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Replication and hematological characterization of human platelet reactivity genetic associations in men from the Caerphilly Prospective Study (CaPS)

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Abstract Platelet reactivity, an important factor in hemostasis and chronic disease, has widespread inter-individual variability with a substantial genetic contribution. Previously, our group performed a genome-wide association study of platelet reactivity identifying single nucleotide polymorphisms (SNPs) associated with ADP- and epinephrine- induced aggregation, including SNPs in *MRVI1, PIK3CG*, *JMJD1C*, and *PEAR1*, among others. Here, we assessed the association of these previously identified SNPs with ADP-, thrombin-, and shear- induced platelet aggregation. Additionally, we sought to expand the association of these SNPs with blood cell counts and hemostatic factors. To accomplish this, we examined the association of 12 SNPs with seven platelet reactivity and various hematological measures in 1300 middle-aged men in the Caerphilly Prospective Study. Nine of the examined SNPs showed at least suggestive association with platelet reactivity. The strongest associations were with rs12566888 in *PEAR1* to ADP-induced ($p = 1.51 \times 10^{-7}$) and thrombin-induced ($p = 1.91 \times 10^{-6}$) reactivity in platelet rich plasma. Our results indicate *PEAR1* functions in a relatively agonist independent manner, possibly through subsequent intracellular propagation of platelet activation. rs10761741 in *JMJD1C* showed suggestive association with ADP-induced reactivity ($p = 1.35 \times 10^{-3}$), but its strongest associations were with platelet-related cell counts ($p = 1.30 \times 10^{-9}$). These associations indicate variation in *JMJD1C* influences pathways that modulate platelet development as well as those that affect reactivity. Associations with other blood cell counts and hemostatic factors were generally weaker among the tested SNPs, indicating a specificity of these SNPs' function to platelets. Future genome-wide analyses will further assess association of these genes and identify new genes important to platelet biology.

Keywords Platelet aggregation · SNPs · Thrombin · Blood platelets · Platelet count

Abbreviations

SNP	Single nucleotide polymorphism
GWAS	Genome-wide association study
PRP	Platelet rich plasma
CaPs	Caerphilly Prospective Study in men
PLT	Platelet count
MPV	Mean platelet volume
LTA	Light transmission aggregometry
WBC	White blood cell
RBC	Red blood cell
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MAF	Minor allele frequency

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Introduction

Platelet reactivity and aggregation are important aspects of hemostasis and wound healing, as well as common therapeutic targets in primary and secondary prevention of cardiovascular disease [1]. Several endogenous agonists, including epinephrine, ADP, and thrombin, among other activators, cause platelet aggregation. These reactive mechanisms serve as initial steps in thrombosis and clotting that allow for injury healing and repair. Differences in platelet reactivity among individuals have substantial effects on thrombotic function and clinical use of antiplatelet agents. Inter-individual variability in platelet reactivity does have a substantial genetic component, with heritability estimates ranging from 40–60 % [2].

Despite this considerable genetic influence, relatively few genome-wide genetic scans have been conducted on platelet reactivity traits in cohort studies, possibly due to logistical and technical difficulties of accurately collecting such data [2]. Our group conducted the largest genome-wide association study (GWAS) on ADP- and epinephrine-induced reactivity in platelet-rich plasma (PRP) and collagen lag time [3]. There, we identified and replicated novel associations with single nucleotide polymorphisms (SNPs) in or near *MRVI1, SHH, PIK3CG* and *JMJD1C*, as well as strengthened prior associations of SNPs in or near *ADRA2A*, *PEAR1* and *GP6* [3]. There were also several loci with suggestive associations, including some with responses to two or more agonists in the same direction of effect (e.g., *ADAMTS2*, *RGS18*, and *SVIL*). The relevance of these associated genes to platelet function in subsequent animal and cellular models reinforced the utility of human genetics in identifying genes important to platelet reactivity. For example, PEAR1 is involved in sustaining αIIβ3 activation following agonist recognition and in attenuating megakaryopoiesis [4, 5]. Additionally, variants in *SVIL* were associated with human shear-dependent platelet function. *SVIL* knockout mice had larger platelets and enhanced thrombus formation under high-shear conditions [6]. Based on these human genetic and subsequent functional studies, we hypothesized that additional assessment of our previously identified SNPs with a variety of agonists and related traits may extend SNP associations and highlight candidates for future functional studies.

