Platelet Glycoprotein IIb/IIa \( \text{PlA}^2/\text{PlA}^2 \) Homozygosity Associated With Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in Young Men

The Copenhagen City Heart Study

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**OBJECTIVES** We tested the hypothesis that platelet glycoprotein (GP) IIb/IIa \( \text{PlA}^2/\text{PlA}^2 \) homozygotes or \( \text{PlA}^1/\text{PlA}^2 \) heterozygotes versus \( \text{PlA}^1/\text{PlA}^1 \) noncarriers have increased risk of ischemic cardiovascular disease and myocardial infarction (MI), stratified for age and gender.

**BACKGROUND** The GP IIb/IIa \( \text{PlA}^1/\text{PlA}^2 \) polymorphism influences aggregation of platelets; however, an association between ischemic cardiovascular disease and heterozygosity remains controversial, and association with homozygosity is largely unexplored.

**METHODS** We genotyped the participants of the Copenhagen City Heart Study, a prospective cardiovascular investigation of the Danish general population (n = 9,149, 22-year follow-up) and assessed the risk of ischemic cardiovascular disease in homozygotes or homozygotes versus noncarriers.

**RESULTS** Of the participants, 70.0%, 27.3%, and 2.7% were noncarriers, heterozygotes, or homozygotes, respectively. Incidence of ischemic cardiovascular disease was 167 and 103 per 10,000 person-years in homozygous and noncarrier men (log-rank: \( p = 0.006 \)), whereas this difference was not observed in women (\( p = 0.33 \) (genotype-gender interaction: \( p = 0.03 \))). In homozygous versus noncarrier men <40 years of age, 40 to 50 years, and >50 years at entry, age-adjusted relative risks (RRs) of ischemic cardiovascular disease were 3.6 (1.4 to 9.0), 2.4 (1.3 to 4.6), and 1.0 (0.6 to 1.8), respectively (age-genotype interaction in men: \( p = 0.04 \)); equivalent multifactorially adjusted RRs were 3.0 (1.1 to 8.0), 2.0 (1.0 to 3.9), and 1.0 (0.6 to 1.8), respectively. The corresponding age-adjusted RR values of MI in men were 5.2 (1.5 to 18), 3.5 (1.6 to 7.5), and 0.5 (0.1 to 1.5), respectively (age-genotype interaction in men: \( p = 0.002 \)); equivalent multifactorially adjusted RRs were 3.8 (1.0 to 15), 3.1 (1.4 to 6.9), and 0.5 (0.2 to 1.5), respectively.

**CONCLUSIONS** \( \text{PlA}^2/\text{PlA}^2 \) homozygosity is associated with a three-fold and four-fold risk of ischemic cardiovascular disease and MI in young men.

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Binding of adhesive plasmaproteins as von Willebrand factor and fibrinogen to membrane-bound glycoprotein (GP) IIb/IIa of platelets plays a pivotal role in platelet aggregation (1). The \( \text{PlA}^1/\text{PlA}^2 \) polymorphism, also called HPA-1a/HPA-1b or Zw(a)/Zw(b), causes a substitution of the wild-type Leu-33 to proline in the Leu33-Pro on platelet aggregation (2), and consequently on the extracellularly positioned conformational change of the \( \beta_2 \)-subunit of GP IIb/IIa, resulting in an extracellularly positioned conformational change of the \( \beta_2 \)-subunit. Therefore, it has been considered biologically plausible to suggest an impact of Leu33-Pro on platelet aggregation (2), and consequently on risk of ischemic cardiovascular disease (3).

In a recent meta-analysis (4) of mainly case-control studies with conflicting results encompassing more than 17,000 individuals, aggregated results showed an odds ratio for ischemic cardiovascular disease in \( \text{PlA}^1/\text{PlA}^2 \) heterozygotes combined with \( \text{PlA}^2/\text{PlA}^2 \) homozygotes versus \( \text{PlA}^1/\text{PlA}^1 \) noncarriers of 1.10 (95% confidence interval [CI] 1.03 to 1.18). Only two prospective studies have been published so far (5,6), and all studies published have been too small to specifically investigate homozygosity, let alone having been stratified for age and gender.

