lectin, and the alternative pathway. Comple-

ment inhibitor factor H is a plasma protein that plays an important part in the inhibition of the alternative pathway; it restricts the action of complement to activating surfaces by binding to C3b, accelerating the decay of the alternative pathway C3-convertase (C3bBb) and acting as a cofactor for the factor-I-mediated proteolytic inactivation of C3b (10). This mechanism allows for activation of the early complement cascade by opsonization and may thereby play a protective role; however, complement activation is limited to the C3 level, and does not lead to full complement activation with cell lysis and ensuing inflammation (8).

Several studies provide evidence for a regulatory role of CFH in the development of atherosclerosis. Complement inhibitor factor H has been found to be associated with severity of coronary luminal narrowing in patients receiving coronary angiography (19). In a study of human atherosclerotic lesions, CFH was observed in a large proportion of the lesions (20). Experimental observations have raised the possibility that interaction between proteoglycans and CFH, which co-localize in the superficial layer of the intima, may inhibit complement activation in the superficial layer of the arterial intima (8). Furthermore, it has been shown that CFH binds to CRP, which may help to inhibit the CRP-dependent alternative complement activation pathway induced by damaged tissue (21,22). C-reactive protein has been found to be associated with both coronary heart disease and age-related macular degeneration (23,24).

Complement inhibitor factor H is encoded by a single gene (HF1) that is part of the regulator of complement activation gene cluster on human chromosome 1q32 that encodes several regulatory proteins of the complement system. Several polymorphisms have been identified in this gene, but their potential influence on the levels of expression