To accomplish this, we used data from the Caerphilly Prospective Study (CaPs), a population sample of approximately 3000 middle aged men from Caerphilly, South Wales. Started in 1979, the primary intent of the study was to identify risk factors for ischemic heart disease [7]. During two of the five phases, platelet reactivity measures in PRP and/or whole blood were collected for ADP, thrombin, and shear stress. We selected 12 promising SNPs from our prior platelet reactivity GWAS and genotyped these in CaPs to assess whether these SNPs were associated with platelet reactivity to several agonists in an independent cohort [3]. Additionally, platelet count (PLT) and mean platelet volume (MPV) as well as other blood cell count and hemostatic factors were collected. We further examined whether these platelet reactivity SNPs were associated with these blood cell counts and hemostatic factors.

Materials and methods

Study sample and approval

CaPs is comprised of unrelated men of European ancestry from Caerphilly, South Wales as previously described [7]. All phenotypes examined presently were collected during Phase 2 or Phase 3 of the study, while genomic DNA was collected in Phase 4. Informed consent was obtained from all participants. Ethical approval for genetic studies in CaPs was granted by the South East Wales Research Ethics Committee (05/WSE02/131) and the Institutional Review Board at the National Hearth Lung and Blood Institute.

Platelet function measures in CaPs

ADP- and thrombin-induced reactivity in PRP

In Phase 2, platelet reactivity in PRP to ADP and thrombin was measured by light transmission aggregation (LTA). Fasting venous blood samples were collected into 0.13 M sodium citrate tubes, and PRP isolated by protocols previously described [8, 9]. Aggregation was measured in duplicate in PRP adjusted to 300,000 platelet/*u*L in autologous platelet poor plasma. The extent of optical density change to 0.725 μM/L ADP in the primary and secondary response wave, as well as the primary response wave to 0.056 unit/mL thrombin (Sigma Chemical Corp., Poole, UK), was measured in a Rubel-Renaud coaguloaggregometer [8].

ADP-induced reactivity in whole blood

In Phase 3, ADP-induced reactivity was determined in whole blood by impedance measures as previously described [10, 11]. In brief, fasting venous blood samples were collected in plastic syringes containing 0.13 M sodium citrate at pH 7.4. Sealed samples were left 30 min. All impedance testing was completed between 30 min and 120 min. The minimal concentration of ADP to produce a 1.5 Ω change in impedance within 2.5 min in a Chronolog 560 Aggregometer (Havertown, PA), was determined by testing 19 serially increasing concentrations of ADP (from

0.1 to 21.5 μM/L) (Sigma Chemical Corp., Poole, UK). The minimal ADP dose required to produce a 21.5 Ω impedance change was measured similarly.

Shear-induced platelet reactivity in whole blood

Retention of platelets after induction of shear stress filtration was measured in Phase 3 as previously described [12]. Briefly, within 5 min of a fasting venous blood draw, heparinized blood was forced at 40 mmHg through a 13 mm diameter filter with fibers ranging from 0.1–3.4 μM. Blood drops passing through filters were measured electronically over time. PLT was measured with a Sysmex K1000 analyzer (Kobe, Japan) in separate pre-filtration and post-blockage samples. Blockage was defined as ≤1 blood drop in 5 s. Pre- and post-blockage PLT were used to calculate the absolute and relative retention values. Four samples were eliminated for having higher post-blockage PLT, and two were eliminated for missing either a pre- or post-PLT .

Other hematological and hemostatic factor measures

Fasting, citrated blood plasma from was stored at −70 °C for assays conducted at the University of Glasgow. Cell counts were obtained on blood samples from Phase 2 (Couter S+, Coulter Corporation, Miami, FL) and Phase 3 (Technicon HI, Bayer, Newbury, UK). These included PLT, MPV, white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophil, lymphocyte, monocyte, eosinophil, basophil, and leukocyte counts. Hemostatic factors included Clauss fibrinogen [13], von Willebrand Factor antigen [14], tissue plasminogen activator antigen [15], plasminogen-activator inhibitor [15], and fibrin D-dimer with both original ELISA assay [15] and Gold assay [16] as previously described.

