

# Low thrombin activatable fibrinolysis inhibitor activity levels are associated with an increased risk of a first myocardial infarction in men

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## ABSTRACT

### Background

Studies on the relation between thrombin activatable fibrinolysis inhibitor (TAFI) and arterial thrombosis have produced conflicting results. TAFI regulates fibrinolysis, but other roles of this inhibitor, including anti-inflammatory properties, have also been demonstrated.

### Design and Methods

We investigated the association between TAFI activity and the risk of myocardial infarction. Additionally, we studied the association of common single nucleotide polymorphisms in the TAFI gene with levels of the TAFI protein and risk of myocardial infarction. We included 554 men under 70 years old with a first myocardial infarction and 643 controls participating in the Study of Myocardial Infarctions Leiden (SMILE), a case-control study.

### Results

We found odds ratios (95% confidence intervals) of a first myocardial infarction of 2.4 (1.6-3.6), 3.2 (2.1-4.7) and 3.4 (2.3-5.1) for subjects whose TAFI levels were in the third, second and first quartiles (lowest TAFI levels), respectively, compared with the fourth quartile, after adjusting for arterial disease risk factors. The rare -438A and 1040T alleles were associated with lower, and the rare 505G allele with higher TAFI levels than the common alleles. Carriers of the -438A allele had an increased risk of myocardial infarction (odds ratio 1.6 (1.0-2.5) for AA; odds ratio 1.2 (0.9-1.5) for AG compared with GG). The other single nucleotide polymorphisms were not associated with myocardial infarction.

### Conclusions

Low TAFI activity levels are associated with increased risk of a first myocardial infarction in men. The results on the association between TAFI single nucleotide polymorphisms and myocardial infarction were inconsistent.

Key words: TAFI, myocardial infarction, genetics, epidemiology.

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## Introduction

Thrombin activatable fibrinolysis inhibitor (TAFI) forms the molecular link between the coagulation and fibrinolytic systems.<sup>1</sup> Various animal models have demonstrated that TAFI regulates both venous and arterial fibrinolysis *in vivo*.<sup>2-5</sup> Furthermore, epidemiological studies have shown elevated TAFI levels to be a mild risk factor for the development of a first or recurrent venous thrombosis.<sup>6-8</sup> However, studies on the association between single nucleotide polymorphisms (SNP) that are associated with TAFI levels and the risk of venous thrombosis have given conflicting results.<sup>9-11</sup>

Epidemiological data on the association between TAFI levels and the risk of arterial disease are inconsistent. Several case-control studies have shown high TAFI levels to be associated with the risk of coronary artery disease.<sup>12-14</sup> However, elevated TAFI levels, or polymorphisms in the *TAFI* gene associated with increased TAFI levels, have been related to a *decreased* risk of myocardial infarction.<sup>15</sup> In one case-control study, high TAFI antigen decreased but high TAFI activity increased the risk of myocardial infarction in young patients.<sup>16</sup> Furthermore, in a prospective study, decreased TAFI levels were associated with refractiveness to medical treatment in patients with unstable angina pectoris.<sup>17</sup>

Recently, biological functions of TAFI distinct from fibrinolysis regulation have been described, including regulation of inflammation, blood pressure, cell migration, and wound healing.<sup>18</sup> These functions may depend on substrates of TAFI other than fibrin, for instance bradykinin, the anaphylatoxins C3a and C5a, annexin II, and osteopontin.<sup>19,20</sup>

The contradictory results from epidemiological studies may be explained by two opposite effects of TAFI on the development of arterial thrombosis. While the association between high levels of TAFI and arterial thrombosis may be the result of a hypofibrinolytic state, the association between low TAFI levels and arterial thrombosis may be explained by a defective regulation of inflammation.

Another explanation of the contradictory results could be the use of different methods for measuring TAFI. Several different antigen assays as well as distinct activity assays have been used in epidemiological studies. With the discovery that some enzyme linked immunosorbent assays of TAFI antigen are dependent on the 1040C/T polymorphism, revealing a lack of reactivity with the 1040T variant,<sup>21</sup> even more caution is required in the interpretation of results.

