CHAPTER 3

MYOCARDIAL INFARCTION OCCURS WITH A SIMILAR 24 H PATTERN IN THE 4G/5G VERSIONS OF PLASMINOGEN ACTIVATOR INHIBITOR-1
ABSTRACT

PAI-1 expression is regulated by a 4G/5G promoter polymorphism. The 4G allele is associated with greater circadian variation of PAI-1 levels. We hypothesized that the 24 h variation of cardiac risk is more pronounced among persons with the 4G4G genotype than among ones with 4G5G and 5G5G genotypes. We assessed the time of onset of symptoms in 623 consecutive patients with acute myocardial infarction (AMI) enrolled in the MISSION! Study between February 1, 2004 and October 29, 2006. All of the patients were genotyped for the PAI-1 4G/5G polymorphism. We quantified the amplitude of the 24 h variation of AMI with a generalized linear model with Poisson distribution. A morning peak, between 06:00 – 11:59 h (n = 197; 32% of all cases), in the onset of symptoms of AMI was observed. The group composed of patients with the 4G4G genotype did not have a more pronounced morning peak than the groups composed of other genotypes; the 24 h variation was 38% (95% confidence interval 12 – 70%) in the group of 4G4G patients and 34% (14 – 58%) and 56% (20 – 100%) in the 4G5G, and 5G5G groups of patients, respectively. Our findings show that 24 h variation of cardiac risk is not more pronounced among the 4G4G genotype of PAI-1.

Key words: PAI-1, genotypes, cardiovascular risk, diurnal, myocardial infarction.

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INTRODUCTION

Plasminogen activator inhibitor 1 (PAI-1), member of the serine proteinase inhibitors family, is a single-chain glycoprotein consisting of 379 or 381 amino acids and a molecular weight of 45 kDa. The human gene of PAI-1 is located on chromosome 7 (q21.3-a22) (Gils & Declerck, 2004). PAI-1 is present in human plasma (Juhan-Vague et al., 1984), platelets (Kruithof et al., 1987), endothelial cells (Booth et al., 1987), and various tumor cell-lines (Andreasen et al., 1986; Coleman et al., 1982; Wagner et al., 1986). PAI-1 is the major component of inhibitors of fibrinolytic activity. Increased PAI-1 plasma concentrations reduce the efficacy of thrombolytic therapy (Booth et al., 1992) and, conversely, monoclonal antibodies developed to inhibit PAI-1 activity have proven their efficiency in a number of in vivo studies where thrombus formation was prevented and lysis of platelet-rich clot was accelerated (Berry et al., 1998; Biemond et al., 1995; van Giezen et al., 1997; Levi et al., 1992; Rupin et al., 2001).

PAI-1 plasma concentrations show a clear circadian oscillation, both in various strains of mice (Ohkura et al., 2007) and humans, with a peak occurring around the commencement of the daily activity span (Angleton et al., 1989; Kapiotis et al., 1997). The transcription factor cycle-like factor (CLIF) forms with clock protein the CLOCK:CLIF heterodimer that regulates the circadian oscillation of PAI-1 gene expression (Maemura et al., 2000). In the PAI-1 promoter, two E-box elements (CACGTG) are responsible for the activation of PAI-1 by the CLOCK:CLIF complex; one of these E-boxes is located at 677 to 672. This overlaps with the sequence of a 4G/5G polymorphism in the PAI-1 promoter. This polymorphism is located 675 bp upstream of the start of transcription of the PAI-1 gene and has been associated with the circadian pattern of PAI-1 plasma concentrations (Dawson et al., 1993). Carriers of the 4G allele have a much more pronounced PAI-1 morning peak than 5G allele carriers (van der Bom et al., 2003; Hoekstra et al., 2002).

