Association between the rs342293 polymorphism and adverse cardiac events in patients undergoing percutaneous coronary intervention

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Summary
The single nucleotide polymorphism (SNP) rs342293 has been shown to influence platelet number and mean platelet volume (MPV). We investigated the association between the rs342293 polymorphism and cardiovascular outcome in a prospective cohort study. The rs342293 polymorphism was analysed in 404 patients with coronary artery disease undergoing percutaneous coronary intervention. The rates of cardiac adverse events were recorded during two years of follow-up. The polymorphism was associated with MPV (median 10.1 fL, interquartile range [IQR]: 9.6 to 10.6 in patients with the CC-allele vs 10.4 fL, IQR: 9.9 to 11.1 in G>C SNP carriers; p<0.001), but not with platelet count. Survival analysis indicated that carriers of the rs342293 G variant had a substantially higher risk to develop cardiac adverse events compared with wild type carriers during two years of follow-up (33% vs 22%; adjusted hazard ratio = 1.63, 95% confidence interval = 1.06–2.52, p=0.027). The rs342293 SNP could explain 2.9% of the variability in MPV (p=0.01). In conclusion, patients undergoing coronary stenting who carry the G-variant of the rs342293 SNP which is associated with larger MPV are at higher risk for adverse cardiovascular outcome.

Keywords
Platelet aggregation, polymorphism, genetic, outcome assessment (healthcare), platelet count, myocardial Infarction

Introduction
Platelets participate in coronary artery thrombosis. A causal relationship between large reactive platelets and myocardial infarction is supported by many clinical studies (1, 2). The population of these anucleate cells, which are unique to mammals, is characterized by a log Gaussian distribution of volume (3). In contrast, all other mammalian cells have a Gaussian cell volume distribution (4). Interestingly, platelets vary more in cellular volume than any other circulating blood cell population, ranging in size from 2 fl to 60 fl (4, 5). Larger platelets are denser and are supposedly more reactive than smaller platelets. Thus, platelet size, usually measured as mean platelet volume (MPV) can be used as an indicator of platelet activity (5). Indeed, several authors described MPV as a marker of platelet reactivity, and increased levels of MPV independently predicted ischaemic events (6-9).

Importantly, MPV is tightly regulated (10). Studies in animals and humans have confirmed that MPV is characterised by a high heritability level (11, 12). Recently, an association between MPV and a single nucleotide polymorphism (SNP), rs342293 on chromosome 7q22.3 has been identified (10). Since a convincing body of evidence suggests a positive correlation between MPV and patients’ outcome in arterial disorders, we investigated whether an association exists between the rs342293 polymorphism and cardiovascular outcome in patients with coronary artery disease undergoing coronary artery stenting. We focused on patients undergoing coronary artery stenting, as it is known that platelet related factors play an important role in this particular patient population (13). Coronary stents represent foreign surfaces that reveal thrombogenic activity (14, 15). Therefore, in patients with stents the inhibition of platelet function is one of the most important pharmacologic interventions during and after stent implantation (16, 17). It has been shown that greater MPV predicts occurrence of adverse events in the settings of coronary stent implantation (18). However, MPV depends on the type of analysis and may vary between centres. Thus, genetic factors could be a valuable supporting information. We hypothesised that the rs342293 polymorphism may represent a prognostic factor for prediction of cardiovascular outcome also in the era of routine use of dual antiplatelet therapies.

Methods
Study design
The study was performed at the Medical University of Vienna. The study protocol of this prospective observational cohort study was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Vienna. Participants were included into the study between 2007 and 2008, and...
followed-up until December 2010 as a part of a platelet function
study (19). Clinical follow-up information was obtained by con-
tacting all patients by phone and/or mail at 3, 6, 9, 12 and 24
months. Source documents of potential events were collected. In
addition, information concerning the cause of death was obtained
from national death registry (Statistics Austria). Inclusion criteria
were: written informed consent obtained before the study entry,
stent implantation, percutaneous coronary intervention (PCI), age
>18 years, treatment with dual antiplatelet therapy, availability of
genetic material. The exclusion criterion was participation in in-
terventional trials. From 437 screened patients 404 patients with
coronary artery disease (CAD) undergoing PCI were included into
the study. Twelve patients were excluded as no genetic material for
the study was available and 21 patients were excluded due to the
participation in other trials. Blood samples from patients were ob-
tained post-PCI. Genotyping was performed after inclusion of the
last participant at the Department of Laboratory Medicine, Medi-
cal University of Vienna, Austria. All analyses were performed by
trained laboratory technicians blinded to the outcome results. All
tests were performed in each participant. The study is reported ac-
cording to the STROBE (strengthening the reporting of observa-
tional studies in epidemiology) standards.