The -438G/A SNP in the promoter region of the *TAFI* gene,<sup>22</sup> the 505G/A SNP (which encodes an Ala to Thr substitution at position 147),<sup>22</sup> and the 1040C/T SNP (encoding a Thr to Ile substitution at position 325)<sup>23</sup> are known to be associated with TAFI plasma antigen levels. In Caucasians, three main haplotypes can be identified ([www.hapmap.org](http://www.hapmap.org)). The -438G/A and 1040C/T are generally located in the same haplotype, but are not in complete linkage disequilibrium.<sup>24</sup> The 505A/G is typical to a second haplotype. A third haplotype is

characterized by a fourth SNP in intron 4 (i4 + 164 A/C). In the Study of Myocardial Infarctions Leiden (SMILE), a case-control study of men with a first myocardial infarction, we investigated determinants of the level of TAFI. In addition we studied the effect of plasma TAFI levels, as determined by a functional assay, and the -438G/A, 505A/G, 1040C/T, and i4 + 164A/C SNP in the *TAFI* gene on the risk of a first myocardial infarction.

## Design and Methods

### Selection of participants

The design of SMILE has been described previously.<sup>25</sup> In brief, the recruited patients were men who had a first myocardial infarction between January 1990 and January 1996, who were below the age of 70. Control subjects were frequency matched on 10-year age groups to the patients and were men without a history of myocardial infarction, without renal disease or severe (neuro)psychiatric problems, with a life expectancy of more than 1 year, and who had not taken any oral anticoagulants in the 6-month period prior to participation in the study. All participants completed a questionnaire and an interview took place prior to collection of blood samples. Questions referred to present and former smoking habits and alcohol use, diabetes, and current use of medications. Blood pressure was measured after a rest of at least 10 min with the person sitting in an upright position. In addition, for patients, information on diabetes and medication use prior to myocardial infarction was retrieved from discharge letters. A person was classified as hypertensive or hypercholesterolemic when he was taking prescription drugs for these conditions. All participants signed an informed consent form. Approval for this study was obtained from the the Medical Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

### Blood collection and laboratory analysis

Fasting blood samples were drawn from the antecubital vein into Sarstedt Monovette tubes (Sarstedt, Nümbrecht, Germany) and were obtained between July 1994 and February 1997. Most blood samples were collected in the morning (at a median of 9:35 am, with 95% before 11:00 am). The time between myocardial infarction and blood sampling ranged from 88 days to 5.8 years with a median of 2.6 years.

Total cholesterol, HDL-cholesterol, triglyceride, and C-reactive protein (CRP) concentrations were measured as described previously.<sup>25</sup>

Plasma TAFI activity levels were determined in citrated plasma with a chromogenic assay (Pefakit TAFI, Pentapharm Ltd., Basel, Switzerland) by converting TAFI into its active form using a reagent containing thrombin-thrombomodulin and subsequently measuring carboxypeptidase activity. Measurements were run on a BCS coagulation analyzer (Dade Behring Inc., Marburg, Germany). TAFI activity was expressed in percentages using a calibration curve of pooled normal

plasma (pool of samples from more than 200 healthy volunteers). The inter-assay coefficients of variation were 5.8% (n=32) at ~100% TAFI concentration and 8.0% (n=31) at ~28% TAFI concentration. For six patients and three control subjects, plasma was unavailable or reliable results were not obtained; therefore 554 patients and 643 control subjects were included in the analysis.

The -438G/A (rs2146881), i4 + 164 A/C (rs3818477), 505G/A (rs3742264), and 1040C/T (rs1926447) polymorphisms were determined by a 5' nuclease assay (Taqman; Applied Biosystems, Foster City, CA) using a standard polymerase chain reaction (PCR) mix (Eurogentec, Seraing, Belgium) and allele-specific fluorescent probes equipped with a minor groove binding moiety (Applied Biosystems, Foster City, CA, USA).

