The association between plasminogen activator inhibitor type 1 (PAI-1) levels, PAI-1 4G/5G polymorphism, and myocardial infarction: a Mendelian randomization meta-analysis

Abstract

Background: The circulating levels of plasminogen activator inhibitor type 1 (PAI-1) are increased in individuals carrying the 4G allele at position –675 of the PAI-1 gene. In turn, overexpression of PAI-1 has been found to affect both atheroma and thrombosis. However, the association between PAI-1 levels and the incidence of myocardial infarction (MI) is complicated by the potentially confounding effects of well-known cardiovascular risk factors. The current study tried to investigate in parallel the association of PAI-1 activity with the PAI-1 4G/5G polymorphism, with MI, and some components of metabolic syndrome (MetS).

Methods: Using meta-analytical Mendelian randomization approaches, genotype-disease and genotype-phenotype associations were modeled simultaneously.

Results: According to an additive model of inheritance and the Mendelian randomization approach, the MI-related odd ratio for individuals carrying the 4G allele was 1.088 with 95% confidence interval (CI) 1.007, 1.175. Moreover, the 4G carriers had, on average, higher PAI-1 activity than 5G carriers by 1.136 units (95% CI 0.738, 1.533). The meta-regression analyses showed that the levels of triglycerides (p=0.005), cholesterol (p=0.037) and PAI-1 (p=0.021) in controls were associated with the MI risk conferred by the 4G carriers.

Conclusions: The Mendelian randomization meta-analysis confirmed previous knowledge that the PAI-1 4G allele slightly increases the risk for MI. In addition, it supports the notion that PAI-1 activity and established cardiovascular determinants, such as cholesterol and triglyceride levels, could lie in the etiological pathway from PAI-1 4G allele to the occurrence of MI. Further research is warranted to elucidate these interactions.

Keywords: 4G/5G polymorphism; Mendelian randomization; meta-analysis; myocardial infarction; plasminogen activator inhibitor type 1 (PAI-1).

Introduction

Myocardial infarction (MI) is usually associated with thrombosis at the site of a ruptured atherosclerotic plaque. The dynamic balance between coagulation and fibrinolysis is an important determinant of the thrombotic response, which, in the setting of atherosclerosis, may influence the development of acute MI [1]. An important factor in the regulation of endogenous fibrinolysis seems to be the plasminogen activator inhibitor type 1 (PAI-1), which blocks both tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) [2]. Besides modulating thrombolysis, fibrinolytic activity likely influences the progression of atherosclerotic lesions.
Overexpression of PAI-1 may also promote the development of weak plaques with thin fibrous caps by inhibiting both u-PA receptor- and integrin-mediated cell adhesion and migration [4, 5]. Therefore, increased PAI-1 levels may play a crucial role in the occurrence of MI by affecting both atheroma and thrombosis.

The association between PAI-1 levels and the risk for MI is equivocal [6]. The interpretation of epidemiological information concerning PAI-1 involvement in cardiovascular outcomes is complicated by the presence of potentially confounding variables. Factors such as obesity, hypertension [7], non-insulin dependent diabetes [8], hypercholesterolemia [9], and smoking [10], which are known to be related to PAI-1 levels and simultaneously affect the incidence of MI, do not allow a definite inference on the causal relationship between PAI-1 and MI risk. The regulation of PAI-1 is multi-factorial and, besides the influence of environmental factors, circulating PAI-1 levels are under genetic control. A sequence length polymorphism in the promoter region of the PAI-1 gene – a single guanosine insertion/deletion – commonly called 4G/5G, at position -675, has been extensively studied and found to be functionally important [11]. It has been suggested that the 4G allele results in higher activity than the 5G allele because the latter contains an additional binding site for a transcriptional repressor [12]. Previous studies on the 4G/5G polymorphism support its role as a risk factor for coronary artery disease (CAD) and MI [6, 13, 14]. It has been proposed, however, that the association between the 4G/5G gene polymorphism and the risk for MI is considerably affected by the underlying metabolic syndrome (MetS) [15]. The evidence regarding the association between the above-mentioned genetic variant and MetS components is contradictory [15–21].

Given the causal relationship between MetS and cardiovascular disease, it remains unclear whether elevation in PAI-1 levels is a real causal risk factor for MI or just a marker of atherosclerosis. The current study takes an advanced meta-analytical approach, by combining information from genotype-disease and genotype-phenotype research and by using Mendelian randomization, to simultaneously evaluate the associations between the PAI-1 promoter 4G/5G polymorphism, the circulating PAI-1 levels, and the incident MI.