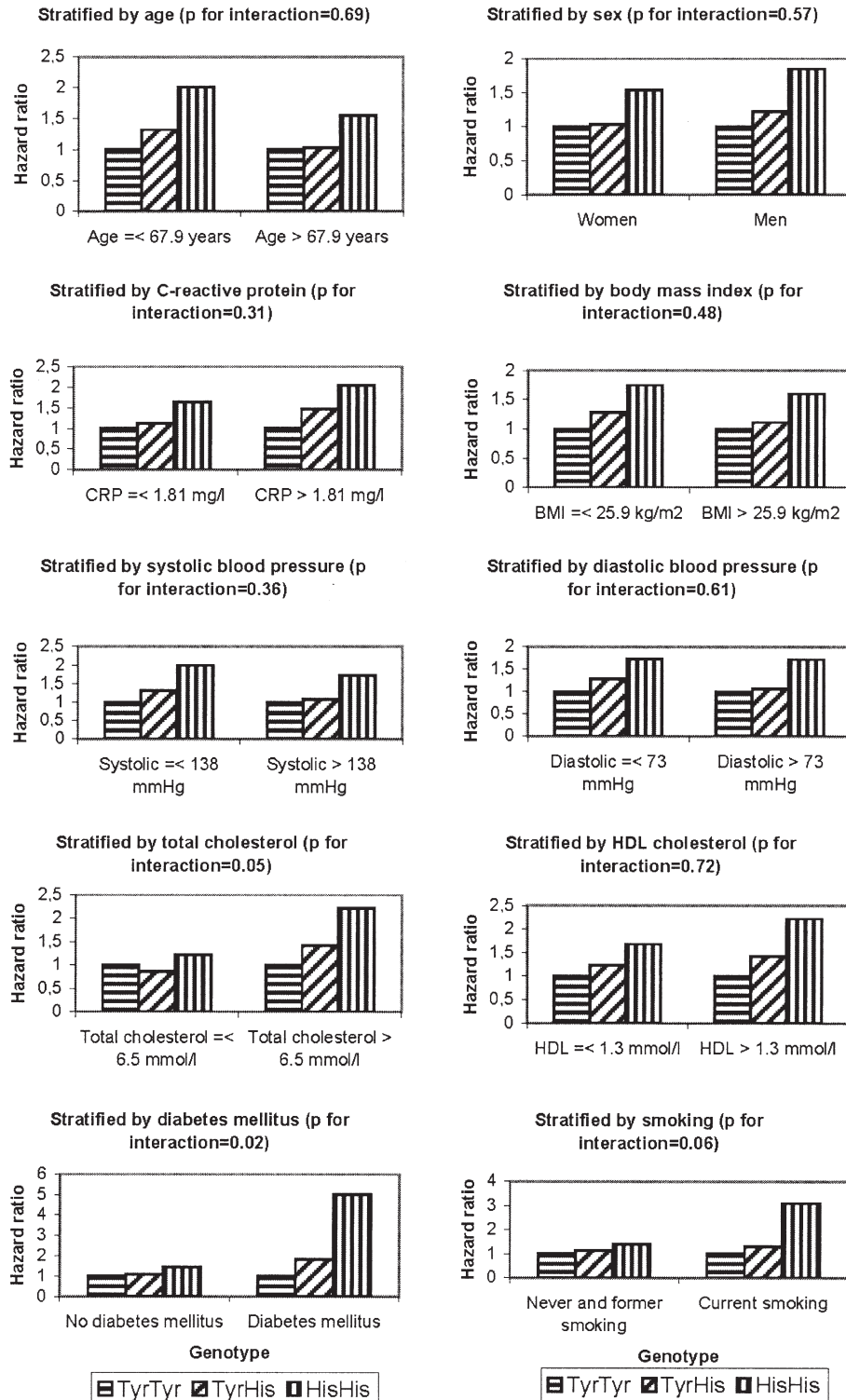


Figure 3. Age- and gender-adjusted hazard ratios for myocardial infarction according to Tyr402His genotype in subgroups, created according to median values for continuous variables. BMI = body mass index; CRP = C-reactive protein; HDL = high-density lipoprotein.

or on the function of CFH is uncertain (10). The Tyr402His polymorphism is located within the cluster of positively charged amino acids implicated in the binding of heparin and CRP. Binding to either of these partners increases the affinity of CFH for the complement protein

C3b (22,25), augmenting its ability to down-regulate complement's effect. The substitution of a positively charged histidine for a non-charged hydrophobic tyrosine in position 402 may alter the binding properties and consequently have functional implications.

Recently, an association of the Tyr402His polymorphism with risk of vascular events could not be demonstrated in a nested case-control study within the Physicians' Health study (26). The authors report that they had the ability to detect, with 80% power, at an alpha of 0.05, a risk ratio of >1.35 for the His variant for vascular events (myocardial infarction, ischemic stroke, deep venous thrombosis/pulmonary embolism, n = 685). Therefore, and also based on the effect estimates and CIs found, as stated by the authors, a modest risk of vascular events associated with the genotypes tested could not be fully excluded.

Several aspects of the present study warrant further consideration. One limitation is that it remains to be investigated whether the Tyr402His variant is the true underlying variant and does not represent a marker in complete or partial linkage disequilibrium, either within the CFH gene itself or in flanking genes. Still, several arguments support the involvement of the CFH gene and in particular the Tyr402His polymorphism in explaining the risk of myocardial infarction. First, the CFH gene is a credible candidate because several studies provide evidence for a regulatory role of CFH in the development of atherosclerosis. Second, the substitution of a positively charged histidine for a non-charged hydrophobic tyrosine theoretically has functional implications for the CFH protein. Finally, haplotype reconstruction has implicated this polymorphism in relation to complement-mediated pathogenesis of age-related macular degeneration (3,7). Despite these arguments, further research is warranted to disclose whether the Tyr402His variant is truly involved in explaining the risk of coronary heart disease. While our results are promising, the potential of other variants in the CFH gene to influence coronary heart disease risk should be further investigated.

Another limitation of the study is found in the follow-up. We performed a thorough follow-up procedure with regard to recognizable myocardial infarction, attaining 94.9% completeness. However, our follow-up did not contain incident silent myocardial infarction, although patients with silent myocardial infarction are in many aspects similar to those with recognized myocardial infarction (27). The result is that incident silent myocardial infarction cases were missed and have been considered as non-cases in the analysis. Because this is non-differential misclassification of the outcome, it should not have influenced our results. We present relative risks, which remain the same. Only the risk difference between the genotypes could have changed due to this type of misclassification (28).

The effect of the Tyr402His polymorphism on risk of myocardial infarction was modified by high total cholesterol levels, presence of diabetes mellitus, and smoking. These factors all have been shown to be pathogens in vessel inflammation (1,29,30). When the alternative complement pathway is activated by such pathogens, it is plausible that subjects with a genetic susceptibility in CFH such as the Tyr402His variant respond with a reduced complement

inhibition and thereby increase their risk of vessel damage, coronary atherosclerosis, and, subsequently, myocardial infarction. Because the Tyr402His polymorphism is located within the cluster of amino acids implicated in the binding of CRP, we also expected CRP level to modify the risk of myocardial infarction. Although the risk was slightly higher in participants with CRP levels above the median, the interaction with CRP was not significant.

In conclusion, we have found an association between the CFH gene Tyr402His polymorphism and myocardial infarction. This suggests that CFH may play an important role in atherosclerosis, and underscores the importance of the alternative complement system in cardiovascular disease.

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