Although acute cardiovascular events occur at all times throughout the 24 h, the occurrence of acute myocardial infarction (AMI) has been shown to exhibit a 24 h pattern with a major peak in the morning (Cannon et al., 1997; Cohen et al., 1997; Marler et al., 1989; Muller et al., 1985, 1987). The morning peak in AMI is seen in large cohorts of patients regardless of sex, age, previous ischemic heart disease, development of Q-wave on electrocardiogram, myocardial infarction location, or survival in the critical care unit (Leiza et al., 2007). Unstable coronary plaques are frequently the locus for non-occlusive thrombus formation. Coronary clots may resolve spontaneously and without clinical consequences due to intervention of the fibrinolytic system, whereas acute coronary syndromes may be triggered when imbalances between fibrinolysis and coagulation are present (Christian et al., 1998; Engel & Lichtlen, 1977; Lee et al., 2001; Swan, 1989). The morning peak of PAI-1 may inhibit fibrinolysis and, as a result, contribute to the morning excess of AMI. We therefore hypothesized that the temporal variation of cardiac risk is more pronounced in persons with the 4G4G genotype than in ones with 4G5G and 5G5G genotypes.
METHODS

Study design and population
We performed a cohort study among 623 patients who had been enrolled in the MISSION! Study between February 1, 2004 and October 29, 2006. The MISSION! Study is a single center study of consecutive AMI patients. Details of the study have been reported elsewhere (Liem et al., 2007). In brief, MISSION! is primarily designed to optimize the acute and chronic care of AMI patients. Its protocol is based on recently established American College of Cardiology/American Heart Association and European Society of Cardiology guidelines for AMI (Antman et al., 2004; Van de Werf et al., 2003) and contains three phases: pre-hospital (focused on the reduction of treatment delay), in-hospital (supporting early and aggressive reperfusion therapy, prescription combination of evidence-based drugs, education, and active involvement of patient in lifestyle modification, as well as early and safe discharge), and outpatient (increasing participation in a cardiac rehabilitation program, systematic monitoring and adjustment of medical therapy, and reinforcement to achieve and maintain lifestyle goals). The study was approved by the ethics committee and conformed to international ethical standards (Portaluppi et al., 2008), and all patients gave written and informed consent. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree with the manuscript as written.

Measurements
MISSION! data are collected from each patient through medical history and medications, symptoms on arrival to the hospital, ECG examination, index times (time of onset of symptoms, time of call for medical help, time of first medical contact, time of hospital arrival, needle time, and time of first balloon inflation), and in-hospital and follow-up records. The time of onset of AMI symptoms was reported by the patient and recorded by the first examining physician. Patients were asked again about the occurrence of the first symptoms of myocardial infarction during their hospital stay. When the time of symptom onset was inconsistent and/or in doubt, a consensus time was established together with the patient, relatives, and attending physicians. Myocardial infarction was documented on the basis of troponin T > 0.1 µg/L and at least one of the following: clinical symptoms ± relevant electrocardiogram (ECG) and ± angiographic evidence, as previously described (Liem et al., 2007). Patients were considered to have dislipidemia, hypertension, and diabetes if they had been diagnosed with such by a physician previous to the present hospital admission for AMI. Patients included in the present analysis were presumed to have been adhering to a normal daytime activity nighttime sleep routine.

4G/5G polymorphism genotyping
Blood was collected in EDTA tubes upon the patient’s admission to the hospital, and genomic DNA was extracted following standard procedures. A multiplex assay
was designed using Assay designer software (Sequenom). All PCR reactions had a final volume of 5 μl and contained standard reagents and 5 ng of genomic DNA. After PCR, a primer extension reaction was performed to introduce mass-differences between alleles, and, after removing salts by adding resin, ~15 nl of the product was spotted onto a target chip with a 384 patches containing matrix. Mass differences were detected using an Autoflex (Bruker) matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF), and genotypes were assigned real-time using Typer 3.1 software (Sequenom). Cluster plots were made of the signals from the low and high mass allele. Two researchers (SCB and DP) carried out the scoring independently. There were neither disagreements nor vaguely positioned dots produced by Genotyper 3.0 (Sequenom Inc.).

Data analysis

Morning peak of myocardial infarctions
We divided the recorded times of AMI symptoms onset into four 6 h intervals starting at 00:00 h. The interval between 06:00 – 11:59 h was defined as the morning interval. The morning peak of AMI was the difference between the proportion of AMIs that occurred during the morning interval and the mean of the proportions of AMIs that occurred during the other three 6 h time intervals.