Materials and methods

The current meta-analysis was conducted according to MOOSE guidelines [22] and the PRISMA statement [23]. The methodology was prespecified and documented in a protocol.

Data sources, search strategy and eligibility criteria

A comprehensive search of MEDLINE and SCOPUS electronic databases was conducted through December 2012. The following keywords or combinations of them were used: “plasminogen activator inhibitor”, “PAI-1”, and “myocardial infarction”. After initial screening of titles and abstracts, only relevant studies remained. Subsequently, full-text articles were critically evaluated for eligibility and their references’ lists were manually scanned to identify further studies for inclusion.

Studies were included in the analysis if: 1) they had examined the association between PAI-1 4G/5G polymorphism and MI using an observational design; and 2) they provided adequate data to calculate an estimate of the relative risk for MI. In the analysis of the mean genotype difference in PAI-1 concentration, which is supposed to be the intermediate phenotype on the causal pathway from PAI-1 4G/5G polymorphism to MI, studies were considered eligible if they had reported measurements of PAI-1 activity across the PAI-1 4G/5G genotypes of healthy populations. Family-based research using the transmission disequilibrium test (TDT) was excluded. To avoid selection bias, no language or quality restrictions were imposed [22, 24]. Furthermore, in an effort to limit the effect of “gray literature”-related bias [25], we also searched for studies published in conference proceedings or as short abstracts.

Data extraction and quality assessment

Data extraction and quality assessment were undertaken by two independent authors (CT and AT). Any discrepancy was resolved by consensus. The following information was extracted from each study and recorded in a datasheet: 1) first author’s name, journal, year of publication, and geographical setting in which the study was undertaken; 2) number of cases and controls; 3) 4G/4G, 4G/5G and 5G/5G genotype distributions in cases and controls; and 4) summary measures of many parameters concerning the participants including age, gender, racial descent, PAI-1 activity, smoking, body mass index (BMI), cholesterol and triglyceride levels, hypertension, and diabetes mellitus. This information was necessary for running meta-regression analyses of the effect estimates against study level parameters and for the Mendelian
Statistical analysis

The odds ratio (OR) was the metric of choice in all comparisons concerning the association of PAI-1 4G/5G polymorphism with MI. The mean difference in PAI-1 activity between the PAI-1 4G/5G genotypes in the control group was used in the continuous data analysis. The available primary information was initially combined using conventional random effects models with inverse variance weights.

In the current research, the interest was in jointly modeling the log-ORs for disease occurrence (MI) and the mean phenotype difference (PAI-1 activity) between the three genotypes (PAI-1 4G/4G, 4G/5G, 5G/5G). The integrated meta-analysis applied here is a Mendelian randomization approach, which appeals to the seemingly random process of genes’ inheritance analogous to treatment allocation in clinical trials and remains unaffected by confounding or reverse causation commonly observed in classical phenotype-disease studies. Therefore, this method can explore the causal role of an intermediate phenotype in the risk of developing a particular disease [26]. In other words, under certain assumptions [27], the Mendelian randomization methods could investigate whether PAI-1 indeed lies on the etiological pathway to MI using as instrumental variable the PAI-1 4G/5G polymorphism, which has been shown in some epidemiological studies to be associated with both PAI-1 levels and MI risk.

Briefly, in our analysis the genotype-disease and genotype-phenotype associations were modeled simultaneously in a multivariate framework. Having access to all genotypes (4G/4G, 4G/5G, and 5G/5G), the multivariate approach of Palmer and coworkers [28], incorporating the genetic model-free method [29, 30], was applied to determine the underlying inheritance model. Subsequently, the genotypes were combined according to the most plausible genetic model. The meta-analytical estimates were obtained from the aforementioned approaches for Mendelian randomization and from another bivariate method proposed by Minelli, Thompson and coworkers [31, 32]. All techniques described above are random-effects methods based on maximum likelihood.

Meta-regression analyses were used to explore the potential association between other factors involved in the development of MI and the risk for MI conferred by the PAI-1 4G allele. It is well known in epidemiology that by adjusting for intermediate variables (or descending proxies for unmeasured intermediates) on the causal pathway to the outcome of interest, the total effect estimate for the exposure under investigation is likely to decrease or even to vanish when the intermediate variables considered comprise the only path between the exposure and the outcome [33]. In other words, controlling for intermediate variables in regression analyses biases the results towards the null. However, at the same time, this property can be used to explore whether a variable is likely to lie or to represent another variable that indeed lies between the exposure and the outcome. In particular, if PAI-1 4G allele (exposure) really exerts its influence on MI risk (outcome of interest) through PAI-1 activity (intermediate variable) or through known risk factors for MI, such as cholesterol or triglyceride levels (intermediate variables or proxy indicators of other unmeasured intermediates), when we insert them in regression models, the estimated OR for the PAI-1 4G allele is expected to diminish.