SNP selection and genotyping

Twelve SNPs were selected primarily on the basis of our prior GWAS results. Eight were selected for having at least suggestive results ($p < 5 \times 10^{-5}$) for associations with ADP- and epinephrine-induced platelet reactivity [3]. We included these because we reasoned they were more likely to influence platelet aggregation in an agonist independent manner, particularly as the overlap between agonists in the prior and current studies was incomplete. These eight SNPs were rs11144351 (*PIP5K1B*), rs12359150 (*CUBN*), rs12566888 (*PEAR1*), rs16876805 (*ATP6V0D2*), rs2479008 (*GMDS*), rs4128880 (*RGS18*), rs467053 (*ADAMTS2*), and rs7940646 (*MRVI1*). Since platelet reactivity to epinephrine was not measured in CaPs, two additional ADP-only associated SNPs, rs2363910 in *SHH* and rs9996294 in *RAPGEF2*, were also selected. Two epinephrine-only associated loci rs10761741 in *JMJD1C* and rs342286 in *PIK3CG* were selected because both have demonstrated pleiotropy with PLT and/or MPV [17]. The selected SNPs and rationale for inclusion are summarized in Table 1 [3]. SNPs were genotyped on genomic DNA stored at LGC Genomics, Inc. (Teddington, UK) using KASP^{TM} genotyping technology. SNP probe information is available in Supplemental Table 1, and SNPviewer (LGC Genomics, Inc., Teddington, UK) was used to review cluster genotype calling.

Statistical analyses

Most traits were normally distributed, but appropriate data transformations were applied when non-normal as noted in the Results. We only adjusted for age in our models as CaPs is entirely comprised of men. We performed analyses in all subjects as well as in a subset of participants not taking anti-platelet medication (e.g., aspirin and dipyridamole) and with greater than 8 h since last food eaten (Supplemental Table 2). Subjects with a genotyping rate less than 80 % were also excluded (n = 37). One SNP, rs16876805 in *ATP6V0D2*, was excluded due to its rare minor allele frequency (MAF = 0.009). The sample size for each phenotype is in Supplemental Table 2. SNPs were tested for association with quantitative measures of platelet reactivity using PLINK [18]. We tested 11 SNPs for association with 7 distinct platelet aggregation measures in our primary analyses. Therefore, we used Bonferroni correction for multiple testing: $0.05/(7 \text{ SNPs} \times 11 \text{ phenotypes}) = 6.49 \times 10^{-4}$. Bonferroni correction is overly conservative, as these SNPs have prior GWAS associations with our primary outcome and the phenotypes are correlated. We also present associations with $p < 0.01$ in the main text and full results for additive models in the supplement (Supplemental Tables 3–8). We also performed two separate secondary analyses: (1) platelet-related cell count (PLT/MPV) and (2) non-platelet cell count and hemostatic factors. Associations with $p < 0.01$ in these secondary analyses are reported.

Results

Genotype quality was high for all SNPs with an average call rate of 98.1 %. Allele frequencies in CaPs ranged from rare (rs16876805, 0.9 % MAF) to common (rs4128880, 47.0 % MAF), and were similar to reference European ancestry allele frequencies (Table 1). Demographics for subjects included in analyses are summarized in Supplemental Table 9.

Table 1 Candidate SNPs, rationale, and genotype frequencies in CaPs and 1000 Genomes Project

Marker	Gene/SNP function	Prior rationale	Minor allele	HWE (P) CaPs	MAF CaPs	MAF 1000G CEU
rs10761741	*JMJD1C*/intron	Epinephrine[d], PLT	T	0.29	0.38	0.48
rs11144351	*PIP5K1B*/intron	ADP[c], epinephrine[a]	G	0.30	0.14	0.13
rs12359150	*CUBN*/intron	ADP[a], epinephrine[a]	A	0.53	0.10	0.07
rs12566888	*PEAR1*/intron	ADP[d], epinephrine[d]	T	0.006	0.08	0.05
rs16876805[e]	*ATP6VOD2*/Ile336Val	ADP[a], epinephrine[a]	C	1	0.009	0.008
rs2363910	*SHH*/intergenic	ADP[d]	T	0.27	0.10	0.08
rs2479008	*GMDS*/intergenic	ADP[a], epinephrine[a]	T	0.57	0.14	0.17
rs342286	*PIK3CG*/intergenic	PLT, epinephrine[d]	G	0.31	0.45	0.45
rs4128880	*RGS18*/intergenic	ADP[b], epinephrine[a]	T	0.70	0.48	0.42
rs467053	*ADAMTS2*/intron	ADP[a], epinephrine[a]	C	0.95	0.41	0.38
rs7940646	*MRVI1*/intron	ADP[b], epinephrine[b]	T	0.90	0.30	0.23
rs9996294	*RAPGEF2*/intergenic	ADP[b]	G	0.30	0.12	0.09