### Statistical analysis

Mean TAFI levels were calculated with 5<sup>th</sup> and 95<sup>th</sup> percentiles for categories of arterial risk factors. Quartiles of continuous variables (blood pressure, total cholesterol, HDL-cholesterol, triglyceride, and CRP) were defined based on the distribution among control subjects. A multiple linear regression analysis was performed among the control subjects to determine the independent effect of arterial risk factors on TAFI levels. As the association between lipid levels or CRP and TAFI is not linear, these variables were entered in the model using quartiles.

The association between TAFI levels and the risk of a first myocardial infarction was investigated by calculating odds ratios (OR), as an approximation of relative risks, and 95% confidence intervals (CI). TAFI levels were grouped into quartiles based on the distribution among the control subjects. The highest quartile (highest TAFI levels) was taken as the reference group. OR adjusted for age and other potential confounders were calculated using multivariate unconditional logistic regression. Age, levels of triglycerides, cholesterol, CRP, and body mass index (BMI) were used as continuous variables in the model. Levels of triglycerides and CRP were entered into the model after  $10_{\log}$  transformation because these variables were not normally distributed. Genotypic frequencies for each of the four loci were determined from the observed genotypic counts, and  $\chi^2$  analysis was used to check for departures from the Hardy-Weinberg equilibrium. The association between the four TAFI SNP and risk of myocardial infarction was also assessed by means of unconditional logistic regression. The homozygous genotype of the common allele was taken as the reference group. SPSS 14.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

## Results

The mean age of the 554 patients with myocardial infarction was 56.4 years (5<sup>th</sup>-95<sup>th</sup> percentiles, 40.4-68.8 years) and that of the 643 control subjects was 57.3 (5<sup>th</sup>-95<sup>th</sup> percentiles, 34.7-72.1 years). Arterial risk factors such as smoking, obesity, diabetes, hypertension, and hypercholesterolemia were more prevalent in patients

than in control subjects (Table 1). The mean TAFI level in patients was 84% (5<sup>th</sup>-95<sup>th</sup>, percentile 56-112%) and 90% in controls (5<sup>th</sup>-95<sup>th</sup>, percentile 58-128%). Age, BMI, presence of diabetes, smoking, or alcohol use had no or little effect on TAFI levels (Table 2). Cholesterol and triglyceride levels were positively associated with TAFI levels. TAFI levels were 83% in subjects with low cholesterol levels (first quartile) and 98% in those with high cholesterol levels (4<sup>th</sup> quartile). Similarly, TAFI levels increased from 84 to 94% when comparing individuals with low and high triglyceride levels. TAFI levels also increased with CRP levels (85% for those with low and 96% for individuals with high CRP). No apparent association was present with HDL cholesterol or blood pressure. Multiple linear regression was performed to determine the independent effect of CRP, total cholesterol, and triglycerides on TAFI levels. With each increase in quartile of CRP, TAFI increased 2.8% ( $\beta=2.8\%$ /quartile of CRP; 95% CI 1.4-4.4). For total cholesterol this was 3.8%/quartile (95% CI 2.3-5.3) and 1.7% (95% CI 0.2-3.2) for triglycerides. Adding smoking to the model did not change the coefficients (*data not shown*).

The association between TAFI levels (in quartiles) and the risk of myocardial infarction is shown in Table 3. The fourth quartile (highest TAFI levels) was taken as the reference group. The risk of myocardial infarction increased with each decreasing quartile. Men with the lowest TAFI levels (first quartile) had a 2.8-fold age-adjusted increased risk of myocardial infarction compared with men with TAFI levels in the fourth quartile