The Begg’s rank correlation method [34], the Egger’s fixed-effects regression method [35], and its random-effects analog [36], were used to search for the presence of publication bias. Cumulative meta-analysis [37–39] was used to identify a possible time trend in the overall estimate. For all analyses, Stata 12 was used (Stata Corp., College Station, TX, USA). Statistically significant results were regarded those with a p-value lower than 0.05.

Results

The literature search (Figure 1) yielded 53 studies (Table 1). Among them, 33 studies provided the 4G/5G genotype frequencies for cases and controls, six studies reported the 4G/5G genotype distribution in cases and controls and the mean PAI-1 activity levels by genotype in controls, and the remaining 14 studies had measured the mean PAI-1 activity by genotype in control groups.

The multivariate meta-analytical approach using Mendelian randomization [28] combined with the genetic model-free method [29] suggested that the risk for MI conferred by the 4G/5G polymorphism (measured on the log-OR scale) behaves following an additive model of inheritance.
Taking into account the suggested additive genetic model, the meta-analysis was based on alleles’ comparisons. The conventional univariate per-allele OR indicated an increased risk for MI for people carrying the 4G variant (1.101; 95% CI 1.030, 1.177). In addition, the 4G carriers had, on average, higher PAI-1 activity by 1.132 units compared to 5G carriers (95% CI 0.741, 1.523). The heterogeneity in both meta-analyses (log-ORs for MI and mean differences in PAI-1 activity between carries of 4G and 5G alleles) was substantial (I^2 equal to 68.7% and 89.2%, respectively), and it could not be largely attributed to the racial differences of the participants (i.e., Asian vs. Caucasian) or the geographical setting in which the studies were conducted (European vs. other). The heterogeneity diminished substantially to 51.3% only when the log-OR analysis was restricted to Northern European countries. Applying a previously suggested bivariate method [31, 32], which also takes advantage of Mendelian randomization, and performing a joint modeling of the log-OR for disease along with the mean, across genotypes, difference in phenotype levels, the pooled per-allele estimate for MI risk changed slightly (1.088; 95% CI 1.007, 1.175). The estimated summary mean difference in PAI-1 activity between 4G and 5G carriers in the control population was almost identical to the estimate derived from the traditional random-effects method mentioned before (1.136; 95% CI 0.738, 1.533) (Figure 2).

The meta-regression models (Figure 3), using cholesterol and triglyceride levels, and PAI-1 activity in controls as co-variates, showed the association of these variables with the risk for MI conferred by the PAI-1 4G allele (β=-0.487 with p=0.005 for triglycerides; β=-0.234 with p=0.037 for cholesterol; and β=-0.030 with p=0.021 for PAI-1 activity). However, the BMI in controls was not statistically associated (p>0.05) with the 4G effect on MI risk (Figure 3). It seems thus that at least a fraction of the MI risk conferred by PAI-1 4G allele is mediated through cholesterol/triglyceride levels and PAI-1 activity or through other variables that are highly correlated with them.

The formal statistical tests provided some evidence for the existence of publication bias (p<0.05 for many tests). Cumulative meta-analyses did not reveal any significant trend of the estimates over time (p>0.05 in all cases).