Platelet counts and MPV

Platelet counts and MPV were measured with a cell counter within
1 hour after blood sampling (XE-2100, Sysmex Counter, Milton,
Keynes, UK). MPV was assessed 1) in the acute setting: one day
after coronary stenting and 2) during the stable phase of the dis-
ease 6–12 months after stenting.

Genotyping

Genomic DNA was extracted from anticoagulated blood by stan-
dard procedures with the MagnaPure LC 2.0 DNA isolation sys-
tem (Roche Diagnostics, Basel, Switzerland). Determination of the
rs342293 C>G polymorphism was performed by real-time PCR
with Simple Probes (Tib Mol Biol, Berlin, Germany). PCR and
melting curve analysis was performed in a CFX-96 Real Time sys-
tem (Roche Diagnostics, Basel, Switzerland). Determination of the
mpv from BioRad (Hercules, CA, USA). Genotypes were assigned
with the BioRad Melt Precision Analysis software. An initial de-
naturation at 95°C for 10 minutes (min) was followed by 50 cycles
at 95°C for 10 seconds (s), 60°C for 10 s and 72°C for 10 s. The
reaction was completed with one cycle of 30 s at 95°C and 2 min at
40°C. For melting curve analysis temperature was increased
0.5°C/s from 40°C to 75°C.

Study endpoints

The primary surrogate endpoint was the MPV. The primary clin-
cal end point was the incidence of the composite of adverse cardiac
events (acute coronary syndrome and cardiac death, repeated rev-
vascularisation).

Statistical analysis

We estimated the statistical power to detect an association be-
tween clinical outcome and the rs342293 genotype at the signifi-
cance level of p<0.05. It was a priori decision to compare G>C al-
lele versus CC allele, which is based on power calculation. We
expected a 25% rate of the primary endpoint at two years of follow-
up. Thus, if the hazard ratio [HR] for the primary endpoint in
G>C allele careers was 1.5, the study would have more than 80%
power to demonstrate this relation. Therefore, with 400 patients,

| Table 1: Patient demographics. Data are reported as mean, standard
deviation (SD), number of patients (n) or percentages.*p<0.05; **p<0.01
wild type vs rs342293 polymorphism; SNP, single nucleotide polymorphism. |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Patient demographics; n=404</td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender: male n (%)</td>
</tr>
<tr>
<td>Risk factors / past medical history n (%)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Smoking</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Hyperlipidemia</td>
</tr>
<tr>
<td>Prior myocardial infarction or PCI</td>
</tr>
<tr>
<td>Peripheral arterial occlusive disease</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
</tr>
<tr>
<td>Laboratory data</td>
</tr>
<tr>
<td>Platelets (x10^9/l)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
</tr>
<tr>
<td>Medications n (%)</td>
</tr>
<tr>
<td>ACE inhibitors or ARB</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
</tr>
<tr>
<td>Statins</td>
</tr>
<tr>
<td>Betablockers</td>
</tr>
<tr>
<td>Proton pump inhibitors (PPI)</td>
</tr>
<tr>
<td>PCI data n (%)</td>
</tr>
<tr>
<td>Elective PCI</td>
</tr>
<tr>
<td>Acute PCI due to ACS</td>
</tr>
<tr>
<td>DES</td>
</tr>
<tr>
<td>BMS</td>
</tr>
<tr>
<td>Number of stents per patient</td>
</tr>
<tr>
<td>Total stent length</td>
</tr>
<tr>
<td>Data are reported as mean ± SD, n (number of patients) or percentages; ACE= angiotensin converting enzyme; ACS= acute coronary syndrome; CAD= coronary artery disease; PCI= percutaneous coronary intervention.</td>
</tr>
</tbody>
</table>
Siller-Matula et al. rs342293 SNP is associated with cardiovascular outcome

250 patients with the G>C allele would have 75 primary endpoint events (30% rate), and 150 patients with the CC allele would have 30 primary endpoint events (20% rate).

