The common Y402H variant in complement factor H gene is not associated with susceptibility to myocardial infarction and its related risk factors

Klaus STARK*, Katharina NEUREUTHER*, Kamil SEDLACEK*, Wibke HENGSTENBERG*, Marcus FISCHER*, Andrea BAESSLER*, Silke WIEDMANN*, Andreas JERON*, Stephan HOLMER†, Jeanette ERDMANN‡, Heribert SCHUNKERT‡ and Christian HENGSTENBERG*

*Klinik und Poliklinik für Innere Medizin II, Klinikum der Universität Regensburg, 93053 Regensburg, Germany, †Medizinische Klinik II, Klinikum Landshut, 84034 Landshut, Germany, and ‡Medizinische Klinik II, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, 23538 Lübeck, Germany

ABSTRACT

Recently, the genetic variant Y402H in the CFH (complement factor H) gene was associated with an increased risk for MI (myocardial infarction) in a prospective Caucasian cohort. In another nested case-control study, however, the CFH-Y402H variant did not carry susceptibility to MI. The aim of the present study was to test for an association between the CFH-Y402H variant and MI in a large case-control sample with a familial background for CAD (coronary artery disease). A total of 2161 individuals from the German MI family study were studied by questionnaire, physical examination and biochemical analyses. MI patients (n=1188; 51.4±8.6 years at first MI) were recruited from families with at least two members affected by MI and/or severe CAD. Spouses, sisters-in-law and brothers-in-law respectively, without MI/CAD were included as unaffected controls (n=973; 56.9±9.8 years). Genotyping was performed using a TaqMan assay. The common Y402H variant in the CFH gene was not associated with classical cardiovascular risk factors (diabetes, hypercholesterolaemia, hypertension, obesity, smoking and C-reactive protein serum levels). No association was found between the CFH-Y402H variant and susceptibility to MI. Separate analyses in both men and women revealed no gender-specific influence of the gene variant on cardiovascular risk factors or MI. This investigation was unable to replicate the association between the common CFH-Y402H variant and susceptibility to MI in our large Caucasian population which is enriched for genetic factors. We conclude that the CFH-Y402H variant has no relevant risk-modifying effect in our population.

INTRODUCTION

CAD (coronary artery disease) and MI (myocardial infarction) are the leading causes of morbidity and mortality in the Western world [1]. In epidemiological studies, factors such as smoking, obesity, high blood pressure, elevated cholesterol levels and diabetes have been identified to increase cardiovascular risk. Additionally,

Key words: association study, cardiovascular disease, complement factor H gene (CFH), genetics, myocardial infarction, polymorphism, risk factor.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CFH, complement factor H; CI, confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; GWA, genome-wide association; LDL, low-density lipoprotein; MGB, minor groove binder; MI, myocardial infarction; OR, odds ratio; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

Correspondence: Professor Christian Hengstenberg (email christian.hengstenberg@klinik.uni-regensburg.de).
a strong genetic component has also been documented in the aetiology of CAD [2,3]. To unravel the underlying genes, several genome-wide analyses have been performed and have revealed chromosomal loci with linkage to CAD or MI [4–12]. However, currently only a few genes and variants responsible for prevalence to MI in the general population are known [9,13].

In the pathophysiology of atherosclerosis, inflammation is hypothesized to play an important role for plaque formation and its destabilization, therefore causing CAD and MI [14,15]. CFH (complement factor H), as a part of innate immunity, contributes to the process of inflammation. CFH provides binding sites for C3b, heparin, as well as sialic acids, and also interacts with CRP (C-reactive protein) [16], which has been linked in several studies to CAD, MI and stroke [17]. Recently, an association between a common CFH gene variant and increased risk for MI was reported in a prospective cohort study: an SNP (single nucleotide polymorphism) rs1061170, representing a tyrosine → histidine change at amino acid position 402 (X402H) in the CFH protein, showed hazard ratios up to 1.77 in 226 MI cases during a mean of 8.4 years of follow-up of 5237 individuals from the Rotterdam Study [18]. However, another prospective study with 335 MI cases did not show an influence of the Y402H variant on susceptibility to MI [19].

The German MI family study [4,20–22] provides well-characterized MI patients as well as unrelated healthy controls for association studies. In the present study, we investigated the association between the CFH-Y402H genetic variant and susceptibility to MI and known cardiovascular risk factors, as well as CRP serum levels.