**Table 1. Characteristics of men with a first myocardial infarction and control subjects.**

Characteristics	Patients	Control subjects
Number	554	643
Mean age, years (5 <sup>th</sup> -95 <sup>th</sup> percentile)	56.4 (40.4-68.8)	57.3 (34.7-72.1)
Current smoking, n. (%)		
No	209 (37.7)	429 (66.7)
Yes	345 (62.3)	214 (33.3)
Alcohol use, n. (%)		
Never	85 (15.3)	64 (10.0)
Occasionally	23 (4.2)	21 (3.3)
Regularly	446 (80.5)	558 (86.8)
Body mass index, <sup>1</sup> n. (%)		
Less than 20 kg/m <sup>2</sup>	4 (0.7)	10 (1.6)
20-24 kg/m <sup>2</sup>	151 (27.3)	186 (29.0)
25-29 kg/m <sup>2</sup>	306 (55.3)	340 (53.0)
At least 30 kg/m <sup>2</sup>	92 (16.6)	106 (16.5)
Diabetes, n. (%)		
Absent	528 (95.3)	621 (96.6)
Present	26 (4.7)	22 (3.4)
Hypertension, n. (%)		
Absent	450 (81.2)	537 (83.5)
Present	104 (18.8)	106 (16.5)
Hypercholesterolemia, n. (%)		
Absent	543 (98.0)	632 (98.3)
Present	11 (2.0)	11 (1.7)

*Data for patients refer to the period prior to myocardial infarction, apart from body mass index. <sup>1</sup>Body mass index was unknown for one patient and one control subject.*

(OR 2.8; 95% CI 1.9-4.0). Adjusting for other factors found to be associated with TAFI in the control subjects (CRP, triglycerides, and cholesterol), resulted in similar or slightly higher odds ratios (OR 3.4; 95% CI 2.3-5.1). Further adjustment for BMI, smoking, alcohol use, and diabetes (full model) did not substantially change the estimates.

**Table 2.** Arterial risk factors among control subjects and the association with TAFI levels.

	N. of control subjects (total=643)	Mean TAFI% (5-95 <sup>th</sup> percentile)
Age (years)		
27-39	56	85 (36-124)
40-49	104	91 (40-138)
50-59	179	95 (68-133)
60-69	247	89 (58-124)
70-75	57	85 (52-112)
Body mass index (kg/m <sup>2</sup> ) <sup>1</sup>		
Less than 25	196	87 (58-125)
25-29	340	92 (59-130)
at least 30	106	92 (59-126)
Diabetes		
Absent	621	91 (60-128)
Present	22	84 (50-128)
Current smoking		
No	429	89 (58-124)
Yes	214	93 (60-133)
Alcohol use		
Never	64	87 (54-121)
Occasionally	21	91 (64-127)
Regularly	558	91 (58-129)
Diastolic blood pressure (mmHg) <sup>2</sup>		
80 or less	199	89 (60-125)
85	93	92 (63-128)
90	169	90 (59-131)
At least 95	177	91 (53-133)
Systolic blood pressure (mmHg) <sup>2</sup>		
125 or less	158	88 (58-123)
130-135	113	89 (63-129)
140-150	205	93 (60-137)
At least 155	162	89 (57-125)
Triglycerides (mmol/L) <sup>1</sup>		
Less than 0.90	162	84 (55-122)
0.90-1.24	162	89 (59-120)
1.25-1.82	160	95 (66-129)
At least 1.83	158	94 (58-133)
Total cholesterol (mmol/L) <sup>1</sup>		
Less than 5.17	164	83 (54-122)
5.17-5.81	159	89 (58-128)
5.82-6.59	160	91 (64-126)
At least 6.60	159	98 (64-139)
HDL cholesterol (mmol/L) <sup>3</sup>		
Less than 1.1	160	87 (53-124)
1.1-1.28	159	93 (58-130)
1.29-1.53	163	91 (65-124)
At least 1.54	158	90 (57-130)
CRP (mg/L)		
Less than 0.78	161	85 (58-118)
0.78-1.55	161	89 (58-130)
1.56-3.42	160	92 (64-133)
At least 3.43	161	96 (62-129)

<sup>1</sup>Information about BMI, total cholesterol, and triglycerides was unavailable for one control subject. <sup>2</sup>Information about diastolic and systolic blood pressure was unavailable for five control subjects. <sup>3</sup>Information about HDL-cholesterol was unavailable for three control subjects.

Excluding individuals who were using aspirin at the time of blood sampling (127 patients and 35 control subjects) did not change the results, nor did excluding the 132 patients who used oral anticoagulants at the time of blood collection (*data not shown*).

