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Full Length Article

The *SH2B3* and *KCNK5* loci may be implicated in regulation of platelet count, volume, and maturity

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ABSTRACT

Introduction: In recent genome-wide association studies, coronary artery disease (CAD) and myocardial infarction (MI) have been linked to a number of genetic variants, but their role in thrombopoiesis is largely unknown.
Aim: We investigated the association between CAD and MI-associated genetic variants and five thrombopoiesis-related indices: platelet count (PC), mean platelet volume (MPV), immature platelet count (IPC), immature platelet fraction (IPF), and serum thrombopoietin (TPO).
Methods: We genotyped 45 genome-wide significant CAD/MI-markers in 879 stable CAD patients. A genetic risk score was calculated to assess the combined risk associated with all the genetic variants. Platelet indices were analysed using the Sysmex XE-2100 haematology analyser. TPO was measured by ELISA.
Results: Two variants were nominally associated with several indices; for rs10947789 (KCNK5), the adjusted geometric mean was 2% higher for MPV (95% confidence interval: 1–2%, p = 0.002), 6% for IPC (0–12%, p = 0.033), and 9% for IPF (3–16%, p = 0.004) per CAD risk allele. Moreover, an 11% lower TPO (3–19%, p = 0.010) was observed. Rs3184504 (SH2B3) was associated with a higher adjusted geometric mean of 3% (1–6%, p = 0.003) per CAD risk allele for PC, and an 11% (5–17%, p < 0.001) lower TPO. Furthermore, the adjusted IPC was 5% (0–9%, p = 0.037) lower per CAD risk allele for PC, whereas IPF levels did not vary across genotypes.
Conclusion: As a novel finding, our study suggests a role for KCNK5 in the regulation of platelet size and maturity. Furthermore, our findings confirm an association between the SH2B3-locus and platelet count.

1. Introduction

Coronary artery disease (CAD) is a frequent underlying cause of coronary events such as acute myocardial infarction (MI) [1]. Coronary atherosclerosis is driven by non-modifiable (*e.g.* male gender, age, and genetics) as well as modifiable risk factors (*e.g.* smoking, dyslipidaemia, hypertension, and diabetes) [2]. Platelets are key players in the development of coronary thrombosis. They are formed and released into the bloodstream from megakaryocytes [3], and the circulating platelet pool is held in an equilibrium, balanced by platelet production and consumption [4]. Thrombopoietin (TPO) is considered the primary physiological and most potent regulator of megakaryocytopoiesis and thrombopoiesis [4].

In patients with increased platelet turnover, a higher number of newly formed platelets is present in peripheral blood, often referred to as “immature platelets”. These platelets are characterised by an increased volume and by being enzymatically and metabolically more active than mature platelets [5,6]. Mean platelet volume (MPV) has been proposed as a surrogate marker of platelet turnover. Increased MPV has been reported in the acute phase of MI [7] and predicts adverse outcome in healthy subjects [8] and in patients with prior MI [9]. Recently, increased levels of immature platelets have been found in patients with acute coronary syndrome (ACS) [7,10,11] and they have been associated with an increased risk of cardiovascular death in ACS

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patients [12].

Heritability in platelet-related traits is substantial. It has been estimated that 50–80% of the variation in platelet count (PC) and MPV is caused by genetic factors [13]. Genome-wide association studies (GWASs) have identified several variants associated with increased risk of CAD and MI [14]. Moreover, the burden of concomitantly inherited risk alleles, as measured by genetic risk scores (GRSs), may predict cardiovascular events in individuals with and without established CAD [15,16]. It is largely unknown how these genetic loci influence atherogenesis and coronary thrombosis.

Therefore, we hypothesised that one or more of the CAD/MI-associated SNPs would be associated with thrombopoiesis. We investigated the association between 45 CAD/MI-associated genetic risk variants and five thrombopoiesis-related indices: PC, MPV, immature platelet count (IPC), immature platelet fraction (IPF), and TPO in patients with stable CAD.

2. Methods

2.1. Design and study population

We conducted a cross-sectional study on patients with stable CAD. A detailed description of the cohort is provided elsewhere [17]. In brief, 900 patients with angiographically verified CAD were recruited from the Western Denmark Heart Registry between November 2007 and January 2011. Of these, we included 883 patients with available DNA samples. Patients were stable at the time of enrolment (*i.e.* no revascularization procedure or MI within the last year) and were on mono-antiplatelet therapy with aspirin 75 mg daily.

All patients provided informed written consent. The project was approved by The Central Denmark Region Committees on Health Research Ethics (record number: 1-10-72-210-15) and by the Danish Data Protection Agency (record number: 1-16-02-400-15).

