A functional single nucleotide polymorphism in the thrombin-activatable fibrinolysis inhibitor (TAFI) gene associates with outcome of meningococcal disease

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Summary. In meningococcal sepsis, disseminated intravascular coagulation with deposition of fibrin and formation of microthrombi occurs in various organs and enhanced inhibition of fibrinolysis is associated with adverse outcome. Recently, TAFI (thrombin-activatable fibrinolysis inhibitor) was identified as a link between coagulation and fibrinolysis, as TAFI can be activated by thrombin and once activated potently attenuates fibrinolysis. On the basis of this one would predict that DNA polymorphisms that increase TAFI activity would deteriorate the outcome in meningococcal sepsis. Therefore, we studied the prevalence of the Thr325Ile dimorphism in the TAFI gene, which is associated with increased TAFIa stability and activity in 50 patients who survived meningococcal disease, in 176 first-degree relatives of a consecutive patient series with meningococcal disease and 212 controls from the same geographic region. The TAFI 325 Ile/Ile genotype was slightly more common among parents of patients with meningococcal disease than in controls (11% vs. 7.1%, P = 0.24). This difference was pronounced among the subgroup of parents of non-surviving patients (19.2%, P = 0.03). Patients whose parents were carriers of the TAFI 325 Ile/Ile genotype had a 1.6-fold (95% CI 0.7–3.7) higher risk to contract meningococcal disease and a 3.1-fold (95% CI 1.0–9.5) increased risk to die from the infection compared with all other genotypes. Survivors had a genotype frequency (4.0%) that was lower than in the general population.

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TAFI 325 variants affect the outcome of meningococcal disease.

Keywords: fibrinolysis, meningococcal disease outcome, single nucleotide polymorphism, TAFI.

Meningococcal infections occur as endemic or epidemic disease and remain an important cause of morbidity and mortality in childhood and young adults [1,2]. Despite treatment with appropriate antimicrobial agents and advanced life support, the overall fatality rate of meningococcal disease ranges from 5 to 20%, with a rate up to 50% among patients with meningococcal septic shock. Furthermore, a significant proportion of surviving patients has sequelae attributable to ischemia of the extremities and/or the central nervous system [2].

Coagulopathy with widespread deposition of fibrin and formation of microthrombi in various organs is a consistent feature of meningococcal sepsis and most pronounced in purpura fulminans with severe disseminated intravascular coagulation [3,4]. Circulating meningococcal endotoxin is a potent activator of the extrinsic pathway of coagulation resulting in the generation of thrombin associated with the consumption of the natural coagulation inhibitors (antithrombin III, protein C and S). Counteraction of the coagulation response by the fibrinolytic system is often insufficient due to increased levels of circulating plasminogen activator inhibitor type 1 (PAI-1) and possibly thrombin-activatable fibrinolysis inhibitor (TAFI). Adverse outcome in sepsis and meningococcal septic shock is correlated to high plasma concentrations of PAI-1 [5–7] and the genetic predisposition for high plasma PAI-1 levels is associated with the development of more severe forms with septic shock and poor outcome of meningococcal disease [8,9].

Recently, TAFI was identified as a link between coagulation and fibrinolysis [10,11]. Activation of TAFI is mediated by
thrombin and accelerated in the presence of the cofactor thrombomodulin (TM) [12] and results in the removal of lysine residues from partially degraded fibrin with a concomitant decrease in plasminogen-binding and activation and therefore retardation of clot lysis [13]. A role for TAFI in inflammatory diseases was suggested as TAFI levels correlated with markers of the acute-phase (C-reactive protein and haptoglobin) in plasma of healthy individuals [14]. Although activated TAFI (TAFIa) is a powerful antifibrinolytic enzyme, to date no inhibitor of TAFIa has been identified in human plasma. However, a high intrinsic instability of TAFIa probably defines the mechanism of down regulation and inactivation [15,16].

TAFI antigen levels are in part dependent on genotype [17,18]. Three naturally occurring single nucleotide polymorphisms (SNPs) have been described in the coding region of the TAFI gene. Two of these result in amino acid substitutions, one at position 505A/G resulting in the amino acid substitution Thr147Ala [19], the other at position 1040C/T leading to the substitution Thr325Ile [20]. This latter SNP is of particular interest as the presence of the Ile residue increases the stability resulting in enhanced activity of TAFIa and consequently in an increased antifibrinolytic potential [20].

Because death in severe meningococcal sepsis is due to multi-organ failure, caused in part by thrombotic occlusion of the microvasculature, we set out to examine whether genetic variation in the TAFI gene contributes to the fatal outcome of meningococcal disease.