We also investigated the effect of the combination of low TAFI levels and smoking or increased CRP levels on the risk of myocardial infarction. Table 4 shows the risk of myocardial infarction for quartiles of TAFI in smokers and non-smokers. The reference group was formed of the non-smokers with the highest levels (4<sup>th</sup> quartile) of TAFI, who had the lowest risk of myocardial infarction. Within the non-smokers, decreased TAFI levels (1<sup>st</sup> quartile) were associated with an almost two-fold increased risk of myocardial infarction (OR 1.9; 95% CI 1.1-3.2). In men who smoked and had high TAFI levels (4<sup>th</sup> quartile) an OR of 2.1 was found (95% CI 1.1-3.7). Smokers with TAFI levels in the 1<sup>st</sup> quartile had a risk of myocardial infarction that was increased 9.4-fold (95% CI 5.4-16.4). The odds ratios were 2.8 (95% CI 1.8-4.4) for men with low TAFI levels and a CRP level below the 75<sup>th</sup> percentile, 1.9 (95% CI 1.0-3.5) for those with CRP levels above the 75<sup>th</sup> percentile and high TAFI levels, and the risk of myocardial infarction was increased 8.2-fold (95% CI 4.4-15.2) in men with high CRP levels and low TAFI levels, all compared with men with low CRP and high TAFI levels.

Table 5 shows the risk of myocardial infarction associated with four SNP in the *TAFI* gene as well as mean TAFI level for each genotype. The observed frequency distribution of the *TAFI* genotypes in the control group did not deviate from that predicted for the four SNP under the Hardy-Weinberg equilibrium. The SNP at position -438, 505, and 1040 were strongly associated with TAFI levels. The mean TAFI level in those subjects carrying the -438GG genotype was 94%, while the mean TAFI level in carriers of the -438AA genotype was 76%. Mean TAFI level was 82% for carriers of the 505GG genotype and 107% for those with the 505AA genotype. Individuals with the 1040CC genotype had a mean TAFI level of 94%, which was higher than that of subjects with the 1040TT genotype (mean TAFI level 75%). The association between the i4 + 164A/C SNP and TAFI levels was less clear. Carriers of the -438AA

**Table 3.** Risk of myocardial infarction with decreasing quartile of TAFI level.

Quartile of TAFI-activity level	4 (ref.)	3	2	1
TAFI-activity (range)	102-161	89-101	76-88	19-75
N. of cases	65	132	172	185
N. of control subjects	156	157	168	162
OR <sub>adj</sub> (95% CI) <sup>1</sup>	1	2.1 (1.4-3.0)	2.5 (1.8-3.6)	2.8 (1.9-4.0)
OR <sub>adj</sub> (95% CI) <sup>2</sup>	1	2.3 (1.6-3.4)	3.0 (2.1-4.4)	3.5 (2.4-5.1)
OR <sub>adj</sub> (95% CI) <sup>3</sup>	1	2.4 (1.6-3.6)	3.2 (2.1-4.7)	3.4 (2.3-5.1)

CI: confidence interval; <sup>1</sup>OR<sub>adj</sub>: Odds ratio adjusted for age. <sup>2</sup>OR<sub>adj</sub>: Odds ratio adjusted for age, CRP, triglycerides, and total cholesterol. <sup>3</sup>OR<sub>adj</sub>: Odds ratio adjusted for age, CRP, triglycerides, total cholesterol, BMI, smoking, alcohol use, and diabetes.



genotype, who had low TAFI levels, had an increased risk of myocardial infarction (OR 1.6; 95% CI 1.0-2.5) compared with carriers of the -438GG genotype. The odds ratio for carriers of the -438AG genotype was 1.2 (0.9-1.5). The other SNP appeared not to be associated with risk.

Results of several studies on the association between levels of TAFI and risk of myocardial infarction were, in retrospect, considered not reliable as assays depended on the 1040C/T genotype. To verify whether the association between low TAFI activity and myocardial infarction was independent of the 1040T allele, the association of TAFI levels and myocardial infarction was determined only in those men who carried the 1040CC genotype. Among men carrying the 1040CC genotype, the age-adjusted odds ratio for myocardial infarction among those with TAFI levels in the first quartile was 2.0 (95% CI 1.2-3.3), compared with those with TAFI levels in the fourth quartile. Using the full model, the odds ratio was 2.2 (95% CI 1.2-3.9).