We tested the hypothesis that platelet GP IIb/IIa homozygotes or heterozygotes versus noncarriers have increased risk of ischemic cardiovascular disease and myocardial infarction (MI), overall or stratified for age and gender. For this purpose we used the Copenhagen City Heart Study, a prospective cardiovascular study of the Danish general population with 9,149 participants and a total of 22 years’ follow-up.

**METHODS**

From the Danish general population, by use of the Danish Central Population Register number, we randomly recruited 4,082 men and 5,067 women who participated in the third examination of the Copenhagen City Heart Study, 1991 to 1994 (7–12). Participants were screened for manifestations...

Myocardial infarction was classified according to World Health Organization International Classification of Diseases 8th ed. (ICD-8) code 410, or 10th ed. (ICD-10) codes I21-122. Non-MI ischemic heart disease was ICD-8 codes 411-413 or ICD-10 codes I20 and I25: diagnosis was based on characteristic symptoms of stable angina pectoris according to the guidelines of the European Society of Cardiology (location, character, and duration of pain and the relation of pain to exercise [14]).

Ischemic stroke was ICD-8 codes 432-434 or ICD-10 code I63, excluding computed tomography proven hematoma, subarachnoidal hemorrhage, or transient ischemic attack. Individual diagnoses were verified by experienced cardiologists and neurologists, respectively (15). Participants with disease before study entry were excluded from the study (n = 93).

We had 99.97% follow-up; three individuals were lost. More than 99% were whites of Danish descent. All participants gave written informed consent. The ethical committees of Copenhagen and Frederiksberg approved the study (No. 100.2039/91).

The PlA2-allele, also called HPA-1b or Zw(b), is caused by a T→C substitution in nucleotide 176 after A in the start-codon in the gene integrin β3 (GenBank AccNo NM_000212). This polymorphism was examined as earlier described (16): in short, a 268-bp polymerase chain reaction-fragment covering the whole of exon 3 (GenBank AccNo M32672) was amplified from genomic deoxyribonucleic acid by the use of intronic primers: sense: TTCT-GATTGCGTGGACTTCTCTT; antisense: TCTTCTCCCCACGGGCAAAGAGT. After thermocycling, the polymerase chain reaction was digested with the restriction endonuclease MspI, run on a 3% agarose gel and visualized using ethidium bromide. The PlA1/PlA1 noncarrier genotype produced a 221/38/8-bp-pattern, PlA1/PlA2 heterozygous genotype a 221/177/44/38/8-bp-pattern, and PlA2/PlA2 homozygous genotype a 177/44/38/8-bp-pattern.

Blood samples were drawn in the nonfasting state. Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, fibrinogen (all Boehringer Mannheim, Mannheim, Germany), and lipoprotein(a) total mass (DAKO A/S, Glostrup, Denmark). The diagnosis of diabetes mellitus was assigned according to participants’ own knowledge of their disease status. The status of smoker was assigned to current and former smokers.

The statistical software package SPSS (SPSS Inc., Chicago, Illinois) was used. A p value <0.05 on a two-sided test was considered significant. For characteristics of participants, we used the Student t test on untransformed or log-transformed variables, Mann-Whitney U test, or the Pearson chi-square test. Multifactorial adjustment included all other parameters listed in Table 1 in an analysis of covariance or logistic regression model. Continuous variables were divided in gender-specific tertiles. It was decided a priori to stratify main analyses for gender and age. We plotted cumulative disease incidence against follow-up time using the Kaplan-Meier method, and we tested differences between genotypes using log-rank statistics. Visual inspection of log-minus-log curves was employed to exclude disproportion of hazards over time. Relative risk for disease with 95% CI was calculated using the Cox regression, unadjusted, adjusted for age at entry in decades, or all the factors listed in Table 1 (multifactorial adjustment). We tested for multiplicative interactions among gender, age, and genotype-status in predicting disease, by introducing two-factor interaction terms after inclusion of individual parent terms in the Cox regression model.