SNP, single nucleotide polymorphism; *MAF*, minor allele frequency; *CaPs*, Caerphilly Prospective Study in men; *HWE*, Hardy–Weinberg equilibrium; *1000G CEU*, 1000 genomes CEU population; *PLT*, platelet count; *MPV*, mean platelet volume

[a] $P < 5 \times 10^{-5}$, [b]$P < 5 \times 10^{-6}$, [c]$P < 5.0 \times 10^{-7}$, [d]$P < 5.0 \times 10^{-8}$ for platelet reactivity in Johnson et al. [3]

[e] Marker excluded from association analyses

Nine of eleven tested SNPs showed at least marginal associations with platelet reactivity ($p < 0.05$) (Supplemental Table 10). The strongest associations were with rs12566888 in *PEAR1*. The T allele was associated with reduced primary aggregation to ADP ($\beta = -2.29$, $p = 9.98 \times 10^{-5}$), secondary aggregation to ADP ($\beta = -0.13$, $p = 1.51 \times 10^{-7}$), and aggregation to thrombin ($\beta = -2.07$, $p = 1.91 \times 10^{-6}$) (Table 2). In addition, several other SNPs showed suggestive associations with platelet reactivity traits. The G allele of rs10761741 in *JMJD1C* showed evidence of association with increased ADP dose necessary to incur both a 1.5 Ω impedance ($\beta = 0.44$, $p = 1.35 \times 10^{-3}$) and 21.5 Ω impedance ($\beta = 0.42$, $p = 8.52 \times 10^{-3}$) change (Table 2). These results indicate that the G allele of rs10761741in *JMJD1C* were associated with decreased platelet reactivity. SNPs in *SHH* ($\beta = -0.06$, $p = 6.65 \times 10^{-3}$), *RGS18* ($\beta = -0.04$, $p = 4.35 \times 10^{-3}$), and *MRVI1* ($\beta = 11.01$, $p = 5.04 \times 10^{-3}$) were suggestively associated with secondary aggregation to ADP (Table 2).

Next, we examined whether these 11 SNPs with prior relationships with platelet aggregation were also associated with platelet-related cell count measures, other blood cell counts, and hemostatic factors. Two SNPs, rs10761741 in *JMJD1C* and rs342286 in *PIK3CG*, showed associations with PLT and/or MPV (Table 3). In both phases, the G allele of rs10761741 was associated with decreased PLT (Phase 2: $\beta = -9.37$, $p = 3.50 \times 10^{-5}$; Phase 3:

Table 2 Association of SNPs with platelet reactivity measures ($p < 0.01$)

Marker	Gene	Allele	Phenotype	No exclusions		Exclusions[b]	
				Effect	*p* value	Effect	p value
rs12566888	*PEAR1*	T	1° aggregation ADP	−2.29	9.98×10^{-5}	−1.83	4.71×10^{-3}
			2° aggregation ADP	−0.13	1.51×10^{-7}	−0.11	2.56×10^{-5}
			Thrombin	−2.07	1.91×10^{-6}	−2.17	8.07×10^{-6}
			ADP 1.5 Ω impedance	0.76	2.36×10^{-3}	0.81	3.86×10^{-3}
rs10761741	*JMJD1C*	G	ADP 1.5 Ω impedance	0.44	1.35×10^{-3}	0.46	2.76×10^{-3}
			ADP 21.5 Ω impedance	0.42	8.52×10^{-3}	0.42	1.77×10^{-2}
rs2363910	*SHH*	T	2° aggregation ADP	−0.06	6.65×10^{-3}	−0.04	1.11×10^{-1}
rs4128880	*RGS18*	C	2° aggregation ADP	−0.04	4.35×10^{-3}	−0.04	8.08×10^{-3}
rs7940646[a]	*MRVI1*	C	Absolute shear stress	11.01	5.04×10^{-3}	10.21	1.55×10^{-2}

