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The *ABO* locus is associated with increased platelet aggregation in patients with stable coronary artery disease☆

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ABSTRACT

Background: Genome-wide association studies of patients with coronary artery disease (CAD) suggest that several risk loci increase the risk of CAD and myocardial infarction (MI) equally. In contrast, the *ABO* locus is stronger associated with MI than with CAD, but the underlying mechanisms are unknown.
Purpose: To investigate the association between the *ABO* risk variant and platelet activation and aggregation. Moreover, to explore the effects of other CAD-associated risk variants.
Methods: We included 879 stable CAD patients receiving low-dose aspirin. All patients were genotyped for 45 genome-wide significant CAD risk variants, including rs495828 at the *ABO* locus. A genetic risk score (GRS) was calculated to assess the combined risk of all genetic variants. Serum soluble P-selectin (sP-selectin) and thromboxane B_2 were used as measures of platelet activation, and platelet aggregation was assessed by multiple electrode aggregometry (MEA) using arachidonic acid and collagen as agonists and VerifyNow.
Results: The rs495828 CAD risk allele was associated with higher MEA platelet aggregation; arachidonic acid: 14.9% (6.7–23.7%, p = 0.0002) higher AUC (**Area Under aggregation Curve**) per risk allele, and collagen: 13.1% (5.8%–20.9%, p = 0.0003). Conversely, sP-selectin levels were 7.5% (3.1%–11.7%, p = 0.001) lower per risk allele. Rs495828 genotypes were not associated with aggregation assessed by VerifyNow (p = 0.30) or S-thromboxane B_2 levels (p = 0.98). None of the remaining variants or the GRS were associated with platelet activation or aggregation.
Conclusions: The *ABO* risk allele was associated with increased platelet aggregation as assessed by MEA. This finding may contribute to explain the increased MI risk in *ABO* risk variant carriers.

1. Introduction

Coronary atherosclerosis is the underlying cause of most ischemic cardiac events [1]. In case of atherosclerotic plaque rupture, adenosine diphosphate, thromboxane A_2, thrombin, and von Willebrand factor (vWF) interact with platelet receptors leading to platelet activation and aggregation, which may cause acute myocardial infarction (MI) [2]. Although most MI patients have coronary atherosclerosis, only few patients with coronary atherosclerosis develop MI [1]. This may be the consequence of genetic factors altering plaque progression and vulnerability, or platelet activation, or platelet aggregation [2].

Over the last decade, genome-wide association studies (GWASs) have established associations between coronary artery disease (CAD) including MI and a number of common genetic variants [3]. In general, the effect size of each variant is modest, but combined into genetic risk scores (GRSs) they may predict cardiovascular events as well as effects of medical treatment or lifestyle modification in both primary and secondary preventive settings [4–6].

The majority of CAD risk variants identified from GWAS increase the risk of CAD and MI equally, which may indicate that these SNPs increase MI risk through atherosclerosis development rather than directly affecting plaque vulnerability or thrombus formation [3]. In contrast, one locus located close to the *ABO* gene associates specifically with MI per se [3,7,8]. However, the mechanisms explaining this association remain unknown.

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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We hypothesized that the association of the *ABO* risk allele with MI is mediated through increased platelet aggregation. Therefore, we investigated the association between the lead variant at the *ABO* locus and platelet activation and aggregation. Moreover, we explored the effects on platelet activation and aggregation of other CAD-associated risk variants individually and combined into a GRS.

2. Methods

2.1. Study population

The study population has previously been described in detail [9]. We included 900 stable CAD patients with either previous myocardial infarction (≥12 months ago from time of inclusion), type 2 diabetes, or both. Patient inclusion is outlined in Fig. S1. Exclusion criteria were ongoing medical treatment known to affect platelet function or coagulation (e.g. non-steroidal anti-inflammatory drugs, any anticoagulants or antiplatelet drugs except aspirin), any ischaemic vascular event, PCI, or CABG within the previous 12 months, platelet count < 120 × 109/l or > 450 × 109/L, or inability to give informed consent. All patients received mono antiplatelet therapy with aspirin 75 mg once daily. Patients were recruited from November 2007 to January 2011 from the Western Denmark Heart Registry, which collects data on all interventional procedures performed in the western part of Denmark [10]. DNA was available in 883 patients. The study cohort represents a high-risk population with documented CAD and either prior MI, type 2 diabetes, or both. Platelet aggregation data and the relation to prior MI and stent thrombosis, type 2 diabetes, and renal insufficiency has previously been published [11–14].