RESULTS

We genotyped 9,149 participants from the Danish general population. Of those, 70.0%, 27.3%, and 2.7% were PlA1/PlA1 noncarriers, PlA1/PlA2 heterozygotes, and PlA2/PlA2 homozygotes, respectively. This distribution did not differ from Hardy-Weinberg equilibrium (chi-square test: all, p = 0.81; men, p = 0.97; women, p = 0.77; Table 2). Allele frequencies of the PlA1-allele and PlA2-allele were 83.6/16.4% overall, 83.6/16.4% in men, and 83.7/16.3% in women. Established risk factors for ischemic cardiovascular disease, except for fibrinogen in men, diabetes and hypertension in women, and lipoprotein(a) in both genders, were evenly distributed among the three genotypes (Table 1). Most of these differences were likely due to chance, and none would remain significant after correction for multiple comparison. In contrast, many differences existed in risk factor distribution between men and women (Table 1).

Incidence of ischemic cardiovascular disease in homozygous and noncarrier men was 167 and 103 per 10,000 person-years (log-rank: p = 0.006) (Fig. 1, Table 2). In an age-adjusted Cox regression model, RR of ischemic cardiovascular disease in homozygotes versus noncarriers was 1.5 (95% CI 1.1 to 2.2) and 0.7 (95% CI 0.4 to 1.4) in men and women, respectively (Table 2). In accordance with this gender difference, gender and (homozygous vs. noncarrier) genotype interacted on ischemic cardiovascular disease (p =
end point in either age group in men or women. Unadjusted heterozygosity versus noncarrier status did not predict either disease or MI in either age group (Table 3). Likewise, noncarrier status did not predict ischemic cardiovascular (0.2 to 1.5), respectively. In women, homozygosity versus adjusted RRs were 3.8 (1.0 to 15), 3.1 (1.4 to 6.9), and 0.5 (0.2 to 1.5), respectively. In women, homozygosity versus noncarrier status did not predict either end point in either age group in men or women. Unadjusted and multifactorial adjusted models gave results similar to age-adjusted models (Table 3).

Fibrinogen, a well-recognized predictor of MI (17,18) directly interacting with platelet GP IIb/IIIa in aggregation of platelets, is in our study higher among PI^{A2}/PI^{A2} men than among PI^{A1}/PI^{A2} heterozygous or PI^{A1}/PI^{A1} noncarrier men (Table 1). Accordingly, fibrinogen level could confound the association between disease and homozygosity in men; however, after multifactorial adjustment (including fibrinogen), the RR for homozygous men remained elevated (Tables 2 and 3).

Age at first MI was 61 ± 2.5 years (mean ± SE) and 66 ± 0.5 years in homozygous and noncarrier men (Table 4; Mann Whitney U test: p = 0.03); equivalent values for ischemic cardiovascular disease were 64 ± 2.0 and 67 ± 0.5 years (p = 0.07). This difference was not observed between homozygotes and noncarrier women, or between heterozygotes and noncarriers of either gender (Table 4).

The unadjusted RRs of death due to MI with seven years of follow-up (1991 to 1994 through 1998) for noncarriers, heterozygotes, and homozygotes were 1.0, 0.8 (95% CI 0.5 to 1.3), and 1.8 (95% CI 0.7 to 5.0) for men and 1.0, 1.0 (95% CI 0.5 to 2.2), and 1.1 (95% CI 0.2 to 8.4) for women.