Amplitude of the 24 h variation
We used a generalized linear regression model with log link and Poisson distribution to model the incidence of AMI according to the time of symptoms onset in the different groups of patients categorized by PAI-1 genotype. The recorded times of symptoms onset were categorized into twelve 2 h time intervals, with values ranging from 0 for AMIs that occurred between 00:00 and 01:59 h to 11 for AMIs that occurred between 22:00 and 23:59 h. The circadian variation was expressed by the sinusoidal functions, Sin(2πI/12) and Cos(2πI/12), where I is the value of the 2 h intervals, and treated in the regression as two terms (i.e., variables). The combined functions allow the peak size to occur at any time of the day. The amplitude of the 24 h variation of the incidence of AMI and its standard error were calculated from the regression model in the following formulas:

Amplitude of 24 h variation of AMI incidence = \sqrt{\beta_1^2 + \beta_2^2}

Standard error = \left(\sqrt{\beta_1^2 + \beta_2^2}\right) = \sqrt{\frac{s_1^2 \beta_1^2 + 2s_{12} \beta_1 \beta_2 + s_2^2 \beta_2^2}{\beta_1^2 + \beta_2^2}}

The formula for the standard error of the amplitude was derived using the delta method (Oehlert, 1992). Finally, the amplitude of 24 h variation and 95% confidence interval (CI) in the incidence of AMI in the respective groups of patients were transformed from log to normal scale.
Sensitivity analysis
In order to examine if race, patient concomitant diseases, or medication, positive familial history, or smoking status confounded our findings, we repeated the analyses in three selected groups of patients:
1. Caucasians only;
2. Caucasians and non-Caucasians without any of the following: diabetes mellitus, current smoking status, hypertension, dislipidemia, familial history of cardiovascular disease, prior myocardial infarction, prior percutaneous coronary intervention (PCI), prior coronary artery by-pass graft (CABG), or concomitant medications, such as oral anticoagulants, aspirin, clopidogrel, beta-blockers, calcium-channel blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor (AT II) blockers, statins, oral antidiabetics, or insulin; and
3. only Caucasians matching the exclusion criteria from Group 2.

RESULTS

Patient characteristics
A total of 623 AMI patients between 22 and 88 yrs of age were included. Baseline characteristics of the study population according to the three different genotypes are presented in Table 1.

There were a few minor differences between patients within the three PAI-1 genotypes. There were more men with the 5G5G genotype than with the other two genotypes. Caucasians were 93% (174/188) in the 4G4G genotype and 91% (278/307) and 85% (109/128) in the 4G5G and 5G5G genotypes, respectively. Patients with known hypertension, dislipidemia, and diabetes mellitus were more frequent among the 4G4G genotype and a higher percentage of 4G4G patients was treated with anti-hypertensive medication (36% vs. 33% and 20%, respectively) compared with the other two genotypes, though the differences were not statistically

Table 1. Baseline characteristics for the study group (n = 623).

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>PAI-1 genotype</th>
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<tbody>
<tr>
<td></td>
<td>4G4G (n = 188)</td>
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<tr>
<td>Age (±SD) (yrs)</td>
<td>61 (12)</td>
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<tr>
<td>Men (%)</td>
<td>139 (74)</td>
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<tr>
<td>Current smoker (%)</td>
<td>88 (47)</td>
</tr>
<tr>
<td>Known hypertension (%)</td>
<td>66 (35)</td>
</tr>
<tr>
<td>Dislipidemia (%)</td>
<td>45 (24)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>15 (8)</td>
</tr>
</tbody>
</table>

Values (rounded) are mean (±SD) or number (%).
significant. Statins (15%, 14%, and 13%, respectively) and antithrombotics (12%, 13%, and 12% respectively) were used in a similar frequency by patients with the 4G4G, 4G5G, and 5G5G genotypes. Approximately 6% of the patients in every 4G/5G genotype had had a previous AMI.

**Morning peak of AMI**

Of the 623 AMI patients, 92 patients (15%) presented the first symptoms between 00:00 – 05:59 h, 197 patients (32%) between 06:00 – 11:59 h, 179 patients (29%) between 12:00 – 17:59 h, and 155 patients (25%) between 18:00 – 00:00 h (Figure 1, Panel 1). The morning peak in AMI events compared to the average of the rest of the day for the total study population was 9% (95% CI: 4 to 14%). Panels 2 – 4 in Figure 1 present the observed 24 h variation in AMI incidence according to the three different genotypes. The group of patients homozygous for the 4G allele did not have a more pronounced morning peak of myocardial infarction than the other genotypes; their proportional excess of AMI was 11% (2 to 20%), whereas among patients with the 4G5G and 5G5G genotypes it was 4% (-3 to 11%) and 18% (7 to 29%), respectively.