2.2. Platelet count, volume, and maturity

Blood samples for haematological analyses were collected in 3.0 mL EDTA tubes (Terumo, Leuven, Belgium). Only patients with platelet count within the normal range (120–450 10^9/L) were included. In order to minimise and standardise time-dependent swelling of platelets, haematological analyses were performed within 60 min of blood sampling. Haematology parameters were measured using the Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan) with upgraded software (XE IPF Master, Sysmex) allowing flow cytometric detection of immature platelets as previously described [18,19]. Absolute immature platelet count (IPC) was obtained, and immature platelet fraction (IPF) was calculated as the ratio of immature platelets to the total PC. Platelet volume parameters were derived from the platelet volume distribution. MPV was calculated by dividing the platelet crit by platelet impedance count.

2.3. Thrombopoietin

Whole blood was allowed to clot at room temperature for 30 min before serum was separated by centrifugation at 1000*g* for 15 min and stored at − 80 °C until analysis. TPO concentrations were analysed in duplicate for all patients and the mean of the two results was included in the statistical analyses. The coefficient of variance was 9%. TPO analyses were performed by ELISA according to the manufacturer's instructions (Human Tpo Immunoassay, R & D Systems Europe Ltd., Abingdon, UK).

2.4. Candidate SNPs and genotyping

Selection of SNPs and genotyping has previously been described in detail [20]. Briefly, 46 SNPs reported to be genome-wide significantly associated with CAD in populations of European ancestry were selected for genotyping [14]. DNA was obtained from whole blood and genotyping was performed on a Fluidigm Biomark HD (Fluidigm Corp. South San Francisco, CA, USA). One SNP (rs17114036) failed on all chips and four samples with < 50% of SNPs successfully genotyped, were removed. Therefore, the final dataset comprised 45 SNPs and 879 samples. The overall call rate was 99.5% (39,375/39,555) and consistently high for all SNPs, except rs964184 with a call rate of 84.7% (745/879). All genotypes were successfully called in 82.9% of the samples (729/879), whereas ≥ 43 genotypes had been called in 99.4% (874/879) of the samples. None of the SNPs deviated significantly from the Hardy-Weinberg equilibrium (Pearson chi-square test; Bonferroni-corrected p-value of 0.0011 [0.05/45 SNPs]).

2.5. Statistical analysis

Patient data are reported as number (percentage), mean ± standard deviation (SD), or median (interquartile range), as appropriate. The genotypes were coded as 0, 1, or 2 depending on the number of CAD risk alleles present in the patient. To evaluate the combined effects of all SNPs, a GRS was calculated as the sum of the number of risk alleles weighted by the effect size reported by the original discovery paper. For the analyses, the GRS was standardised. A log-linear regression model was used to analyse the relationship between the SNPs or the GRS and PC, MPV, IPC, IPF, and TPO. Predefined covariates including age, sex, diabetes, prior MI, current smoking, body mass index, and impaired renal function (estimated glomerular filtration rate ≤ 60 mL/min) were simultaneously added to the model. For reporting, the beta-coefficients were back-transformed. Therefore, the presented effect size for a SNP corresponds to the adjusted factorial change in the geometric mean of the variable per increase in the number of CAD risk alleles. Similarly, for the GRS the reported effect size corresponds to the adjusted factorial change in the geometric mean per SD increase in the GRS. Correlations for rs10947789 and rs3184504 were computed using Spearman's rank correlation coefficient. The regression model was validated by inspecting quantile-quantile plots of residuals and by plotting residuals against fitted values and explanatory variables. To account for multiple testing (associations between 45 SNPs, the GRS, and five thrombopoiesis-related indices), we applied a conservative Bonferroni-corrected p-value of 2.2×10^{-4} (0.05/[46 × 5]) as the threshold of statistical significance. All analyses were performed using STATA version 13.1 (StataCorp, College Station, TX, USA).

3. Results

Patient characteristics are presented in Table 1. The mean age was 65 ± 9 years and 78% of the patients were men. Prior MI, diabetes, and impaired renal function were present in 777 (88%), 240 (27%), and 169 (19%) of patients.

Associations between the SNPs and the five thrombopoiesis-related indices are presented in Table 2. None of the SNPs met the Bonferroni-corrected threshold of significance. However, looking at nominal p-values, two SNPs (rs10947789 and rs3184504) were significantly associated with several indices (Table 2 and Figs. 1 and 2).

Rs10947789 is located in the *KCNK5* gene. In adjusted analyses, this variant was associated with a 2% higher geometric mean MPV (95% confidence interval [CI]: 1–2%, p = 0.002), 6% higher IPC (95% CI: 0–12%, p = 0.033), and 9% higher IPF (95% CI: 3–16%, p = 0.004) per CAD risk allele. Moreover, an 11% lower geometric mean TPO (95% CI: 3%–19%, p = 0.010) was observed.

Rs3184504 is part of a large linkage disequilibrium block on chromosome 12 spanning over several genes. This locus was associated with an adjusted 3% (95% CI: 1–6%, p = 0.003) higher geometric mean PC, and an 11% (95% CI: 5–17%, p < 0.001) lower TPO. The IPC was also 5% higher (95% CI: 0–9%, p = 0.037), per CAD risk allele, while IPF levels did not vary across genotypes.