Table 1. Characteristics of Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Noncarriers (PI^{A1}/PI^{A1})</th>
<th>Heterozygotes (PI^{A1}/PI^{A2})</th>
<th>Homozygotes (PI^{A2}/PI^{A2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>Age at entry (yrs)</td>
<td>Fibrinogen (mg/l)</td>
</tr>
<tr>
<td></td>
<td>2,854 (68.7)</td>
<td>44.5 ± 0.21</td>
<td>3.06 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>1,118 (28.6)</td>
<td>44.7 ± 0.35</td>
<td>3.07 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>110 (2.6)</td>
<td>45.5 ± 1.8</td>
<td>3.33 ± 0.11§</td>
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<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE or frequencies. Heterozygotes and homozygotes were compared with noncarriers of the same gender using the Mann-Whitney U test, the Pearson chi-square test, or Student t test on untransformed or log-transformed parameters. *p < 0.05. †p < 0.01. Equivalent multifactorial adjusted comparison including all other covariants listed in the Table using analysis of covariance or logistic regression; ‡p < 0.05. §p < 0.01. Women and men were compared using the Mann-Whitney U test, the Pearson chi-square test, or Student t test on untransformed or log-transformed parameters.

HDL = high-density lipoprotein.

0.03). For the end point of MI, the results were similar to those for ischemic cardiovascular disease; however, statistical significance was not reached (Table 2). Heterozygotes did not differ from noncarriers with respect to incidence or risk of ischemic cardiovascular disease or MI in either gender (Table 2). Unadjusted and multifactorial adjusted models gave results similar to age-adjusted models (Table 2).

In homozygous versus noncarrier men aged <40 years, 40 to 50 years, and >50 years at entry, age-adjusted RR values of ischemic cardiovascular disease were 3.6 (1.4 to 9.0), 2.4 (1.3 to 4.6), and 1.0 (0.6 to 1.8), respectively (genotype-age interaction in men: p = 0.04; Table 3); equivalent multifactorially adjusted RR values were 3.0 (1.1 to 8.0), 2.0 (1.0 to 3.9), and 1.0 (0.6 to 1.8), respectively. The corresponding age-adjusted RRs of MI in men were 5.2 (1.5 to 18), 3.5 (1.6 to 7.5), and 0.5 (0.1 to 1.5), respectively (age-genotype interaction in men: p = 0.002); equivalent multifactorially adjusted RRs were 3.8 (1.0 to 15), 3.1 (1.4 to 6.9), and 0.5 (0.2 to 1.5), respectively. In women, homozygosity versus noncarrier status did not predict ischemic cardiovascular disease or MI in either age group (Table 3). Likewise, heterozygosity versus noncarrier status did not predict either end point in either age group in men or women. Unadjusted
respectively. Only 81 (59 noncarriers, 18 heterozygous, and 4 homozygous) men and 33 (23 noncarriers, 9 heterozygous, and 1 homozygous) women died of MI during this short period; therefore, the statistical power is much less in these analyses on mortality compared with the analyses on morbidity.

**DISCUSSION**

This study reports an increased risk of ischemic cardiovascular disease and MI in young men homozygous for the PlA2/PlA2 and MI