**MATERIALS AND METHODS**

**Study sample**

The study sample consisted of individuals from the German MI family study. Selection criteria have been described previously [4,20]. All participants were studied using a standardized questionnaire, physical examination and biochemical analyses at inclusion (n = 2161) and 5-year follow-up (n = 1780). Baseline characteristics of the 2161 investigated participants in the present study at the time point of inclusion are summarized in Table 1. The present analyses included independent MI cases (n = 1188) with a positive family history (at least one additional family member who had suffered from MI or severe CAD, defined as treatment with percutaneous coronary intervention or coronary artery bypass graft). Control individuals (n = 973) consisted of married-in spouses, sisters-in-law and brothers-in-law. Of these controls, 617 were confirmed to be free of any cardiovascular symptoms and events during the follow-up period. Of the remaining 356 control individuals, follow-up examination was not completed by the time of the present study. Consanguineous individuals were excluded. The research was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Written informed consent was obtained from all subjects, and the Ethics Committee of the University of Regensburg approved the study.

**Table 1 Characteristics of study sample from the German MI family study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MI cases (n = 1188)</th>
<th>Controls (n = 973)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-year follow-up available (n)</td>
<td>1163</td>
<td>617</td>
</tr>
<tr>
<td>Age at first MI (years)</td>
<td>51.4 ± 8.6 (24–77)</td>
<td>–</td>
</tr>
<tr>
<td>Age at inclusion (years)</td>
<td>58.7 ± 8.6 (29–87)</td>
<td>56.9 ± 9.8 (29–80)</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>68.4 (813)</td>
<td>34.6 (337)*</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>82.9 (985)</td>
<td>39.7 (386)*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>227.2 ± 47.1</td>
<td>238.2 ± 42.8*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>50.1 ± 13.2</td>
<td>60.7 ± 15.4*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>151.3 ± 63.3</td>
<td>146.9 ± 34.9*</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dl)</td>
<td>199.6 ± 139.0</td>
<td>152.5 ± 109.3*</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>93.7 (1113)</td>
<td>57.0 (554)*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.6 ± 19.5</td>
<td>133.8 ± 17.7*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.2 ± 10.1</td>
<td>82.0 ± 9.8</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>22.6 (269)</td>
<td>18.5 (180)*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 ± 3.6</td>
<td>26.7 ± 4.2*</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>16.8 (199)</td>
<td>5.4 (53)*</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>69.3 (823)</td>
<td>49.7 (484)*</td>
</tr>
</tbody>
</table>

Table values are means ± S.D. with range in parentheses or percentage (n) unless indicated otherwise. To convert values for total cholesterol, HDL (high-density lipoprotein) cholesterol and LDL cholesterol into millimoles per litre, divide by 38.66. See the Materials and methods section for definitions of risk factors.

*Significant difference between the groups (P < 0.05).

**Definition of risk factors**

Diabetes was defined as a history of diabetes mellitus or intake of antidiabetic medication. Individuals with a former or current smoking habit were classified as smokers. Obesity was defined as a BMI (body mass index) ≥ 30 kg/m². Study subjects receiving antihypertensive therapy or with a SBP (systolic blood pressure) ≥ 140 mmHg or DBP (diastolic blood pressure) ≥ 90 mmHg were classified as hypertensive. Hypercholesterolaemia was defined as LDL (low-density lipoprotein) cholesterol ≥ 160 mg/dl or the use of a lipid-lowering therapy.

**Genetic analysis**

Genomic DNA was isolated from whole blood samples using the PureGene DNA Purification System Blood Kit (Gentra). DNA samples were genotyped using 5′- exonuclease TaqMan® technology (Applied Biosystems) with different fluorescence-labelled probes, including non-fluorescence quencher and MGB (minor groove binder) [23,24]. For SNP rs1061170, a Custom TaqMan® SNP Genotyping Assay (Applied Biosystems) was used.
with: forward primer, 5′-CTT TAT TTA TTT ATC ATT GTT ATG GTC CTT AGG AAA ATG TTA TTT -3′; reverse primer, 5′-GGC AGG CAA CGT CTA TAG ATT TAC C -3′; probe 1, VIC-5′-TTT CTT CCC TAA TTT TG -3′-MGB; probe 2, FAM-5′-TTT CTT CCA TGA TTT TG -3′-MGB (VIC and FAM are the fluorophores VIC® and 6FAM™ respectively; the SNP position is indicated as bold and underlined; probes were designed on the reverse chromosomal strand).