## Discussion

This case-control study shows that individuals with low TAFI levels, as measured with a functional assay, had an increased risk of a first myocardial infarction compared with those with high TAFI levels. In accordance with these results, individuals carrying the A allele of the -438A/G SNP in the promoter region of the TAFI gene, who have low TAFI levels, had an increased risk of myocardial infarction, compared with individu-

als carrying the GG genotype. The 505G/A and 1040C/T polymorphisms, which are also associated with levels of TAFI activity, were not associated with the risk of myocardial infarction.

The increased risk of myocardial infarction in men with lower TAFI levels remained after adjusting for several arterial risk factors, some of which (i.e., triglycerides and total cholesterol) were shown to be strongly associated with TAFI levels. The increased risk was found to be independent of the 1040T allele. A dose-response association was found between TAFI levels and myocardial infarction, but the risk difference between the third and fourth quartile was most pronounced while the odds ratios for the first, second and third quartiles were similar. In other words, there might be a threshold, above which levels of TAFI protect against myocardial infarction.

The results of our study partly contrast those of previous studies and are not in line with what would be expected based on the role of TAFI in the regulation of fibrinolysis. Several case-control studies have found increased TAFI levels to increase the risk of coronary artery disease or myocardial infarction<sup>13,14,16</sup> or did not find any association between TAFI and arterial thrombosis.<sup>26</sup> Explanations for these differences could be different outcome measures,<sup>15,14,26</sup> inclusion of men and women,<sup>13,14,16,26</sup> restriction to a relatively young population,<sup>16</sup> or blood sampling during the acute phase of the disease.<sup>14</sup> In some studies comparisons were only made between mean TAFI levels of patients and controls and thus extreme values were not studied and adjustments for possible confounders that could have influenced the

**Table 4.** Combined risk of myocardial infarction by quartiles of TAFI and smoking status.

	TAFI (% range)	N. of cases	N. of control subjects	OR <sub>adj</sub> (95% CI) <sup>1</sup>
<b>Smoking</b>				
No	102-161	25	88	1 (ref.)
	89-101	47	112	1.5 (0.8-2.6)
	76-88	75	113	2.3 (1.4-4.0)
	19-75	62	116	1.9 (1.1-3.2)
Yes	102-161	40	68	2.1 (1.1-3.7)
	89-101	85	45	6.6 (3.7-11.8)
	76-88	97	55	6.2 (3.6-10.8)
	19-75	123	46	9.4 (5.4-16.4)
<b>CRP</b>				
<75 <sup>th</sup> percentile	102-161	35	104	1 (ref.)
	89-101	84	110	2.4 (1.5-3.8)
	76-88	112	130	2.7 (1.7-4.3)
	19-75	125	138	2.8 (1.8-4.5)
>75 <sup>th</sup> percentile	102-161	30	52	1.9 (1.0-3.5)
	89-101	48	47	3.4 (1.9-5.9)
	76-88	60	38	5.3 (3.0-9.3)
	19-75	60	24	8.2 (4.4-15.2)

<sup>1</sup>OR<sub>adj</sub> =Odds ratio adjusted for age; CI=confidence interval.