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**Table 2. Incidence and Relative Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in PlA1/PlA2 Heterozygotes or PlA2/PlA2 Homozygotes Versus PlA1/PlA1 Noncarriers by Cox Regression Analysis**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Hardy-Weinberg Equilibrium</th>
<th>First Events</th>
<th>Incidence (95% CI)/10,000 Person-Years</th>
<th>Relative Risk (95% CI) Adjusted</th>
<th>First Events</th>
<th>Incidence (95% CI)/10,000 Person-Years</th>
<th>Relative Risk (95% CI) Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>2,854</td>
<td>0.97</td>
<td>470</td>
<td>103 (95–114)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>1,118</td>
<td>p = 0.97</td>
<td>190</td>
<td>107 (93–124)</td>
<td>1.1 (0.9–1.2)</td>
<td>1.1 (0.9–1.3)</td>
<td>1.0 (0.9–1.2)</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>110</td>
<td></td>
<td>29</td>
<td>167 (112–239)</td>
<td>1.7 (1.2–2.4)</td>
<td>1.5 (1.1–2.2)</td>
<td>1.5 (1.0–2.1)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>3,550</td>
<td>0.77</td>
<td>347</td>
<td>58 (52–65)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>1,379</td>
<td>p = 0.77</td>
<td>139</td>
<td>58 (49–69)</td>
<td>1.0 (0.8–1.2)</td>
<td>1.0 (0.8–1.2)</td>
<td>0.9 (0.8–1.2)</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>138</td>
<td></td>
<td>10</td>
<td>43 (21–79)</td>
<td>0.7 (0.4–1.4)</td>
<td>0.7 (0.4–1.4)</td>
<td>0.6 (0.3–1.2)</td>
</tr>
<tr>
<td>Gender-genotype interaction test</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygotes vs. noncarriers</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.62</td>
<td>p = 0.59</td>
<td>p = 0.67</td>
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<tr>
<td>Homozygotes vs. noncarriers</td>
<td></td>
<td></td>
<td></td>
<td>p = 0.02</td>
<td>p = 0.03</td>
<td>p = 0.02</td>
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Multifactorial adjustment included all factors listed in Table 1.
CI = confidence interval.
Table 3. Incidence and Relative Risk of Ischemic Cardiovascular Disease and Myocardial Infarction in Pt*1/Pt*2 Heterozygotes or Pt*2/Pt*2 Homozygotes Versus Pt*1/Pt*1 Noncarriers by Cox Regression, Stratified by Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>Participants (n)</th>
<th>Incidence (95% CI)/10,000 Person-Years</th>
<th>Relative Risk (95% CI)</th>
<th>Myocardial Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardy-Weinberg equilibrium</td>
<td>First Events (n)</td>
<td>None</td>
<td>Age</td>
</tr>
<tr>
<td>Men</td>
<td>&lt;40 yrs</td>
<td>Noncarriers</td>
<td>999</td>
<td>999</td>
<td>28 (21-39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>414</td>
<td>414</td>
<td>33 (20-50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygotes</td>
<td>37</td>
<td>37</td>
<td>102 (33-238)</td>
</tr>
<tr>
<td></td>
<td>40-50 yrs</td>
<td>Noncarriers</td>
<td>917</td>
<td>917</td>
<td>93 (78-109)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>319</td>
<td>319</td>
<td>89 (67-122)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygotes</td>
<td>30</td>
<td>30</td>
<td>210 (101-366)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Noncarriers</td>
<td>938</td>
<td>938</td>
<td>176 (158-199)</td>
</tr>
<tr>
<td>&gt;50 yrs</td>
<td>Noncarriers</td>
<td>305</td>
<td>305</td>
<td>187 (156-222)</td>
<td>1.0 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>385</td>
<td>385</td>
<td>126 (100-154)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygotes</td>
<td>43</td>
<td>43</td>
<td>14 (9-23)</td>
</tr>
<tr>
<td>Women</td>
<td>&lt;40 yrs</td>
<td>Noncarriers</td>
<td>1,123</td>
<td>1,123</td>
<td>12 (8-19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>409</td>
<td>409</td>
<td>16 (8-30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygotes</td>
<td>41</td>
<td>41</td>
<td>0 (0-70)</td>
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<tr>
<td></td>
<td>40-50 yrs</td>
<td>Noncarriers</td>
<td>1,036</td>
<td>1,036</td>
<td>42 (33-53)</td>
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<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>399</td>
<td>399</td>
<td>47 (32-66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygotes</td>
<td>43</td>
<td>43</td>
<td>26 (3-95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Noncarriers</td>
<td>1,391</td>
<td>1,391</td>
<td>256 (98-108)</td>
</tr>
<tr>
<td>&gt;50 yrs</td>
<td>Noncarriers</td>
<td>571</td>
<td>571</td>
<td>88 (73-109)</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygotes</td>
<td>54</td>
<td>54</td>
<td>76 (33-150)</td>
</tr>
</tbody>
</table>