Normal distribution was tested with the Kolmogorov-Smirnov test. Data are expressed as mean, standard deviation (SD), 95% confidence intervals (CI) median or interquartile range (IQR), as appropriate. Statistical comparisons were performed by t-test, the Mann-Whitney U test and the Chi²-test when applicable. Kaplan-Meier curves with the Breslow test were used for survival analyses. Spearman’s rank correlation was used to examine the correlation between MPV and platelet count. Univariate and multivariate Cox regression analysis was used to estimate independent variables responsible for clinical outcome. The covariates included in the multivariate analyses were chosen a priori. The multivariate model included: rs342293 G>C polymorphism, use of beta-blockers, total stent length, age, sex, platelet count and MPV, all assessed at study entry. For identification of possible predictors for MPV we used a multivariable linear regression model. The percentage of variability of MPV that could be attributable to independent variables was derived from R² calculated by multivariable linear regression analysis with MPV as dependent variable and the following variables as fixed factors: rs342293 G>C genotype, platelet count, sex, age, C reactive protein (CRP), and presence of hypertension, hy-

Table 2: Frequency of the rs342293 polymorphism.

<table>
<thead>
<tr>
<th>Frequency n (%)</th>
<th>Patients n=404</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild type C/C</td>
<td>151 (37)</td>
</tr>
<tr>
<td>rs342293 heterozygous carrier C/G</td>
<td>179 (45)</td>
</tr>
<tr>
<td>rs342293 homozygous carrier G/G</td>
<td>74 (18)</td>
</tr>
</tbody>
</table>

Figure 1: Mean platelet volume (MPV) in patients with (G>C allele) or without the rs342293 polymorphism (CC allele). A) In the acute setting: one day after coronary stenting and B) during the stable phase of the disease 6–12 months after coronary stenting.
perlipidaemia, diabetes mellitus and adipositas. All statistical calculations were performed using commercially available statistical software (SPSS Version 21.0; Chicago, IL, USA).

Results

Patient demographics

Some minor differences in the demographic data were seen between patients with and without the rs342293 G allele (▶ Table 1). Patients with the rs342293 G variant used more often beta-blockers (80% vs 67%; \(p=0.007\)) and received a shorter total stent length compared to patients with the C allele (31 mm vs 33 mm; \(p=0.039\)).

Frequencies of the rs342293 C>G alleles

The genotype distribution followed the Hardy-Weinberg equilibrium. In the study population 37% carried the homozygous C allele (wild type), 45% were heterozygous and 18% were homozygous for the G variant of rs342293 (▶ Table 2). The allele frequency distribution matches with the reported frequencies (10).

Association between the rs342293 C>G variant and MPV

There was a trend towards significance of the association between rs342293 SNP and MPV in the acute setting of PCI, and the association became statistically significant in the chronic phase of the disease (▶ Figure 1). In the acute setting of PCI, the median MPV was 10.2 fl (IQR: 9.7 to 10.8) in patients with the wild-type