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. Genotyping and construction of the genetic risk score

Selection of SNPs and genotyping has previously been described in detail [4]. Briefly, we selected all 46 lead SNPs for genotyping that were reported to be genome-wide significantly associated with CAD in European populations at the time of study initiation [15]. DNA was obtained from whole blood and specific target amplification followed by direct genotyping was performed on a Fluidigm Biomark HD platform. The lead SNP rs579459 at the *ABO* locus failed assay design and instead rs495828 was included as a perfect proxy (HapMap 3, release 2: 699 base pair distance; $r^2 = 1.00$). For lead SNP rs17514846 at the *FURIN* locus, genotyping was obstructed due to adjacent variants within a 30 base pair distance and rs8039305 was chosen as a proxy (HapMap 3, release 2: 5993 base pair distance; $r^2 = 0.93$). One SNP (rs17114036; *PPAP2B* locus) was excluded because the genotypes failed to separate clearly into clusters on all chip runs. Four samples with <50% of SNPs successfully genotyped were excluded. Therefore, the final dataset consisted of 45 SNPs genotyped in 879 patients. The overall call rate was 99.5% (39375 of 39555 genotypes successfully called). The Pearson chi-square test was used to test if the observed genotype distributions differed significantly from Hardy-Weinberg equilibrium. No significant deviation from Hardy-Weinberg equilibrium was found for rs495828 ($p = 0.16$) or any of the remaining 44 SNPs (Bonferroni-corrected threshold of $p = 0.0011$ [0.05/45 SNPs]).

The genotypes were coded as 0, 1, or 2 depending on the number of CAD risk alleles. To assess the combined effects of all 45 SNPs, we used a GRS calculated by summing the number of CAD risk alleles (0, 1, or 2) weighted by the logarithm of the odds ratio for CAD for each of the SNPs [16]. The odds ratios were obtained from the original discovery GWAS papers, as was the method used by Ripatti et al. [17]. In the rare case of a missing genotype, an average of the genotype value in the cohort (fractional value between 0 and 2) was used to calculate the GRS. The reason for this was to compensate for the missing values so that cases who failed genotyping were not given a lower score. Given the arbitrary scale, the GRS was standardized i.e. the mean score was subtracted from the value of each score and the difference between the individual's score and the mean was divided by the standard deviation. Platelet activation and aggregation.

Blood for platelet aggregation analyses was drawn 1 h after aspirin intake and rested at room temperature for 30 to 120 min before analysis. Whole blood platelet aggregation was evaluated with two different instruments; Multiplate Analyzer and VerifyNow Aspirin. For multiple electrode aggregometry (MEA) analyses (Multiplate Analyzer, Roche Diagnostics International LDT, Rotkreuz, Switzerland), 3.6 mL tubes containing 3.2% sodium citrate (Terumo, Leuven, Belgium) were used. Platelet aggregation was induced with arachidonic acid 1.0 mM (ASPI test, Triolab AS, Brøndby, Denmark) or collagen 1.0 μg/mL (Horm, Medinor, Nycomed, Austria). Aggregation was reported as area under the aggregation curve (AUC, aggregation units * min). For VerifyNow Aspirin analyses (Accriva Diagnostics, San Diego, CA, USA), blood was collected in 2.7 mL tubes containing 3.2% sodium citrate (Terumo, Leuven, Belgium). Arachidonic acid was used as agonist (VerifyNow Aspirin). Platelet aggregation was reported as Aspirin Reaction Units (ARU). Coefficients of variation on both tests have previously been reported by our group [18].

For measurements of serum sP-selectin and thromboxane B_2, non-siliconized 5.0 mL tubes (Terumo, Leuven, Belgium) without anticoagulants were used. Blood was allowed to clot for 30 min at room temperature before serum was separated by centrifugation at 1500*g* for 15 min. Serum was stored at −80 °C before analysis. Concentrations of sP-selectin and thromboxane B_2 were measured using ELISA (R&D Systems, Minneapolis, MN, USA, and Thromboxane B_2 EIA Kit, Cayman Chemical, Michigan, MI, USA).

2.3. Statistical analysis

Data are presented as number (percentage), mean ± standard deviation (SD), or median (interquartile range [IQR]). Differences in baseline characteristics between the three rs495828 genotype carriers were evaluated by the chi-square test, one-way analysis of variance, or the Kruskal-Wallis test. Under the assumption of additive genetic effects, a log-linear regression model was used to assess the association between each SNP (independent variables) and the measures of platelet activation and aggregation (dependent variables), respectively. Predefined adjustment variables were age, sex, diabetes, prior MI, body mass index, smoking status, and impaired renal function (estimated glomerular filtration rate ≤ 60 mL/min). All adjustment variables were simultaneously added to the model. Therefore, the calculated effect size of a SNP corresponds to the adjusted change in the geometric mean of the dependent variable (reported in percent) per increase in the number of CAD risk alleles. As with the SNPs, a log-linear regression model was used to assess the association between the GRS and platelet activation and aggregation, where the calculated effect size of the GRS corresponds to the adjusted change in the geometric mean of the dependent variable per SD increase in the GRS. The regression models were validated by inspecting quantile-quantile plots of residuals and by plotting residuals against fitted values and independent variables, respectively. The models were judged to be valid in all cases. Correlations between rs495828 and the dependent variables were computed using Spearman's rank correlation coefficient. To account for the number of statistical tests performed we applied a Bonferroni-corrected threshold of statistical significance. In our primary analysis investigating the effects of rs495828 at the *ABO* locus, p-values ≤ 0.01 were considered significant (0.05/[1 SNP × 5 measures of platelet activation and aggregation]). When evaluating the remaining 44 SNPs and the GRS a p-value of 2.2×10^{-4} (0.05/[45 SNPs and the GRS × 5 measures of platelet activation and aggregation]) was considered significant. All data analyses were performed using STATA version 13.1 (StataCorp, College Station, TX, USA).