Patients and methods

Between January 1989 and February 1994, 80 patients with meningococcal infection were admitted to the Leiden University Medical Center, Netherlands [8]. The diagnosis was based on clinical presentation and bacterial cultures from blood and cerebrospinal fluid. In four cases, the diagnosis was made on the basis of the clinical picture alone, since bacterial cultures remained negative because of previous administration of antibiotics. More than 90% of isolates were Neisseria meningitidis serogroup B, the remainder were group C. Sixteen patients died.

Details of the study were published previously [8]. The original study included 50 patients who survived meningococcal infection and 212 controls; 14 patients did not respond to the invitation to participate in the study. The controls originated from the same geographic region as the patients and were enrolled without selection based on health. To prevent bias of the data by selective mortality of patients with meningococcal infection, the study also included 138 first-degree relatives (75 parents) of 45 patients who survived, and 52 first-degree relatives (28 parents) of 16 patients who died. All the families of patients who had died from meningococcal disease participated whereas five families of the patients who survived refused to participate in the study, leaving a total of 61 families for analysis. For the present investigation DNA was available for all 50 patients, and for 176 relatives (for various technical reasons 14 samples are missing from the original patient series). The local hospital ethics committee approved the study and informed consent was obtained from all patients or their parents.

DNA was extracted from blood samples employing standard techniques. A 406-bp fragment of the coding region of the TAFI gene containing the 1040C/T transition was amplified by PCR using primers ACCTTATTTATTGGCTTTTAGAT (F) and CTGGTGTCAGCATTTGCATACG (R), and a restriction digestion protocol using the enzyme SpeI was employed to define genotypes [20]. For technical reasons, one first-degree relative was not genotyped.

Allele frequencies were calculated by gene counting and compared by means of $\chi^2$ analysis. Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were estimated by cross-tabulation.

Results

The gene frequencies of the TAFI 325 Thr/Ile polymorphism in the first-degree relatives of patients with meningococcal disease, the surviving patients, and the controls are shown in Table 1. Allele frequencies for the Thr/Ile polymorphism were similar between controls and relatives of patients. The distribution of genotypes was in Hardy–Weinberg equilibrium for each of the groups.

The frequency of the TAFI 1040T/I genotype, corresponding to the homozygous TAFI 325 Ile/Ile variant, was more common, although not significantly, in parents of patients who contracted meningococcal disease than in the control group [11.0% vs. 7.1%, OR 1.6 (95% CI 0.7–3.7), $P = 0.24$] (Table 2). This

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 212)</th>
<th>Relatives of patients with MD (n = 176)</th>
<th>Survivors (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>325 Thr/Thr</td>
<td>108 (50.9%)</td>
<td>90 (51.1%)</td>
<td>25 (50.0%)</td>
</tr>
<tr>
<td>325 Thr/Ile</td>
<td>128 (42.0%)</td>
<td>71 (40.3%)</td>
<td>23 (46.0%)</td>
</tr>
<tr>
<td>325 Ile/Ile</td>
<td>15 (7.1%)</td>
<td>15 (8.5%)</td>
<td>2 (4.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls (n = 212)</th>
<th>Relatives of patients with MD (n = 176)</th>
<th>Survivors (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>325 Thr</td>
<td>305 (72.0%)</td>
<td>251 (71.0%)</td>
<td>73 (73.0%)</td>
</tr>
<tr>
<td>325 Ile</td>
<td>119 (28.0%)</td>
<td>101 (29.0%)</td>
<td>27 (27.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>325 Ile/Ile</th>
<th>Other</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>15 (7.1%)</td>
<td>197</td>
<td>1*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Parents of patients with MD</td>
<td>11 (11.0%)</td>
<td>89</td>
<td>1.6</td>
<td>0.7–3.7</td>
<td>0.24</td>
</tr>
<tr>
<td>who died from MD</td>
<td>5 (19.2%)</td>
<td>21</td>
<td>3.1</td>
<td>1.0–9.5</td>
<td>0.03</td>
</tr>
<tr>
<td>who survived MD</td>
<td>6 (8.1%)</td>
<td>68</td>
<td>1.2</td>
<td>0.4–3.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Patients who survived MD</td>
<td>2 (4.0%)</td>
<td>48</td>
<td>0.5</td>
<td>0.1–2.5</td>
<td>0.43</td>
</tr>
</tbody>
</table>

OR = odds ratio. *Reference category (OR = 1.0).

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difference was pronounced and significant in the subgroup of parents of patients who died of meningococcal disease [19.2% vs. 7.1%, OR 3.1 (95% CI 1.0–9.5), P = 0.03]. Among the 50 survivors of meningococcal disease two patients (4.0%) were carriers of the TAFI 325 Ile/Ile variant and this genotype frequency was almost half of that observed in the control group (7.1%, difference not statistically significant) (Table 2).