**Table 5.** Effect of TAFI SNP on functional TAFI levels and on risk of myocardial infarction.

SNP	Cases (%) (n=554)	Controls (%) (n=643)	TAFI (%) <sup>1</sup> , mean (5-95 <sup>th</sup> percentile)	OR <sub>adj</sub> (95% CI)
<b>-438</b>				
GG	297 (53.6)	378 (58.8)	94 (66-131)	1 <sup>2</sup>
AG	215 (38.8)	231 (35.9)	86 (58-120)	1.2 (0.9-1.5)
AA	42 (7.6)	34 (5.3)	76 (58-98)	1.6 (1.0-2.5)
<b>i4 + 164</b>				
AA	203 (36.6)	237 (36.9)	93 (60-137)	1 <sup>2</sup>
AC	274 (49.5)	301 (46.8)	88 (57-124)	1.1 (0.8-1.4)
CC	77 (13.9)	105 (16.3)	89 (68-118)	0.9 (0.6-1.2)
<b>505</b>				
GG	264 (47.7)	297 (46.2)	82 (55-111)	1 <sup>2</sup>
AG	236 (42.6)	282 (43.9)	95 (68-127)	0.9 (0.7-1.2)
AA	54 (9.7)	64 (10.0)	107 (39-153)	0.9 (0.6-1.4)
<b>1040</b>				
CC	269 (48.6)	310 (48.2)	94 (66-133)	1 <sup>2</sup>
CT	233 (42.1)	285 (44.3)	88 (58-123)	0.9 (0.7-1.2)
TT	52 (9.4)	48 (7.5)	75 (54-99)	1.2 (0.8-1.9)

OR<sub>adj</sub>: Odds ratio adjusted for age; CI: confidence interval. <sup>1</sup>TAFI levels in control subjects. <sup>2</sup>reference group.

results were not performed.<sup>14,16</sup> In one case-control study TAFI antigen levels were decreased in patients with myocardial infarction but activity levels of TAFI were increased, a difference that could not be explained by the 1040C/T SNP.<sup>16</sup> In the PRIME-study, a large prospective cohort study set up to investigate potential reasons for the difference in incidence of coronary heart disease in France and Northern Ireland, no clear association was found between baseline TAFI levels and angina pectoris or myocardial infarction and coronary death. However, in this study TAFI was measured with an antigen and not activity assay. Surprisingly, the 505A allele, which is associated with increased TAFI levels, was associated with an increased risk of coronary heart disease in France but with a decreased risk in Northern Ireland.<sup>27</sup> The HIFMECH study, a European multicenter case-control study, found elevated TAFI levels to be associated with a decreased risk of myocardial infarction, confirmed by a higher frequency of the 505A allele in patients than in controls.<sup>15</sup> The TAFI antigen assay used in the HIFMECH study was later found to be dependent on the 1040C/T genotype.

Although TAFI has been shown to inhibit fibrinolysis *in vivo*, recent studies suggest that TAFI plays an important role in regulating complement-mediated vascular inflammation *in vivo*. TAFI inhibits inflammation *in vivo* by inactivating the inflammatory mediators bradykinin and the anaphylatoxins C3a and C5a.<sup>19</sup> Furthermore, TAFI<sup>-/-</sup> plasminogen<sup>+/-</sup> mice expressed increased leukocyte migration compared with their wild-type counterparts in a model of peritoneal inflammation.<sup>28</sup> In another study, TAFI-deficient mice, primed by lipopolysaccharide, showed increased mortality after infusion of cobra venom factor, which is a potent non-specific activator of complement proteins.<sup>29</sup> Based on these results, one might expect that TAFI-deficient mice would have a more rapid progression of atherosclerosis, in which inflammation plays a pivotal role. However, to our knowledge, no data on atherosclerosis progression in TAFI knockout mice are available. As inflammation plays an important role in the development of atherosclerosis and arterial thrombosis,<sup>30</sup> it is not implausible that increased TAFI levels decrease the risk of myocardial infarction by decreasing the inflammatory response. These two counteracting effects may also explain the conflicting study results on TAFI and arterial thrombosis, and, because inflammation is less important in venous disease, the consistent relation between high TAFI levels and venous thrombosis risk. There is also evidence that TAFI binds to collagen resulting in reduced platelet adhesion to collagen under flow conditions,<sup>31</sup> which could also contribute to the protective effect of TAFI.

The high risk of myocardial infarction found in smokers with low TAFI levels is also in line with an excessive inflammatory response. It is known that smoking leads to an inflammatory state,<sup>32</sup> a fact also supported by increased CRP levels in smokers compared to non-smokers in this study.<sup>25</sup> It is possible that the effect of smoking on risk of myocardial infarction is most pronounced in those individuals with decreased TAFI levels, who have reduced potential to neutralize

this inflammatory response. This hypothesis is also supported by the 8.2-fold increased risk in men with high levels of CRP, indicating elevated inflammatory state, and low TAFI levels. In addition to smoking and increased CRP levels, hypercholesterolemia is also associated with an increased inflammatory state.<sup>33</sup> A large number of patients, however, were using lipid-lowering drugs at the time of blood sampling, making an analysis on the combination of cholesterol and TAFI levels unreliable. The data on hypercholesterolemia based on the use of lipid-lowering drugs prior to myocardial infarction (Table 1) were insufficient for such an analysis.