3. Results

Patient characteristics are shown in Table 1. Mean age was 65 ± 9 years, and 689 (78%) were males. Although the study patients had stable CAD at the time of enrolment, they were considered at high risk of cardiovascular events, since 777 (88%) had a prior MI and 240 (27%) had type 2 diabetes. Rs495828 CAD risk allele (T) frequency was 22.6%. There were no significant differences (all p-values > 0.05) in the distribution of baseline characteristics across rs495828 genotypes except for statin treatment, which differed slightly between the groups (statin treatment in G/G carriers: 471 (89%); G/T carriers: 275 (95%); T/T carriers: 47 (90%), $p = 0.023$).

Table 1
Patient characteristics (n = 879).

Age (years)	65 ± 9
Male	689 (78%)
Prior MI	777 (88%)
Prior PCI/CABG	832 (95%)
Prior stroke[a]	49 (6%)
Diabetes	240 (27%)
Impaired renal function[b,c]	169 (19%)
Antihypertensive treatment[b]	809 (92%)
Statin treatment[d]	795 (91%)
Current smoking[e, f, g]	192 (22%)
Platelet count (10^9/L)[b]	226 (193–267)
MEA, 1.0 mM arachidonic acid (AUC)[h]	164 (100–238)
MEA, 1.0 μg/mL collagen (AUC)[i]	266 (172–396)
VerifyNow (ARU)[j]	426 (409–453)
S-Thromboxane B_2 (ng/mL)	0.97 (0.53–1.82)
sP-Selectin (ng/mL)[a]	74 (58–91)

Data are presented as mean ± standard deviation, median (interquartile range), or number (%).
Abbreviations: ARU, Aspirin Reaction Units; AUC, area under the aggregation curve; CABG, coronary artery bypass graft surgery; MEA, multiple electrode aggregometry; MI, myocardial infarction; PCI, percutaneous coronary intervention.
[a] Data missing in 6 patients.
[b] Data missing in 2 patients.
[c] Defined as an estimated glomerular filtration rate ≤ 60 mL/min.
[d] Data missing in 3 patients.
[e] Data missing in 1 patient.
[f] Data missing in 30 patients.
[g] Data missing in 176 patients.
[h] Data missing in 7 patients.
[i] Data missing in 4 patients.
[j] Data missing in 5 patients.

Table 2
Association between rs495828 (*ABO* locus) and measures of platelet activation and aggregation.

	Genotype G/G geometric mean (95% CI)	Genotype G/T geometric mean (95% CI)	Genotype T/T geometric mean (95% CI)	Adjusted effect size per risk allele (95% CI)	p-Value
MEA, 1.0 mM arachidonic acid (AUC, AU * min)	141 (133–150)	156 (145–168)	183 (158–212)	14.9% (6.7%–23.7%)	0.0002
Multiplate, 1.0 μg/mL collagen (AUC, AU * min)	237 (224–249)	261 (243–280)	310 (264–364)	13.1% (5.8%–20.9%)	0.0003
VerifyNow Aspirin (ARU)	433 (429–436)	435 (431–440)	432 (423–441)	0.5% (−0.4%–1.4%)	0.30
S-Thromboxane B_2 (ng/mL)	1.00 (0.92–1.09)	1.06 (0.94–1.19)	0.82 (0.67–1.02)	0.1% (−8.9%–10.0%)	0.98
sP-Selectin (ng/mL)	72.1 (69.6–74.7)	66.2 (63.1–69.5)	60.9 (52.9–70.0)	−7.5% (−11.7%–3.1%)	0.001

The effect size for a SNP corresponds to the change in % in the geometric mean of the variable per increase in the number of CAD risk alleles. Effect sizes were adjusted for age, sex, diabetes, prior myocardial infarction, body mass index, smoking status, and impaired renal function (estimated glomerular filtration rate ≤ 60 mL/min). Abbreviations: ARU, aspirin reaction units; AUC, area under the aggregation curve; CAD, coronary artery disease; CI, confidence interval; MEA, multiple electrode aggregometry; MI, myocardial infarction.

Platelet activation and aggregation measurements are summarized in Table 1. Compliance with aspirin intake was confirmed by S-thromboxane B_2 levels below 30 ng/mL (range 0.02–26.4 ng/mL) in all patients corresponding to >95% inhibition of cyclooxygenase (COX)-1 activity [19].

3.1. Rs495828 (ABO locus) and platelet activation and aggregation

The association of rs495828 with measures of platelet activation and aggregation is shown in Table 2. Rs495828 was associated with high platelet aggregation by MEA with both arachidonic acid (14.9% [95% CI 6.7%–23.7%] higher geometric mean AUC per risk allele, p = 0.0002) and collagen (13.1% [95% CI 5.8%–20.9%] higher geometric mean AUC per risk allele, p = 0.0003) as agonists (Fig. 1B and C). No association was observed between rs495828 and platelet aggregation by VerifyNow Aspirin (0.5% [95% CI −0.4%–1.4%] higher geometric mean ARU per risk allele, p = 0.30). sP-selectin levels showed an inverse association with the number of rs495828 CAD risk alleles(7.5% [95% CI 3.1%–11.7%] lower geometric mean serum levels per risk allele, p = 0.001), while there was no association between rs495828 and S-thromboxane B_2 (0.1% [95% CI −8.9%–10.0%] higher geometric mean serum levels per risk allele, p = 0.98).