Age-genotype interaction test

Men

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (95% CI)/10,000 Person-Years</th>
<th>Relative Risk (95% CI)</th>
<th>Myocardial Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygotes vs. noncarriers</td>
<td>p = 0.85</td>
<td>p = 0.87</td>
<td>p = 0.93</td>
</tr>
<tr>
<td>Homozygotes vs. noncarriers</td>
<td>p = 0.03</td>
<td>p = 0.04</td>
<td>p = 0.08</td>
</tr>
</tbody>
</table>

Women

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (95% CI)/10,000 Person-Years</th>
<th>Relative Risk (95% CI)</th>
<th>Myocardial Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygotes vs. noncarriers</td>
<td>p = 0.47</td>
<td>p = 0.47</td>
<td>p = 0.89</td>
</tr>
<tr>
<td>Homozygotes vs. noncarriers</td>
<td>p = 0.64</td>
<td>p = 0.60</td>
<td>p = 0.64</td>
</tr>
</tbody>
</table>

Multifactorial adjustment included all factors listed in Table 1.
CI = confidence interval.
PlA1/PlA1 young men over 22 years of follow-up in a large
women.

Men

PlA2/PlA2 Homozygotes, and PlA1/PlA1 Noncarriers

platelet GP IIb/IIIa PlA2/PlA2 compared to noncarrier
PlA1/PlA1 young men over 22 years of follow-up in a large
Danish cohort.

The more than 30 case-control studies published about
this polymorphism and risk of ischemic cardiovascular
disease reach divergent results (3,4). Two factors complicate
a direct comparison of our results with the findings of
others. Most importantly, the majority of previous studies
combine PlA1/PlA2 heterozygotes and PlA2/PlA2 homozy-
gotes versus PlA1/PlA1 noncarriers in their calculations
in order to obtain sufficient statistical power, and thus do not
study homozygotes alone. Second, most previous studies did
not investigate associations stratified on gender or age,
which proved essential in our work. Our study suggests an
association between homozygosity and ischemic cardiovas-
cular disease in men, but not in women. This association is
most pronounced in men <40 years of age, an age group
where very few women are affected. Among the group 40 to
50 years of age, we do find cases among women and also an
elevated relative risk for ischemic cardiovascular disease in
homozygous men, but not in homozygous women, thus
supporting the gender-specific association.

Contrary to most other reports, we do not find any
increased risk of ischemic cardiovascular disease among
PlA1/PlA2 heterozygotes versus PlA1/PlA1 noncarriers. Sev-
eral factors could contribute to this discrepancy: publication
bias toward positive results in small studies (4), different
ethnicity between studies, differences in study design, and
chance alone. Our study is the largest so far and, together
with the two other published prospective studies (5,6), agree
that PlA1/PlA2 heterozygosity does not increase the risk of
ischemic cardiovascular disease.

Of the many in vitro studies examining the impact of the
PlA2-allele on platelet function, two (19,20) investigated all
three genotypes separately. In these studies, PlA2 was
associated with increased platelet aggregability, and thus
the propensity to initiate thrombus formation, in a gene-dose
dependent manner. Biologically, the observation of in-
creased risk of ischemic cardiovascular disease in PlA2/PlA2
homozygotes therefore seems reasonable. However, this
remains an untested correlation that may or may not explain
the enhanced risk of homozygotes reported in our study.

Interestingly, when exposed to physiologic concentrations
of estrogen, the aggregability of PlA1/PlA2 heterozygous
platelets was inhibited more than PlA1/PlA1 noncarrier
platelets (21). This accords with our data, which demon-
strate an association between PlA2/PlA2 homozygosity and
ischemic cardiovascular disease in men only.