Table 3: Multivariate linear regression model for MPV (mean platelet volume) assessed at the time point of coronary stenting. CRP, C-reactive protein.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B coefficient</th>
<th>Standard error</th>
<th>p value</th>
<th>incremental R2</th>
<th>R2 for the model</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs342293</td>
<td>0.295</td>
<td>0.113</td>
<td>0.010</td>
<td>0.029</td>
<td>0.20</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.005</td>
<td>0.001</td>
<td>0.000</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.284</td>
<td>0.142</td>
<td>0.047</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.060</td>
<td>0.121</td>
<td>0.622</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.138</td>
<td>0.122</td>
<td>0.258</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>0.148</td>
<td>0.140</td>
<td>0.294</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.014</td>
<td>0.166</td>
<td>0.933</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>CRP levels</td>
<td>-0.002</td>
<td>0.016</td>
<td>0.920</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.006</td>
<td>0.005</td>
<td>0.287</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Adipositas</td>
<td>0.035</td>
<td>0.075</td>
<td>0.644</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Time to event analysis for the composite of cardiac adverse events (acute coronary syndrome, cardiovascular death and repeated revascularisation) in patients with (G>C allele) or without the rs342293 polymorphism (CC allele) during two years follow-up.
CC-allele, and 10.6 fl (IQR: 9.9 to 11.2) in carriers of the rs342293 SNP G>C allele (Figure 1A; p=0.064). In the chronic phase of disease (6–12 months after PCI), the difference in the median MPV between CC and G>C allele carriers of rs342293 was statistically significant: 10.1 fl (IQR: 9.6 to 10.6) in patients with the CC-allele versus 10.4 fl (IQR: 9.9 to 11.1) in G>C carriers (Figure 1B; p=0.001). The MPV at the time point of coronary stenting was generally higher than 6–12 months after the procedure (median 10.5 fl vs 10.3 fl; p=0.02, respectively). Interestingly, the MPV was higher in patients presenting with an ACS compared with patients with stable coronary artery disease (median 10.7 fl vs 10.3 fl; p=0.003, respectively).

There was no association between rs342293 and platelet count (data not shown).

We extended our calculations and estimated the individual contribution of several variables to the variation of MPV in a linear regression model entering MPV as a continuous variable. We found that rs342293 explains 2.9% of the variability in MPV (p=0.01), whereas all determinants together explain 20.0% (p<0.001); (data not shown).

Regarding the impact of the rs342293 on single endpoints, we identified a significant increase in the rates of cardiac adverse events between carriers of CC vs G>C allele of rs342293 during two years of follow-up (the composite of an acute coronary syndrome, cardiovascular death and repeated revascularisation; 22% vs 33%; p=0.022; Table 4). In the univariate Cox regression analysis the rs342293 GG genotype was associated with a 1.6-fold higher risk for the composite of cardiac adverse events (HR=1.60, 95%CI=1.07–2.42, p=0.023; Table 4). The multivariate Cox regression model confirmed this association (HR=1.63, 95%CI=1.06–2.52, p=0.027; Table 4). In this model, the platelet count emerged as a weak but independent predictor of the composite of cardiac adverse events (HR=0.995, 95%CI=0.991–0.999, p=0.007; Table 4).

Table: Univariate and multivariate Cox regression analyses on impact of genetic and clinical variables on secondary endpoints. PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; SNP, single nucleotide polymorphism.

<table>
<thead>
<tr>
<th>Event</th>
<th>CC allele</th>
<th>G&gt;C SNP</th>
<th>Univariate Cox regression analysis HR (95%CI) p</th>
<th>Multivariate Cox regression analysis HR (95%CI) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute coronary syndrome (ACS)</td>
<td>12 (8.6%)</td>
<td>36 (15.5%)</td>
<td>1.82 (1.05–3.50) 0.038</td>
<td>2.00 (1.09–4.07) 0.045</td>
</tr>
<tr>
<td>Repeated revascularisation (PCI or CABG)</td>
<td>27 (18.6%)</td>
<td>73 (29.9%)</td>
<td>1.62 (1.04–2.52) 0.033</td>
<td>1.76 (1.09–2.83) 0.021</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>7 (4.9%)</td>
<td>19 (7.9%)</td>
<td>1.80 (0.71–4.52) 0.215</td>
<td>1.72 (0.67–4.37) 0.550</td>
</tr>
</tbody>
</table>

Table 5: Event rates and the results of the Cox regression analysis in patients with and without the rs342293 polymorphism during two years follow-up for secondary endpoints. PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; SNP, single nucleotide polymorphism.