**24 h variation of AMI occurrence**

The overall amplitude of the 24 h variation in AMI occurrence was 39% (95% CI: 24 – 55%), indicating the difference between the highest and lowest myocardial infarction occurrence during the day. In accordance with the findings on the morning peak, the 24 h variation was not more pronounced in the group of patients with the 4G allele than among the others. Among patients with the 4G4G genotype, the 24 h variation was 38% (12 – 70%), and among those with the 4G5G and 5G5G genotypes, it was 34% (14 – 58%) and 56% (20 – 100%), respectively.

**Sensitivity analysis results**

The symptoms onset of AMI displayed a similar 24 h pattern (see Appendix Table 1), morning excess (see Appendix Table 2), and relation with the PAI-1 genotypes in Caucasians (see also Appendix Figure 1), patients previously non-medicated and apparently healthy (see also Appendix Figure 2), and Caucasians previously non-medicated and apparently healthy (see also Appendix Figure 3) as in the whole study group (see Figure 1). However, the 4G4G patients previously non-medicated and apparently healthy (see Appendix Figure 2) and the 4G4G Caucasian patients previously non-medicated and apparently healthy (see Appendix Figure 3) presented a 24 h pattern with a non statistically significant evening excess for symptoms onset of AMI.
Figure 1 (panels 1-4). Occurrence of AMI according to time of the day presented per 6 h intervals (boxes) and 2 h intervals (dots). The 4G4G patients did not display a more pronounced morning peak or 24 h variation of AMI occurrence when compared with the 4G5G and 5G5G patients.
DISCUSSION

In our cohort of 623 consecutive patients, we observed a clear morning peak in AMI occurrence. Our findings did not support the proposition that persons with the 4G4G PAI-1 genotype have a more pronounced morning peak in the occurrence of AMI than persons with the 4G5G or 5G5G genotypes.

Several arguments support the validity of our findings. First, our study protocol involved a relatively large cohort and had applied very strict and standardized criteria for the diagnosis of AMI (Liem et al., 2007); therefore, only patients with a definite diagnosis of AMI were entered into the investigation. Second, we identified a similar morning peak in AMI symptoms onset as previously presented in a meta-analysis by Cohen et al. (1997). Third, we conducted reliable genotyping and found a similar frequency of the 4G/5G genotypes with that already reported in two previous studies in the healthy Dutch population (Hermans et al., 1999; Westendorp et al., 1999) and in one myocardial infarction study (Sibbing et al., 2005).

The role of peak PAI-1 in the morning occurrence of AMI, as to be explained by decreased fibrinolytic response, was also proposed by Haus (2007). This author considered that differences in zygosity of the PAI-1 gene may explain the differences among individuals in the risk of developing coronary events during the peak time of PAI-1 activity (Haus, 2007). Our study is the first to investigate the association between the PAI-1 4G/5G genotype and the time of AMI symptoms onset. In the present study, the mendelian (Davey & Ebrahim, 2003; Keavney, 2002) inference made was that the 4G4G genotype, which is known to be associated with a higher PAI-1 morning peak (van der Bom et al., 2003), would translate into a larger variation and a higher morning excess of daily AMI incidence in a group of such patients.

We did not measure PAI-1 plasma concentrations, because these are known to be affected by the presence of cardiovascular diseases. We presumed all participants to have a normal daily rhythm. Differences between groups were considered unlikely to appear because of the mendelian inference. The mendelian approach is known to limit confounding of the findings, as genetic variants are not influenced by behavioral, socioeconomic, or physiological factors (Davey & Ebrahim, 2005). The sensitivity analyses confirm that our findings appear not to be affected by the established, potentially confounding factors. However, among previously non-diseased and non-medicated persons, the AMI symptoms onset peak appears in the last 6 h interval of the day for the 4G4G subgroups. Other population-based studies have found that the 24 h pattern of angina pectoris, AMI, sudden cardiac death and even stroke shows two peaks: a generally, more prominent morning peak and a second, generally, less prominent peak around early evening, in presumably day-active samples of persons (Smolensky et al., 2007). It is interesting to speculate that in previously non-diseased and non-medicated individuals the 4G4G genotype may be associated with a different AMI timing; however, due to the low number of cases (25 patients) a clear conclusion may not be drawn in the present study. Components of the fibrinolytic system, tissue-type plasminogen activator (tPA) and PAI-1, have been shown to exhibit a marked
circadian variation in the plasma (Andreotti & Kluft, 1991). Several studies have found tPA and the plasma tPA/PAI-1 complex to be predictive of myocardial infarction (Nordenhem et al., 2005; Ridker et al., 1993). Although the complex between tPA and PAI-1 is known to have a much less circadian variation than PAI-1 (Nordenhem et al., 2005), one cannot exclude that tPA and the tPA/PAI-1 complex play a role in morning peak of myocardial infarction.