In a previous study on the same cohort we reported on the association between common SNPs in platelet-related genes, of which only rs12041331 (*PEAR1*) was associated with MEA-induced platelet aggregation [20]. However, the *ABO* and *PEAR1* genes are located on chromosomes 9 and 1, respectively, and therefore a possible genetic interaction is unlikely. In accordance, we observed no association between the genotypes of rs495828 and rs12041331 (p = 0.13) when performing additional analyses using the *PEAR1* data from our previous paper. Furthermore, adjusting for rs12041331 genotypes did not alter the effect of rs495828 on platelet aggregation and activation.

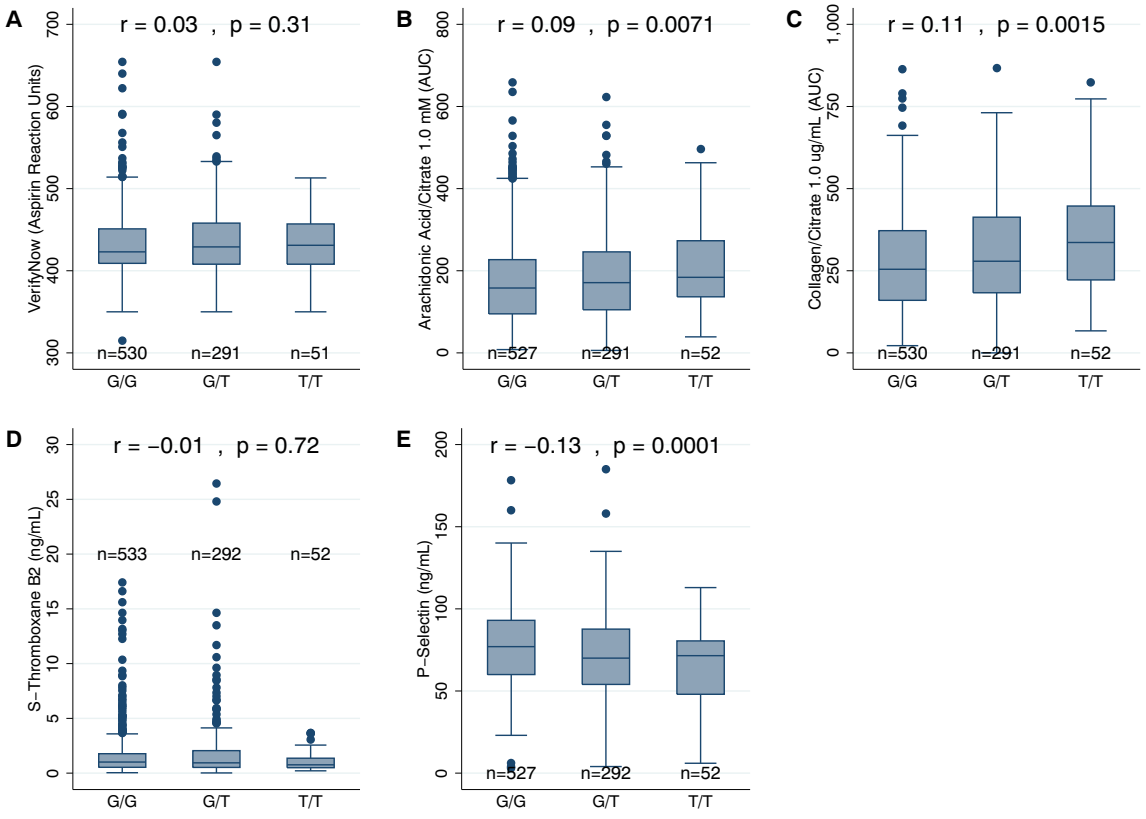

Fig. 1. Distribution of platelet activation and aggregation measurements according to the genotypes of rs495828 (*ABO* locus). Boxes and whiskers represent quartiles and adjacent values. Values outside the range of adjacent values are plotted as outliers. Abbreviations: AUC, area under the aggregation curve.

Table 3
Association between CAD-associated risk variants and measures of platelet activation and aggregation.