We could perform follow up analyses in 389 patients, 15 patients (3.7%) were lost to follow-up. Kaplan-Meier curves showed a separation of the rates of cardiac adverse events between carriers of CC vs G>C allele of rs342293 during two years of follow-up (the composite of an acute coronary syndrome, cardiovascular death and repeated revascularisation; 22% vs 33%; p=0.022; Figure 2).
Discussion

The central finding of this study is that the rs342293 polymorphism predicts MPV and cardiovascular outcome in patients undergoing coronary artery stenting. Our study confirms the results of a genome wide association study, which has identified a significant association between rs342293 on chromosome 7q22.3 and MPV in healthy individuals (10). Apparently, the sequence variation in the 7q22.3 region exerts its effect on molecular events downstream of the GPV/Fc receptor chain complex (10). It was shown that the rs342293 polymorphism influences the transcript levels of PICK3CG which seems to affect the platelet volume (10). PICK3CG encodes for pi3/pi4-kinase a key protein in megakaryopoiesis. It controls signal transduction cascades leading to the release of calcium from the intracellular stores, controls cellular movement, adhesion, and contraction (20). Furthermore, the rs342293 G allele was associated with decreased binding of annexin V to platelets. Annexin V exerts antithrombotic activity by binding to phosphatidylinerine (PS), an important contributor to platelet procoagulant activity (21, 22).

In our study, we analysed only one polymorphism, rs342293. In another report, however, an association between the MPV with three other SNPs was reported: rs7961894 located within intron 3 of WDR66 on chromosome 12q24.31, rs12485738 upstream of the ARHGEF3 on chromosome 3p13-p21, and rs2138852 located upstream of TAOK1 on chromosome 17q11.2 (23). Indeed, the discovered genes could influence the process of platelet formation. WDR66 might be involved in proplatelet formation (24), ARHGEF3 plays an important role in the regulation of cell morphology and cytoskeletal rearrangements (25) and TAOK1 is an regulator of mitotic progression (26). However, the three quantitative trait loci rs7961894, rs12485738 and rs2138852 together accounted only for 5% of the variance in MPV (23). In contrast, in our study population rs342293 alone contributed in 2.9% to the MPV variance, which is higher than previously reported (7, 8). Recently, 68 genomic loci associated with platelet count and volume were reported (27). To our knowledge, however, the impact of these polymorphisms on patient's outcome has not been investigated yet.

Undoubtedly, additional genetic and non-genetic variables are involved in the modulation of MPV. The inter-individual variability in MPV has been related to common risk factors for cardiovascular diseases such as diabetes mellitus, hyperlipidaemia, hypertension and cigarette smoking as well as to the age, sex, dietary habits or ongoing inflammation (28). In our multivariate model, three variables: the rs342293, sex and platelet count explained 20% of the MPV variability, of which the platelet count had the largest effect size (14%).

Our study confirms previous findings, which have shown that MPV was significantly higher in patients with myocardial infarction compared to patients with stable angina or healthy controls (18, 29-32). Our results also demonstrate that even though all patients were treated with dual antiplatelet therapy consisting of aspirin and clopidogrel, the rs342293 SNP and MPV contributed to prediction of cardiac ischaemic events. Our observations correspond well with previous reports, showing that higher MPV in patients with acute myocardial infarction predicts adverse events even if dual antiplatelet therapies and inhibitors of the glycoprotein IIb/IIIa receptor are applied (33-36).

We presented evidence that the effect of the rs342293 genotype on MPV contributes to the clinical phenotype of coronary artery disease. Our finding is of relevance as determination of MPV is method dependent while genotyping is not. In this regard, further research on this association is needed, as this might provide new potential avenues in drug development.

Limitations

The main limitation of our study is the fact that we focused on one single polymorphism which, however, has been shown to have important functional consequences. Furthermore, the sample size was relatively small for a genetic study; however, we reached a power >80 which is generally considered adequate. Accordingly, limited power of our study did not allow to investigate possible differences in the effects of the rs342293 homo- and heterozygous SNP. We are aware that previous comparisons that reported controversial results (e.g. impact of PCI setting on MPV), could also be due to a limited power. Despite these limitations, our results are conclusive and are supported by previous reports.

Conclusion

Our study underlines the association between the rs342293 SNP with the intermediate phenotype MPV and with adverse cardiac outcomes. The finding emphasises that genetic variables influencing the production of large platelets should, therefore, be further studied.
investigated, as understanding of this issue might help to prevent adverse events in patients undergoing coronary stenting.

Conflicts of interest
None declared.

References