We found a higher risk in individuals carrying the -438A allele than in those carrying the -438G allele, which supports our finding that decreased TAFI levels increase the risk of myocardial infarction. No clear increase in risk was found for those carrying the 1040T allele compared with the 1040C, while both the -438A and 1040T alleles appeared to decrease TAFI levels. It is known that the 1040 T for C substitution results in a TAFIa species with a prolonged half-life at 37°C.<sup>34</sup> Possibly, this increased stability of TAFIa compensates for the decreased levels of the protein.

Surprisingly, the 505G/A SNP (or Ala147Thr), which has the largest effect on TAFI levels, was not associated with the risk of myocardial infarction. It could be that this SNP has an unknown function that counteracts the protective effect of the increased levels. The TAFI crystal structure shows that the amino acid at position 147 is solvent exposed.<sup>35</sup> Thus, theoretically, this residue, which is located close to the Arg145 that is known to be one of the essential residues for substrate peptide anchoring,<sup>36</sup> might contribute to ligand binding and the 505G/A polymorphism might affect this process. Although the 505G/A polymorphism does not affect TAFI fibrinolytic activity,<sup>34</sup> the effect of the polymorphism on the catalytic activity towards other molecular substrates such as bradykinin and anaphylatoxins has, to our knowledge, not yet been tested. A decreased activity of the A variant towards TAFI substrates involved in inflammation could plausibly explain why no association is found between the 505G/A SNP and the risk of myocardial infarction. Another possibility is that it is not the 505G/A SNP itself that is the functional variant but that this SNP is in strong linkage disequilibrium with another functional SNP. It has been suggested that the 505G/A SNP is not directly related to TAFI levels but that the 1583T>A SNP, which is in strong linkage disequilibrium with the 505G/A SNP, is actually the functional variant.<sup>24</sup> Similarly, the -438G/A might itself not be functional.<sup>37</sup> However, the linkage between SNP in the *TAFI* gene is so strong that the results of the SNP measured in our study are reliable indicators of the effects of the putative functional SNP.

It is possible that reduced TAFI levels are not causally linked to an increased risk of myocardial infarction, but that TAFI levels are reduced by another causal factor preceding the myocardial infarction. We are not aware of any risk factor for myocardial infarction that would reduce TAFI levels. The only disease state that could lead to low TAFI levels would be liver disease,

which was only present in two cases and three controls, who had normal TAFI levels. Furthermore, TAFI levels were found to increase with risk factors for myocardial infarction, such as lipid levels and CRP, rather than decrease. As TAFI is also suggested to be an acute phase protein<sup>38</sup> it is not likely that decreased TAFI levels are a result of some other underlying factor. Samples were frozen and stored for approximately 10 years. This could have influenced the levels of TAFI. However, if TAFI activity decreases during storage this effect would be equal in patients and controls so no bias of the results is expected, or at most an attenuation of the observed effects.

In conclusion, our study provides evidence that decreased TAFI levels are associated with an increased risk of a first myocardial infarction in men. The results on the association between TAFI SNP and myocardial infarction were inconsistent.

## Authorship and Disclosures

MEM designed the study, analyzed and interpreted the data, and drafted the manuscript; CJMD designed the overall study, collected and interpreted the data, and critically reviewed the analyses and the manuscript; PGdG interpreted the data and critically reviewed the analyses and the manuscript; JCMM designed the present study, was responsible for performing the assay, interpreted the data and critically reviewed the analyses and the manuscript; FRR designed the overall study, interpreted the data, and critically reviewed the analyses and the manuscript; TL designed the study, interpreted the data, critically reviewed the analyses and participated in writing the manuscript.

The authors declare that they have no conflicts of interest.

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