Locus	SNP	Nearby genes	Effect MEA, arachidonic acid (95% CI)	p-Value	Effect MEA, collagen (95% CI)	p-Value	Effect VerifyNow Aspirin (95% CI)	p-Value	Effect S-thromboxane B_2 (95%CI)	p-Value	Effect sP-selectin (95%CI)	p-Value
6p21.2	rs10947789	*KCNK5*	1.7 (−6.1–10.3)	0.67	3.8 (−3.5–11.7)	0.32	0.5 (−0.4–1.5)	0.28	−3.8 (−13.1–6.6)	0.46	1.6 (−3.4–6.9)	0.54
7q22	rs10953541	*BCAP29*	−0.3 (−7.2–7.2)	0.94	2.0 (−4.5–8.8)	0.56	0.0 (−0.9–0.9)	0.99	1.2 (−7.6–10.9)	0.79	−3.3 (−7.6–1.1)	0.14
1p32.3	rs11206510	*PCSK9*	−6.7 (−13.9–1.0)	0.088	−7.8 (−14.2 to −0.9)	**0.027**	−0.7 (−1.7–0.3)	0.16	1.5 (−8.2–12.3)	0.77	3.8 (−1.2–9.1)	0.14
19p13.2	rs1122608	*LDLR*	5.0 (−2.9–13.5)	0.22	−0.3 (−7.1–7.1)	0.94	0.0 (−0.9–1.0)	0.94	5.0 (−4.9–16.0)	0.33	−1.7 (−6.4–3.2)	0.49
7q32.2	rs11556924	*ZC3HC1*	1.2 (−5.4–8.2)	0.74	−0.1 (−6.0–6.2)	0.98	−0.6 (−1.5–0.2)	0.12	3.6 (−4.9–12.9)	0.41	2.0 (2.2–6.5)	0.35
6q23.2	rs12190287	*TCF21*	−1.7 (−8.2–5.2)	0.61	2.2 (−3.9–8.7)	0.49	0.2 (−0.7–1.0)	0.68	3.8 (−4.8–13.2)	0.39	3.3 (1.0–7.8)	0.13
10q24.3	rs12413409	*CYP17A1*	2.8 (−7.8–14.7)	0.62	−3.4 (−12.5–6.6)	0.49	0.9 (−0.5–2.2)	0.20	5.9 (−7.8–21.6)	0.42	6.7 (−0.3–14.2)	0.063
6p24.1	rs12526453	*PHACTR1*	−2.8 (−9.3–4.1)	0.42	−1.6 (−7.5–4.6)	0.60	0.2 (−0.7–1.0)	0.68	−2.9 (−11.0–5.9)	0.51	−2.1 (−6.2–2.2)	0.33
17p11.2	rs12936587	*RASD1, SMCR3, PEMT*	−5.2 (−11.2–1.2)	0.11	−4.6 (−10.1–1.2)	0.12	−0.1 (−0.9–0.7)	0.79	1.3 (−6.7–10.0)	0.76	2.6 (−1.5–6.9)	0.21
9p21.3	rs1333049	*CDKN2BAS (ANRIL)*	−1.3 (−7.4–5.2)	0.69	2.2 (−3.6–8.2)	0.47	0.7 (−0.1–1.5)	0.08	2.9 (−5.1–11.5)	0.49	−3.2 (−7.0–0.7)	0.11
10q23	rs1412444	*LIPA*	−0.2 (−6.5–6.5)	0.94	−0.4 (−6.1–5.6)	0.89	−0.2 (−1.0–0.5)	0.54	4.8 (−3.5–13.8)	0.26	0.5 (−3.5–4.7)	0.80
2p11.2	rs1561198	*VAMP5, VAMP8*	−4.2 (−10.0–2.0)	0.18	−1.8 (−7.2–4.0)	0.54	0.1 (−0.6–0.9)	0.70	−4.3 (−11.6–3.6)	0.28	0.9 (−2.9–5.0)	0.64
1q41	rs17465637	*MIA3*	−2.5 (−9.6–5.1)	0.51	−2.3 (−8.8–4.6)	0.50	−0.4 (−1.3–0.5)	0.37	8.5 (−1.3–19.4)	0.092	4.6 (−0.2–9.7)	0.059
6p21.31	rs17609940	*ANKS1A*	−0.9 (−8.2–7.0)	0.81	8.3 (1.1–16.0)	**0.024**	0.0 (−0.9–1.0)	0.94	−5.7 (−14.4–3.9)	0.23	−1.9 (−6.5–2.9)	0.44
4q31.22	rs1878406	*EDNRA*	−8.9 (−16.7 to −0.3)	**0.044**	−7.2 (−14.4–0.6)	0.072	−0.8 (−1.8–0.3)	0.16	6.4 (−5.0–19.2)	0.28	3.1 (−2.5–9.1)	0.28
7p21.1	rs2023938	*HDAC9*	−1.7 (−11.7–9.5)	0.76	−4.8 (−13.6–5.0)	0.33	1.1 (−0.2–2.4)	0.11	−1.2 (−13.8–13.3)	0.86	−0.5 (−7.0–6.5)	0.88
19q13	rs2075650	*APOE*	3.9 (−4.4–13.0)	0.36	−4.0 (−11.0–3.5)	0.28	−1.0 (−2.0–0.0)	0.059	−0.6 (−10.6–10.4)	0.91	1.5 (−3.7–6.9)	0.59
17p13.3	rs216172	*SMG6*	−2.5 (−8.7–4.1)	0.45	−1.0 (−6.7–5.1)	0.75	0.1 (−0.7–0.9)	0.81	0.7 (−7.4–9.4)	0.88	−2.2 (−6.2–1.9)	0.29
2q22.3	rs2252641	*ZEB2*	−2.5 (−8.6–4.0)	0.44	0.6 (−5.1–6.6)	0.84	0.1 (−0.7–0.9)	0.76	−0.6 (−8.4–7.8)	0.88	−0.2 (−4.1–3.9)	0.93
10p11.23	rs2505083	*KIAA1462*	0.7 (−5.7–7.6)	0.84	0.6 (−5.3–6.8)	0.84	0.0 (−0.8–0.8)	0.90	−0.8 (−8.7–7.9)	0.86	−3.9 (−7.8–0.2)	0.060
8p21.3	rs264	*LPL*	−1.0 (−9.8–8.6)	0.83	−1.0 (−9.0–7.8)	0.82	0.0 (−1.1–1.1)	0.95	2.9 (−8.6–15.8)	0.64	2.4 (−3.4–8.6)	0.43