For each genotyping experiment 10 ng of DNA was used in a total volume of 5 µl containing 1 x TaqMan® Universal PCR Master Mix (Applied Biosystems). The PCR reaction and post-PCR end point plate reading was carried out according to the manufacturer’s protocol using the Applied Biosystems 7900HT Real-Time PCR System. Sequence Detection System software version 2.2 (Applied Biosystems) was used to assign genotypes applying the allelic discrimination test [24]. Case and control DNA were genotyped together on the same plates. Duplicates of samples were employed to assess intraplate and interplate genotype quality. No genotyping discrepancies were detected. Assignment of genotypes was performed by a person without knowledge of the affection status.

Statistical analysis
Genotype distribution within the groups of cases and controls respectively, was compared with values predicted by Hardy–Weinberg equilibrium using the $\chi^2$-test. Differences in allele frequencies between dichotomous traits were calculated using the same method. Genotype distribution between cases and controls assuming dominant or recessive genetic models were performed using logistic regression analysis. Linear regression analysis was employed for comparison of genotype distributions with continuous variables, whereas ln(CRP) serum levels were used. The potential interaction between each traditional cardiovascular risk factor and genotype was tested in separate logistic regression analyses including the cross-product term. Prevalence ORs (odds ratios) with their 95 % CIs (confidence intervals) were reported. A two-sided $P$ value $\leq 0.05$ was considered statistically significant. All analyses were carried out using JMP IN 5.1 (SAS Institute). Power analysis was performed applying the G’Power program [25].

RESULTS

From 2187 DNA specimens, a total of 2161 individuals were genotyped successfully and therefore the overall call rate was 98.8 %. Baseline characteristics of the present study sample are shown in Table 1. The proportion of women was lower in the patient group ($n = 375$) than in the control group ($n = 636$). As expected, the MI patients ($n = 1188$) had a higher prevalence of classical cardiovascular risk factors (hypercholesterolaemia, hypertension, obesity, diabetes and smoking habit) than the control subjects ($n = 973$). In MI patients, the mean age at first MI was 51.4 ± 8.6 years. Anthropometric and biochemical measurements were performed at the time-point of inclusion at a mean age of 57.9 ± 9.2 years (58.7 ± 8.6 years for MI patients and 56.9 ± 9.8 years for controls).

Genotype distribution of the CFH gene variant Y402H was analysed in the whole population and in sub-groups separately. The Hardy–Weinberg equilibrium was always fulfilled. Hence tests for allele frequency difference and the co-dominant model gave approximately the same $P$ values (results not shown). Therefore in addition to the allele frequency comparisons we have reported results from dominant and recessive genetic models (Table 2). In the present study with 2161 different DNA samples, the frequency of the H allele was 36.7 %. Genotype frequencies in the whole study group were 40.4 %, 45.8 % and 13.8 % for YY, YH and HH respectively.

CFH-Y402H variant and susceptibility to MI and risk factors
The observed allele frequency and genotype distributions of the CFH-Y402H variant were not significantly different between the 1188 MI cases and 973 unaffected controls. In addition, no association was found between the Y402H variant and classical cardiovascular risk factors, namely diabetes, hypercholesterolaemia, hypertension, obesity and smoking habit (Table 2). We also performed adjusted analyses to exclude confounding effects of the differently distributed risk factors between MI cases and controls and found no significant association between the CFH-Y402H variant and MI (results not shown).

Additionally, no association between the CFH-Y402H variant and CRP serum levels at the time of inclusion was observed ($P = 0.16$). Due to the limited sample size with CRP data points at the inclusion date (497 of 2161 individuals; 471 of them classified as MI patients), we used 5-year follow-up values (mean age 62.9 ± 8.7 years), where 1284 CRP values were measured. Again, no association between the CFH-Y402H variant and CRP serum levels could be found ($P = 0.97$). From the follow-up examination, the CRP measurements were available from 855 MI patients (mean age 63.2 ± 8.1 years, i.e. on average of 11.8 years after first MI) and 429 controls (mean age 62.5 ± 9.1 years) respectively. No significant association between CRP serum levels at follow-up examination and MI was observed ($P = 0.09$). To test for gender-specific influences of the CFH-Y402H variant, we performed the analyses in both men and women separately. Neither susceptibility to MI nor cardiovascular risk factors (diabetes, hypercholesterolaemia, hypertension, obesity and smoking habit) had
Table 2