**Discussion**

The current meta-analysis, based on the most updated information (53 studies) and the concept of Mendelian randomization, showed that the PAI-1 4G allele was related to a slightly elevated MI risk (around 10%) as has previously been suggested by other teams (4G allele risk for coronary...
Table 1  Eligible studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Study type</th>
<th>Cases (MI)</th>
<th>Controls (no MI)</th>
<th>Race</th>
<th>Genotypes in cases</th>
<th>Genotypes in controls</th>
<th>PAI-1a activity levels in controls (SD), U/mL</th>
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<tbody>
<tr>
<td>Anderson et al. [40]</td>
<td>1999</td>
<td>USA</td>
<td>Case-control</td>
<td>375</td>
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<td>105 193 77</td>
<td>303 457 218</td>
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<td>2003</td>
<td>USA</td>
<td>Case-control</td>
<td>264</td>
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<td>70 136 58</td>
<td>200 387 166</td>
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<td>Germany</td>
<td>Case-control</td>
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<td>1351</td>
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<td>382 606 226</td>
<td>409 684 258</td>
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<td>2002</td>
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<td>Case-control</td>
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<td>17 32 29</td>
<td>115 187 83</td>
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<td>Mikkelsson et al. [44]</td>
<td>2000</td>
<td>Finland</td>
<td>Prospective (autopsy series)</td>
<td>68</td>
<td>164</td>
<td>Caucasian</td>
<td>18 38 12</td>
<td>29 78 57</td>
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</tr>
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<td>Case-control</td>
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<td>Asian</td>
<td>5 28 33</td>
<td>6 27 29</td>
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<td>Pegoraro and Ranjith [46]</td>
<td>2005</td>
<td>S. Africa</td>
<td>Case-control</td>
<td>195</td>
<td>300</td>
<td>Asian Indian</td>
<td>42 99 54</td>
<td>65 132 103</td>
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<td>Eriksson et al. [12]</td>
<td>1995</td>
<td>Sweden</td>
<td>Case-control</td>
<td>93</td>
<td>100</td>
<td>Caucasian</td>
<td>40 38 15</td>
<td>26 54 20</td>
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<td>1997</td>
<td>USA</td>
<td>Cohort</td>
<td>374</td>
<td>495</td>
<td>Mixed</td>
<td>101 191 82</td>
<td>133 247 115</td>
<td>– – –</td>
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<td>Ardissino et al. [48]</td>
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<td>Italy</td>
<td>Case-control</td>
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<td>200</td>
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<td>38 93 69</td>
<td>32 102 66</td>
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<td>France</td>
<td>Case-control</td>
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<td>Caucasian</td>
<td>48 97 56</td>
<td>64 121 59</td>
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<td>Pastinen et al. [50]</td>
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<td>Cross sectional</td>
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<td>30 80 40</td>
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<td>1210</td>
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<td>Slovenia</td>
<td>Cross sectional</td>
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<td>68 89 37</td>
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<td>2007</td>
<td>Turkey</td>
<td>Case-control</td>
<td>156</td>
<td>392</td>
<td>Asian (Turkish)</td>
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<td>90 182 120</td>
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<td>Martinelli et al. [55]</td>
<td>2008</td>
<td>Italy</td>
<td>Case-control</td>
<td>307</td>
<td>497</td>
<td>Caucasian</td>
<td>92 154 61</td>
<td>152 254 91</td>
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<td>Tassies et al. [56]</td>
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<td>Spain</td>
<td>Prospective</td>
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<td>Case-control</td>
<td>201</td>
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<td>28 89 23</td>
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<td>2007</td>
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<td>Case-control</td>
<td>461</td>
<td>474</td>
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<td>84 150 68</td>
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<td>Case-control</td>
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<td>217</td>
<td>Asian</td>
<td>64 86 79</td>
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<td>Case-control</td>
<td>100</td>
<td>36</td>
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<td>Controls (no MI)</td>
<td>Race</td>
<td>Genotypes in cases</td>
<td>Genotypes in controls</td>
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<td>China</td>
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<td>Germany</td>
<td>Case-control</td>
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<td>5G/5G</td>
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<td>Ireland</td>
<td>Case-control</td>
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*myocardial infarction; †plasminogen activator inhibitor type 1; ‡standard deviation.
Figure 2  Forest plots of multivariate meta-analyses concerning the effect of the PAI-1 4G/5G polymorphism on the risk for myocardial infarction and the mean difference in PAI-1 activity.

PAI-1, plasminogen activator inhibitor type 1.

Figure 3  Meta-regression models using as dependent variable the natural logarithm of the MI-related OR for the PAI-1 4G variant and as independent variables study-level parameters including the concentration of triglycerides and cholesterol, the PAI-1 activity and the BMI in the control population.