The TAFI 325 Ile/Ile variant was found in 8.1% of parents of the 45 patients who survived compared to 19.2% in parents of patients who died of meningococcal disease (Table 2). On the basis of these genotype frequencies patients whose parents were carriers of the TAFI 325 Ile/Ile variant had a 2.7-fold (95% CI 0.8–9.7, P = 0.14) increased risk to die of meningococcal disease compared with all other genotypes.

**Discussion**

This study found a small difference between the gene frequency of the TAFI 325 Ile/Ile variant in parents of patients with meningococcal disease and the general population. This difference was more evident in the subgroup of parents of patients who had died of meningococcal disease. These findings indicate that the TAFI 325 Ile/Ile variant may be involved in the susceptibility of patients for meningococcal disease and in particular related to mortality once meningococcal disease has been contracted.

The importance of the fibrinolytic system is to preserve microcirculation by clearing of fibrin deposits and thrombi from intra- and extravascular sites. The first response to administration of endotoxin in healthy volunteers is the release of tissue-type plasminogen-activator into the circulation followed by thrombin generation through activation of the extrinsic pathway of coagulation [21,22]. A few hours’ postchallenge, high levels of circulating PAI-1 dominate the effects on the fibrinolytic system leading to inadequate fibrinolysis with widespread fibrin deposition. These events are likely to favor bacterial outgrowth and viable microorganisms have been cultured from thrombotic skin lesions of most patients with meningococcal sepsis even after administration of antibiotics [23,24]. A defective fibrinolytic response was shown to be related to development of septic shock, multi-organ failure and adverse outcome in patients with sepsis, septic shock and meningococcal disease [5–9].

It is noteworthy that the surviving patients had a genotype frequency that tended to be lower than that observed in relatives or controls. Thus the carrier state of the TAFI 325 Ile/Ile variant in survivors (4.0%) is not in line with that of their families (8.1%), suggesting selective survival and supporting a relation of this TAFI variant with adverse outcome in meningococcal disease. This finding stresses the importance of studying relatives, especially parents of patients with meningococcal disease, as investigation of survivors only would have lead to biased results.

TAFI represents a link between coagulation and fibrinolysis. So far several SNPs of the TAFI gene have been reported. The SNP 1040C/T encoding the two naturally occurring TAFI variants, TAFI 325 Thr and TAFI 325 Ile is of particular interest as it affects TAFI antigen levels and TAFI stability and functional activity in the opposite direction [20,25]. Our data suggest that although the TAFI 325 Ile/Ile variant generally is associated with lower TAFI antigen levels than the other TAFI variants [25] increased stability and antifibrinolytic activity prevail and result in an enhanced antifibrinolytic activity.

The precise role of TAFI in severe infectious diseases, sepsis and DIC has not yet been established but could be 2-fold. Increased activity of TAFI might contribute to the inactivation of inflammatory mediators, such as anaphylatoxins, bradykinin and complement components [26] thereby reducing the susceptibility for septic shock. On the other hand, elevated activity of TAFI contributes substantially to an increased inhibition of fibrinolysis, which in combination with elevated PAI-1 levels facilitates the deteriorating effects of disseminated intravascular coagulation and thrombotic occlusion of the microvasculature [4]. Thrombin bound to TM accelerates activation of protein C and TAFI suggesting a dual role for TM in the regulation of fibrinolysis [12,27]. By stimulation of TAFI activation (soluble or cellular) TM can attenuate fibrinolysis [12,28], while fibrinolysis is enhanced by activation of protein C, resulting in a decreased rate of thrombin formation and consequently in reduced formation of TAFIa. TAFI activation is propagated at low and attenuated at high TM concentrations [29]. Under baseline conditions highest concentrations of TM are found in the microvasculature, in sepsis and septic shock, however, the cellular density of TM is reduced [30], rendering the microvasculature especially susceptible to TAFI activation. The imbalance between anti-coagulation and inhibition of fibrinolysis is further enhanced by the oxidation of TM Met388 by activated neutrophils thereby blocking protein C activation selectively, without affecting TAFI activation [31].

There are two limitations to this study. First, we were able to include only a relatively small number of surviving patients, resulting in only two carriers of the TAFI 325 Ile/Ile variant in the group of surviving patients. Such small numbers lead to statistical instability of the results. Second, although this study was originally set up to search for novel genetic susceptibility factors for meningococcal sepsis, the decision to examine TAFI polymorphisms was made post hoc. Therefore the results should be considered as hypothesis generating and in need of confirmation in, preferably larger, follow-up studies.

As TAFI is activated by thrombin bound at the site of coagulation and thrombus formation, inhibition of TAFIa might be a mechanism to enhanced thrombolysis with additional fibrin specificity and an interesting target for new therapeutic strategies in treatment of sepsis and septic shock.

**Acknowledgements**

JAKH is supported by a grant from the Swiss National Foundation for Scientific Research. HiC is a Clinical Established Investigator of the Netherlands Heart Foundation.
References