<table>
<thead>
<tr>
<th>CFH-Y402H genotype distribution in MI patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted ORs are given. MAF, minor allele frequency. See the Materials and methods section for definitions of risk factors.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>MI patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant model</td>
<td>Recessive model</td>
</tr>
<tr>
<td>Genotype (n)</td>
<td>Difference in allele frequency (genotype HH + YH versus YY)</td>
</tr>
<tr>
<td>YY</td>
<td>YH</td>
</tr>
<tr>
<td>MI</td>
<td>484</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>104</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>558</td>
</tr>
<tr>
<td>Hypertension</td>
<td>679</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>507</td>
</tr>
<tr>
<td>Obesity</td>
<td>186</td>
</tr>
</tbody>
</table>

a gender-specific association with the Y402H variant. Likewise, no gender-related association between CFH-Y402H genotypes and CRP serum levels from the follow-up examination were found (results not shown). Additional analysis in a age- and sex-matched sub-sample (n = 1200) with 300 cases and 300 controls from both men and women also showed no association between risk factors, MI and CFH-Y402H genotypes (results not shown).

DISCUSSION

Inflammation and components of innate immunity, and therefore potentially complement activation, play a major role in the pathophysiology of atherosclerosis [26]. The CFH gene, encoding a plasma protein essential for regulation of the alternative complement pathway, is a good candidate for genetic susceptibility to MI. Two recent studies have shown inconsistent results regarding the association of the CFH-Y402H variant with MI in prospective cohorts [18,19]. Thus additional data are needed to assess the impact of this variant on the genetic aetiology of MI. In the present case-control association study, the CFH-Y402H variant was neither associated with an increased risk for MI in patients with a strong familial background for cardiovascular disease nor with classical cardiovascular risk factors, such as diabetes mellitus, hypercholesterolaemia, hypertension, obesity and smoking habit (Table 2). Serum CRP levels as a non-specific indicator of inflammation has gained great importance as a risk marker in cardiovascular disease, although the magnitude of its risk stratification potential has been debated [27]. Since the CRP-binding site of the CFH protein is localized within the region of the Y402H variant [28] and its genotype potentially determines CRP levels [29], we analysed a possible association of CFH-Y402H and serum CRP levels in the present study group. At follow-up, serum CRP levels were available from 1284 participants. No association between serum CRP and CFH-Y402H genotypes was detectable, indicating that CRP serum levels are not influenced by the CFH gene variant in a measurable fashion in the present cohort. Additionally, CRP serum levels at follow-up examination were not associated with susceptibility to MI in the present study. However, it has to be noted that, in the present study group, baseline as well as follow-up values for serum CRP were measured after a mean of 7.3 and 11.8 years respectively, after suffering from MI. It is thus questionable whether these values can be related to the pathophysiology of the MI event. Within the CFH gene the amino acid position 402 is encoded in exon 9. This region is part of a strong LD (linkage disequilibrium) block that does not cover the whole gene with respect to an r² value of 0.8 by analysing data from the HapMap project [30]. However,
the Y402H variant is the only one so far having a positive association with MI in a prospective cohort study [18]. We are currently carrying out a GWA (genome-wide association) study on MI using an Affymetrix® GeneChip® Human Mapping 500K Array Set, but this chip does not contain the SNP rs1061170. Altogether, information on 18 SNPs within the 95.5-kb chip does not contain the SNP rs1061170. Altogether, this information will be available from these GWA studies. Recent studies on the CFH gene investigating the association between the Y402H variant and age-related macular degeneration have shown that other polymorphisms within the CFH gene also contribute to disease susceptibility independent of the Y402H genotype [31,32]. This emphasizes the relevance of analysing the Y402H variant separately. Additionally, in the background of GWA studies, future focus should be given on the remaining part of the CFH gene as a potential candidate locus for MI susceptibility.

Our non-replication of the recently reported association between the CFH-Y402H variant and MI [18] is unlikely to be a result of insufficient power. The sample size with 1188 MI cases and 973 controls has greater than 96% power to detect a weak-to-moderate gene effect with an effect size equal to 0.2 at a significance level of 0.05.

A possible limitation of the present study is the recruitment of our MI patients: only patients who survived at least one MI and had an affected sibling with CAD or MI were included. Hence there was a selection bias for both MI-surviving patients and the familial form of the disease. On the other hand, however, due to the family-based character of our patient selection, the genetic background for the aetiology of MI is high in the present study group thus enabling us to investigate even small genetic effects. Using married-in spouses or their relatives as control subjects, the risk of population stratification between affected MI patients and unaffected individuals is minimized. Additionally, a main part of the control group was validated as being free of any cardiovascular symptoms and events during the 5-year follow-up period.

In a similar manner to other candidate genes, analyses of CFH should be included in forthcoming association studies of CAD and MI to reveal more information about the contribution of this gene and its variants to the genetic aetiology of the disease.

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