5q31.1	rs273909	*SLC22A4*	−3.8 (−13.2–6.7)	0.46	2.1 (−7.0–12.1)	0.66	0.7 (−0.5–2.0)	0.24	2.7 (−9.8–16.9)	0.68	−0.9 (−7.1–5.7)	0.78
14q32.2	rs2895811	*HHIPL1*	−2.9 (−8.9–3.5)	0.37	−0.4 (−6.1–5.5)	0.88	0.7 (0.0–1.5)	0.064	−3.3 (−10.8–4.9)	0.43	−2.8 (−6.6–1.2)	0.17
8q24.13	rs2954029	*TRIB1*	−1.3 (−7.5–5.4)	0.70	−1.4 (−7.0–4.6)	0.65	0.2 (−0.5–1.0)	0.54	3.9 (−4.4–12.9)	0.37	−0.1 (−4.1–4.1)	0.98
12q24.12	rs3184504	*SH2B3*	7.0 (0.5–14.0)	**0.036**	7.5 (1.6–13.8)	**0.013**	0.4 (−0.4–1.2)	0.31	2.1 (−5.7–10.7)	0.61	−1.3 (−5.1–2.7)	0.52
6q25.3	rs3798220	*LPA*	1.5 (−19.4–27.9)	0.90	0.6 (−18.4–24.0)	0.95	3.4 (0.6–6.4)	**0.019**	2.8 (−23.4–37.9)	0.85	−1.9 (−15.2–13.4)	0.79
15q25.1	rs3825807	*ADAMTS7*	−0.2 (−6.5–6.4)	0.95	−5.1 (−10.5–0.6)	0.080	0.0 (−0.8–0.8)	0.98	2.7 (−5.4–11.4)	0.53	−1.5 (−5.4–2.6)	0.47
6p21.33	rs3869109	*HLA-C, HLA-B*	−5.8 (−11.6–0.3)	0.064	0.7 (−4.9–6.6)	0.82	−0.3 (−1.1–0.4)	0.37	−1.1 (−8.7–7.2)	0.79	−0.2 (−4.1–3.9)	0.92
6q26	rs4252120	*PLG*	5.5 (−1.7–13.1)	0.14	3.8 (−2.6–10.6)	0.25	0.2 (−0.7–1.0)	0.66	−7.8 (−15.6–0.79)	0.072	0.9 (−3.4–5.4)	0.69
17q21.32	rs46522	*UBE2Z*	−8.4 (−14.1 to −2.3)	**0.0077**	−5.6 (−11.0–0.0)	0.052	−0.6 (−1.4–0.1)	0.11	1.0 (−6.9–9.6)	0.81	−1.2 (−5.1–2.9)	0.56
13q34	rs4773144	*COL4A1*	−1.6 (−7.9–5.0)	0.62	3.1 (−2.8–9.4)	0.30	−0.2 (−1.0–0.6)	0.58	3.1 (−5.0–12.0)	0.46	−0.1 (−4.1–4.0)	0.95
1q21.3	rs4845625	*IL6R*	−1.2 (−7.2–5.3)	0.72	0.5 (−5.1–6.4)	0.87	−0.1 (−0.9–0.7)	0.80	−2.1 (−9.7–6.1)	0.60	1.0 (−2.9–5.1)	0.61
10q11.1	rs501120	*CXCL12*	−0.3 (−9.2–9.4)	0.95	−0.5 (−8.5–8.3)	0.91	0.3 (−0.8–1.5)	0.57	−0.5 (−11.7–12.0)	0.93	−0.7 (−6.3–5.3)	0.82
2p24.1	rs515135	*APOB*	−2.0 (−9.9–6.5)	0.63	−2.6 (−9.7–5.1)	0.50	−0.2 (−1.3–0.8)	0.64	−4.6 (−14.2–6.0)	0.38	2.4 (−2.8–7.9)	0.37
1p13	rs599839	*SORT1*	2.4 (−5.3–10.7)	0.56	0.0 (−6.9–7.3)	0.99	0.3 (−0.7–1.2)	0.57	1.8 (−7.7–12.4)	0.72	1.7 (−3.1–6.8)	0.49
2p21	rs6544713	*ABCG8*	−4.1 (−10.6–2.8)	0.24	−3.3 (−9.3–3.0)	0.30	0.3 (−0.5–1.2)	0.47	1.0 (−7.5–10.4)	0.82	−2.4 (−6.6–1.9)	0.27
2q33.1	rs6725887	*WDR12*	−0.5 (−8.6–8.4)	0.92	−0.2 (−7.6–7.9)	0.97	−0.4 (−1.4–0.6)	0.46	5.3 (−5.5–17.4)	0.35	3.2 (−2.2–8.9)	0.25
4q32.1	rs7692387	*GUCY1A3*	6.2 (−2.7–15.8)	0.18	6.1 (−1.9–14.7)	0.14	0.5 (−0.5–1.6)	0.34	3.5 (−7.3–15.5)	0.54	−0.6 (−5.9–4.9)	0.82
15q26.1	rs8039305	*FURIN*	3.0 (−3.4–9.9)	0.37	−0.7 (−6.3–5.3)	0.82	0.1 (−0.7–0.9)	0.85	−7.0 (−14.3–0.9)	0.080	1.3 (−2.7–5.4)	0.54
13q12.3	rs9319428	*FLT1*	−5.9 (−11.9–0.5)	0.070	−2.9 (−8.6–3.1)	0.33	−0.3 (−1.1–0.5)	0.44	−3.4 (−11.1–5.1)	0.42	−2.6 (−6.5–1.6)	0.22
11q23.3	rs964184	*ZNF259, APOA5, APOA1, APOC3*	1.8 (−7.6–12.1)	0.71	−0.3 (−8.9–9.1)	0.95	−1.0 (−2.2–0.2)	0.11	−7.1 (−17.9–5.1)	0.24	0.4 (−4.9–6.1)	0.87
11q22.3	rs974819	*PDGFD*	4.2 (−3.1–11.9)	0.27	2.2 (−4.2–9.0)	0.51	0.2 (−0.7–1.1)	0.68	−3.1 (−11.5–6.2)	0.50	−0.1 (−4.5–4.5)	0.97
3q22.3	rs9818870	*MRAS*	5.9 (−2.4–14.9)	0.17	5.8 (−1.8–13.9)	0.14	−0.1 (−1.1–0.8)	0.77	3.9 (−6.3–15.2)	0.47	−1.5 (−6.5–3.7)	0.56
21q22.1	rs9982601	*MRPS6*	−1.2 (−9.4–7.8)	0.79	−1.0 (−8.5–7.1)	0.80	0.0 (−1.1–1.1)	1.00	0.9 (−9.7–12.7)	0.88	2.7 (−2.7–8.5)	0.33