BMI, body mass index; MI, myocardial infarction; OR, odds ratio; PAI-1, plasminogen activator inhibitor type 1.
The fibrinolytic system probably influences the progression of atherosclerotic lesions through several pathophysiological mechanisms [3]. Many investigators have noted that PAI-1 is in excess in atherosclerotic plaques in humans [90–92]. Whether PAI-1 has indeed a casual nature in MI or MetS, or is only a bystander, is difficult to assess in humans. One way to address the research hypothesis is to simultaneously look for associations between PAI-1 4G/5G polymorphism, PAI-1 expression or activity, MI, and components of MetS. Therefore, in this meta-analysis, we tried not only to identify studies exploring the relationship between PAI-1 4G/5G polymorphism and MI risk, but we also searched for measurements of PAI-1 activity in the 4G/4G, 4G/5G, and 5G/5G genotypes. Moreover, in all studies, we retrieved, if available, information on MetS parameters. The Mendelian randomization approaches used the genetic information as an instrumental variable to adjust for confounding effects or reverse causation bias and allowed the simultaneous modeling of the associations between PAI-1 4G/5G polymorphism (genotype), MI risk (disease), and PAI-1 activity (phenotype). Importantly, in all models, the 4G allele carriers had higher PAI-1 activity and faced an elevated MI risk. This is considerable evidence that PAI-1 activity is indeed on the causal pathway between PAI-1 4G/5G polymorphism and the occurrence of MI. In addition, meta-regression analyses showed that controlling for PAI-1 activity in controls, the risk for MI conferred by the PAI-1 4G allele decreases. This reduction of effect size towards the null is observed when an intermediate variable on the pathogenetic mechanism is included in a regression model that also contains the initial exposure. There is thus another piece of evidence supporting the hypothesis that PAI-1 4G allele heightens the risk for MI by changing PAI-1 activity. Interestingly, two parameters of the metabolic syndrome, i.e., cholesterol and triglyceride levels in controls, also attenuated the risk for MI conferred by the PAI-1 4G allele in the meta-regression analysis. To avoid confusion, this finding should not be translated as an opposition to the current state of knowledge that accepts the association between increased cholesterol or triglyceride levels and poor cardiovascular health. Instead, it is an indication that the impact of the PAI-1 4G allele on MI development is mediated not only by PAI-1 levels but is also related with MetS elements or other unmeasured covariates that are highly correlated with them.

Our hypothesis that the increase in the risk of MI conferred by the 4G allele could be associated with MetS components seems to have biological basis. Insulin, proinsulin-like molecules, glucose and very low-density lipoprotein (VLDL) have been suggested to stimulate PAI-1 transcription and secretion in endothelial cells [93]. Several reports have suggested that PAI-1 activity correlates significantly with serum triglycerides [20, 84], and these associations were influenced by attributes in the region of the PAI-1 gene promoter. The 4G allele was also associated with cholesterol levels in CAD patients [9], and the levels of cholesterol were significantly higher in the 4G/4G genotype than in other groups [18]. Another recent study reported an association of large low-density lipoprotein (LDL) particles with homozygous 4G patients experiencing recurrent events [94]. Allison et al. reported that triglyceride-enriched LDL is a potent activator of PAI-1 protein and mRNA [95]. Finally, an identified VLDL responsive element adjacent to the 4G/5G polymorphism site is thought to serve as a basis for allele-specific effects of triglyceride-rich lipoproteins on PAI-1 blood levels [96]. A prior study suggested that elevated PAI-1 activity might be only the consequence of increased body fat that secretes excess PAI-1 [97]. However, the inverse relation has been proposed by an experimental study, which supported the concept that overexpression of PAI-1 could be involved causally in the development of obesity [98].

Doubtless, although circulating levels of PAI-1 could determine MI risk through two separate mechanisms, i.e., atherogenesis and occlusive thrombosis, it seems that the influence of the 4G/5G polymorphism through its intermediate phenotype on atherogenesis, either directly or through some of the components of MetS, is probably greater than that on coronary thrombosis. This is in accordance with the findings of a recent meta-analysis that reported a positive association between the PAI-1 4G genotype and cardiac disease but a lack of relationship with stroke [14]. Similarly, we did not demonstrate a significant association between the 4G/5G polymorphism and ischemic stroke under basal conditions [99]. Stroke is not as clearly related to MetS as CAD is. Rupture of an atherosclerotic plaque is considered a central event in the pathogenesis of MI. On the contrary, in the case of stroke, thromboembolism without significant underlying large vessel disease is a more common cause of cerebral ischemia [100].

Our analysis is probably limited by the inclusion of studies which had, in most cases, recruited only MI survivors. Therefore, it cannot be excluded that hypofibrinolysis due to this genetic variant may be associated with more severe MIs and a larger number of early deaths, and the
effect on the risk of MI may have been underestimated. Secondly, meta-analysis cannot correct or adjust for biases in primary research. Third, the Mendelian randomization approach has certain assumptions and their potential violation might affect the validity of the results [28]. Finally, in our analysis, there is considerable heterogeneity of the estimates across studies and publication bias is likely.

In conclusion, although MI is considered a multifactorial and polygenic disease, there is evidence that the 4G allele could affect the risk for MI by increasing the concentration of PAI-1 and the 4G-related risk of MI is also associated with the levels of cholesterol and triglycerides. However, further research is necessary. Future individual studies on the association between PAI-1 4G and MI should always collect information on the intermediate phenotype (PAI-1) and on other MI risk factors, which can be later used in causation models. If future analyses clearly show that raised plasma PAI-1 levels lie on the etiological pathway to MI then PAI-1 will have important clinical and public health implications, and methods to reduce its levels would be worthy.

Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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