In conclusion, this study found that the 4G4G genotype of PAI-1 was not related to the amplitude of the 24 h temporal pattern in AMI, suggesting that 4G4G individuals do not carry a higher risk for morning cardiac events than do the 4G5G and 5G5G individuals.

ACKNOWLEDGEMENTS

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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and after platelet aggregation. Blood 70:1645-1653.


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**Appendix Table 1.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Genotypes combined</th>
<th>4G4G</th>
<th>4G5G</th>
<th>5G5G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian*</td>
<td>40.4 (24.6; 58.3)</td>
<td>41.8 (14.4; 75.8)</td>
<td>33 (12.3; 57.4)</td>
<td>71 (29.3; 126.3)</td>
</tr>
<tr>
<td>Pre-AMI†</td>
<td>72.3 (25.7; 136.2)</td>
<td>48.3 (-20; 162.5)</td>
<td>130.7 (43; 272.9)</td>
<td>193.9 (32.6; 551.6)</td>
</tr>
<tr>
<td>Pre-AMI Caucasian‡</td>
<td>72 (24.2; 138)</td>
<td>38.5 (-23; 146.7)</td>
<td>129.5 (39.9; 276.7)</td>
<td>227.7 (40; 667)</td>
</tr>
</tbody>
</table>

24 h (%) variation (95% CI) of AMI incidence according to the PAI-1 4G5G genotype. *Caucasian patients only (n = 561)†Caucasian and non-Caucasian patients previously non-diseased and non-medicated (n = 86)‡Caucasian patients previously non-diseased and non-medicated (n = 81).

**Appendix Table 2.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Genotypes combined</th>
<th>4G4G</th>
<th>4G5G</th>
<th>5G5G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian*</td>
<td>8.8 (3.6; 14)</td>
<td>11.1 (1.8; 20.4)</td>
<td>3.1 (-3.6; 10.4)</td>
<td>19.3 (7.4; 31.1)</td>
</tr>
<tr>
<td>Pre-AMI†</td>
<td>17.8 (4.5; 31.2)</td>
<td>-12 (-34.7; 10.7)</td>
<td>22.5 (3.6; 41.3)</td>
<td>48.1 (20.8; 75.5)</td>
</tr>
<tr>
<td>Pre-AMI Caucasian‡</td>
<td>19.3 (5.6; 33.1)</td>
<td>-11.1 (-34.4; 12.2)</td>
<td>23.3 (3.8; 42.9)</td>
<td>52.9 (25.5; 80.3)</td>
</tr>
</tbody>
</table>

Percent morning excess variation (95% CI) of AMI incidence according to the PAI-1 4G5G genotype. Morning excess represents the difference between incidence of AMI in the 06:00 – 11:59 h interval and the mean value of the other three 6 h intervals. *Caucasian patients only (n = 561)†Caucasian and non-Caucasian patients previously non-diseased and non-medicated (n = 86)‡Caucasian patients previously non-diseased and non-medicated (n = 81).
Appendix Figure 1. 24 h variation of AMI symptoms onset per 6 h and 2 h intervals in Caucasian patients only (n = 561).
Appendix Figure 2. 24 h variation of AMI symptoms onset per 6 h and 2 h intervals in patients previously non-diseased and non-medicated (n = 86).
Appendix Figure 3. 24 h variation of AMI symptoms onset per 6 h and 2 h intervals in Caucasian patients previously non-diseased and non-medicated (n = 81).