The effect size for a SNP corresponds to the change in % in the geometric mean of the variable per increase in the number of CAD risk alleles. Effect sizes were adjusted for age, sex, diabetes, prior myocardial infarction, body mass index, smoking status, and impaired renal function (estimated glomerular filtration rate ≤ 60 mL/min). Nominally significant p-values are displayed in bold. Abbreviations: CAD, coronary artery disease; MEA, multiple electrode aggregometry.

3.2. Other CAD-associated SNPs and platelet activation and aggregation

There were no significant associations between any of the remaining 44 CAD-associated SNPs and platelet activation or aggregation (Table 3). However, rs3184504 (*SH2B3*) was nominally associated with platelet aggregation measured by MEA. The GRS was not associated with MEA (adjusted effect per SD for arachidonic acid: −1.4% [95% CI −5.7%–3.1%], p = 0.54, adjusted change per SD for collagen: 0.3% [95% CI −3.6%–4.5%], p = 0.87), VerifyNow Aspirin (adjusted change per SD: 0.3% [95% CI −0.3%–0.8%], p = 0.36), sP-selectin (adjusted effect per SD: −0.5% [95% CI −3.3%–2.3%], p = 0.70), or S-thromboxane B_2 (adjusted change per SD: 3.5% [95% CI −2.3%–9.5%], p = 0.24) measurements.

4. Discussion

In the present study, we investigated the association between platelet activation and aggregation and CAD-associated genetic variants. The *ABO* risk allele (T-allele) was significantly associated with high platelet aggregation levels as assessed by MEA, but not by VerifyNow Aspirin. The other CAD-associated risk variants were not associated with platelet activation or aggregation.

Currently, the ABO locus is the only CAD-associated locus that has been shown to increase the risk of MI on top of the increased risk associated with coronary atherosclerosis. In one of the first GWAS studies, allele frequencies in CAD-patients with and without prior MI were compared, and among CAD-associated genetic loci, only the *ABO* locus was significantly associated with MI [7]. This finding was subsequently confirmed in a large GWAS by the CARDIoGRAMplusC4D Consortium [3], and in the UK Biobank [8], suggesting that this locus may specifically increase the risk of plaque rupture and/or thrombosis. Extending this finding, the increased platelet aggregation observed in the present study provides a possible explanation for the increased risk of MI in *ABO* risk allele carriers. However, aggregation by VerifyNow Aspirin was not affected. This corresponds with previous results from our group and may reflect that the two platelet aggregation tests are based on different test principles [21,22]. The most optimal test to evaluate platelet aggregation is a topic of continuous discussion. Both MEA [23] and VerifyNow [24] have been shown to predict clinical outcome and are recommended for platelet function testing [25].