Prothrombotic conditions like increased platelet aggrega-
bility is believed by some investigators (22,23) to outweigh
the importance of atherosclerosis in premature MI, mainly
because of their findings of fewer stenoses on coronary
angiography in young patients when compared with old
patients with MI. Therefore, having the above-mentioned
in vitro studies on PlA-genotype and platelet aggregability in
mind (19–21,24), our finding of age-dependency in men of
the effect of the PlA2/PlA2-genotype on MI could simply be
caused by increased platelet aggregability induced by the
PlA2/PlA2-genotype operating at an early age.

In a series of autopsy studies on men, Mikkelsson et al.
(25–28) reported lower incidence of atherosclerotic lesions
but higher incidence of coronary thrombosis among PlA2-
carriers than among PlA1/PlA1 noncarriers for participants
<60 years, but not for those >60 years. Although the
stratification on genotype and age in these studies (age
under or above 60 years, genotype noncarriers vs. heterozy-
gotes, and homozygotes combined) differs from our work,
those studies nevertheless support our finding of higher risk
of disease among younger homozygous men. Unfortunately,
no data on the extent of atherosclerosis (including results
from coronary angiographies) of the participants of our
study are available. Therefore, we do not know whether the
young homozygous men in our study have more or less
atherosclerosis than expected.

We cannot totally exclude that our findings represent
chance findings simply because no other studies have
demonstrated this association in young men. However, the
association was found in those <40 years old and in 40–
50-year-old men, and the association decreased stepwise
with increasing age.

Another potential limitation of our study is that partici-
pants underwent genotyping only if they attended the 1991
to 1994 third examination. The absence of a correlation
between PlA2/PlA2 homozygosity and ischemic heart disease
in older men could be the result of a dropout of homozygous
men at an early age because of premature death or disability.
However, the stable Hardy-Weinberg equilibria throughout
the age groups (Table 3) do not support such a hypothesis.
Furthermore, age percentiles for noncarrier and homozy-
gous men display a linear relationship, as would be expected
with no selection against homozygotes (data not shown).
Thus, we do not consider selection bias against homozy-
gotes very likely, but if this does apply for our study, it
would rather result in a conservative estimate concerning
association of the PlA2-genotype with ischemic cardiovas-
cular disease, and thus cannot explain our results.

### Table 4. Age at First Event in PlA1/PlA2 Heterozygotes,
PlA2/PlA2 Homozygotes, and PlA1/PlA1 Noncarriers

<table>
<thead>
<tr>
<th>Ischemic Cardiovascular Disease</th>
<th>Myocardial Infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age at First Event (yrs)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>67 ± 0.5</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>61 ± 1.0</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>64 ± 2.0</td>
</tr>
<tr>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>Noncarriers</td>
<td>71 ± 0.5</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>71 ± 1.0</td>
</tr>
<tr>
<td>Homozygotes</td>
<td>74 ± 2.5</td>
</tr>
</tbody>
</table>

Age shown as mean ± SE. The p values are by Mann-Whitney U test versus noncarriers.
Systematic misclassification of genotypes in this study is unlikely, because of agreement with the Hardy-Weinberg equilibrium and because the PCR assay included a restriction enzyme site in each person assayed. Misclassification of ischemic cardiovascular disease in the Copenhagen City Heart Study is very low, as we have 99.97% follow-up, and because all hospital admissions as well as deaths are registered systematically throughout Denmark.

Medication given to prevent ischemic cardiovascular disease and MI might overcome an underlying genetic risk. Therefore, another limitation of the present study is that we do not have complete information on all preventive medications given to all participants during the entire 22-year follow-up; however, addition of the use of antihypertensive and cholesterol-lowering medications at the 1991 to 1994 examination to the multifactorially adjusted models only resulted in trivial changes in the RR estimates.

Finally, the main finding of our prospective study in the Copenhagen City Heart Study, with a total follow-up of 163,987 person-years, is the three-fold and four-fold 22-year risk of ischemic cardiovascular disease and MI in men <40 years homozygous for platelet GP IIb/IIIa PlA2/PlA2.

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