The *ABO* gene encodes a glycosyltransferase, which mediates the glycosylation of the H-antigen present on a large variety of cell types, including erythrocytes [26]. Variants in the coding sequence of the gene alter the specificity for the sugar substrate forming the basis of the blood types of the ABO blood type system. Of the three allelic forms (A, B and O), the A/B polymorphism arises from several SNPs in *ABO*, which result in A and B transferases that differ by four amino acids. The O allele arise from a one base deletion (rs8176719) in exon 6, resulting in a frameshift, and an inactive glycosyltransferase that leaves the ABO antigen precursor (the H antigen) unmodified. Rs495828 is positioned in the upstream region of the *ABO* gene. It is a tissue specific eQTL, with the T-allele associated with lower *ABO* transcription levels in blood, and higher transcription in adipose tissue and mucosa [27], opening the possibility that differences in *ABO* transcript level play a role in platelet aggregation.

The *ABO* locus is also significantly associated with venous thromboembolism (VTE). In a large meta-analysis comprising 38 studies and 10,305 patients, non-O blood type (A, B, and AB) was associated with two times higher odds of VTE compared with O blood type carriers [28]. In fact, ABO blood type status is estimated to explain 20% of all VTEs in the general population [29]. In a recent UK Biobank GWAS, rs495828 was associated with deep venous thrombosis with a p-value of 1.58×10^{-52}, with the broad *ABO* locus being the second most strongly associated locus after the F5 gene [30], underscoring the importance of the ABO locus in thrombus formation. It is currently believed that the increased thrombotic risk in non-O carriers is mediated primarily by an ABO glycosyltransferase modification of the vWF protein, resulting in reduced vWF proteolysis and higher vWF plasma levels [31]. In our study, there was moderate linkage disequilibrium ($r^2 = 0.42$) between rs495828 and rs8176719 (O type polymorphism). Hence, the observed association between *ABO* and platelet aggregation may possibly represent an effect of one or more of the variants defining the non-O blood types.

The role of platelet aggregation in VTE is uncertain. Recently, in the community-based Framingham Heart Study cohort (2831 participants, median follow-up of 20.4 years), baseline platelet aggregation was only weakly associated with incident VTE, and in a paradoxical direction [32]. However, in the INSPIRE study [33], aspirin after anticoagulant treatment reduced the overall risk of VTE recurrence by more than a third in patients with a first unprovoked VTE suggesting involvement of platelet aggregation in VTE formation as well.

Higher levels of sP-selectin may promote atherosclerosis development and predict cardiovascular events [34,35]. Nonetheless, we found that carriers of the *ABO* CAD risk allele had lower levels of sP-selectin than non-carriers, and is in contrast to the increased MEA platelet aggregation observed in risk allele carriers. The same counterintuitive relationship between the CAD risk allele and sP-selectin has previously been documented in population-based cohorts without known cardiovascular disease [36,37]. The *ABO* locus has also been associated with multiple other traits including pancreatic cancer [38], angiotensin-converting enzyme activity [39], type 2 diabetes [36], and low-density lipoprotein cholesterol levels [40]. Therefore, a possible explanation might be that the *ABO* locus exerts simultaneous protective and damaging effects on CAD risk through separate pathways. Importantly, the absolute difference in sP-selectin levels between homozygous wildtype and risk allele carriers was relatively small, and hence, the clinical importance may be modest.

4.1. Strengths and limitations

The main strengths of the study include the standardized sampling procedure and the large population of stable CAD patients receiving mono antiplatelet therapy with low-dose aspirin. However, some limitations have to be considered. ABO blood type status was not determined and none of the ABO determining variants were genotyped. Likewise, vWF was only available in 124 patients and were therefore not included in the analyses. Hence, it is not possible to determine whether the increased MEA aggregation may be explained by the blood type and whether this effect could be partly attributed to higher vWF plasma levels. Cholesterol levels and alcohol consumption could potentially affect platelet function, but we did not register these parameters. Since the *ABO* risk allele has also been linked to a number of other cardiovascular risk factors, risk allele carriers may have been more likely to receive primary preventive aspirin treatment for several years prior to their CAD event. Possible differences in length of therapy between carriers and non-carriers might potentially have influenced the platelet aggregation measured, although it should be noted that all patients had received aspirin therapy for a minimum of one year prior to the study. Except for the *ABO* variant, we did not observe any significant associations between CAD-associated risk variants and platelet activation or aggregation. Although this is consistent with previous GWASs, our study was not powered to observe small differences given the defined Bonferroni-corrected significance levels of these analyses. The number of identified CAD risk variants has recently increased beyond what has been genotyped in the present study [8]. Therefore, some additional loci may potentially affect CAD and MI risk through platelet aggregation. In particular, the newly identified *MRVI1* locus has recently been linked to both CAD and platelet aggregation [41,42].

5. Conclusion

In stable CAD patients treated with low-dose aspirin, the CAD risk allele of the *ABO* locus was associated with increased MEA platelet aggregation. This finding may provide a possible mechanistic explanation for the increased MI risk in *ABO* CAD risk variant carriers.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.01.090.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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