[a] Under dominant model

[b] Exclusions included taking anti-platelet medications and fasting for <8 h before blood draw

Table 3 Platelet count (PLT) and mean platelet volume (MPV) associations ($p < 0.01$)

Marker	Gene	Allele	Phenotype	Effect	p value
rs10761741	*JMJD1C*	G	MPV Phase 2	1.17	5.07×10^{-3}
			MPV Phase 3	2.25	1.30×10^{-9}
			PLT Phase 2	−9.37	3.50×10^{-5}
			PLT Phase 3	−8.71	9.05×10^{-5}
rs342286	*PIK3CG*	G	MPV Phase 2	1.39	9.43×10^{-4}
			MPV Phase 3	1.09	3.46×10^{-3}

Table 4 Red blood cell, white blood cell count, and hemostatic factor associations ($p < 0.01$)

Marker	Gene	Allele	Phenotype	Effect	p value
rs9996294	*RAPGEF2*	G	RBC Phase 2	−6.04	9.97×10^{-3}
			D-dimer Phase 2	0.42	9.50×10^{-3}
			Gold D-dimer Phase 2	0.38	7.40×10^{-3}
			Hemoglobin Phase 2	−2.49	5.12×10^{-4}
			Hematocrit Phase 2	−0.69	1.07×10^{-3}
rs342286	*PIK3CG*	G	Hemoglobin Phase 2	−1.25	9.30×10^{-3}
			Monocyte count Phase 2[a]	0.04	1.07×10^{-3}
			RBC Phase 3	−4.65	3.54×10^{-3}
rs12359150	*CUBN*	A	Monocyte count Phase 3[a]	0.25	3.91×10^{-5}
rs4128880	*RGS18*	C	Hematocrit Phase 3[a]	−0.60	4.38×10^{-3}

RBC, red blood cell count

[a] Under recessive model

$\beta = -8.71$ $p = 9.05 \times 10^{-5}$) and increased MPV (Phase 2: $\beta = 1.17$, $p = 5.07 \times 10^{-3}$; Phase 3: $\beta = 2.25$ $p = 1.30 \times 10^{-9}$) (Table 3). Similarly, rs342286 in *PIK3CG* associated with increased MPV (Phase 2: $\beta = 1.39$ $p = 9.43 \times 10^{-4}$; Phase 3: $\beta = 1.09$ $p = 3.46 \times 10^{-3}$) (Table 3). However, no other SNP, including rs12566888 in *PEAR1*, showed evidence of association with PLT or MPV ($p > 0.05$).

Associations with non-platelet blood cell count and hemostatic factors were generally weak. The most intriguing associations were rs9996294 in *RAPGEF2* and rs342286 in *PIK3CG* with RBC-related traits (Table 4). These included nominal associations of (1) rs9996294 with RBC ($\beta = -6.04$, $p = 9.97 \times 10^{-3}$), hemoglobin ($\beta = -2.49$, $p = 5.12 \times 10^{-4}$), hematocrit ($\beta = -6.90$, $p = 1.07 \times 10^{-3}$), and D-dimer ($\beta = 0.38$, $p = 7.40 \times 10^{-3}$) and (2) rs342286 with RBC ($\beta = -4.65$, $p = 3.54 \times 10^{-3}$) and hemoglobin ($\beta = -1.25$, $p = 9.30 \times 10^{-3}$).

Discussion

In this investigation, our primary goal was to assess the associations of 12 SNPs identified by GWAS with a variety of platelet reactivity measures in the independently ascertained CaPs. We further implicate rs12566888 in *PEAR1* with ADP-induced platelet reactivity and expand its association to thrombin-induced platelet reactivity. We also provide suggestive evidence for association of 8 other SNPs, including rs10761741 in *JMJD1C*, rs2363910 in *SHH*, rs4128880 in *RGS18*, and rs7940646 in *MRVI1*. A majority of the platelet reactivity associated SNPs were not associated with platelet-related cell count traits. Of the SNPs examined, only rs10761741 in *JMJD1C* and rs342286 in *PIK3CG* showed association with PLT and/or MPV. Associations with non-platelet blood cell counts and hemostatic factors were fewer and weaker. Nonetheless, rs9996294 in *RAPGEF2* and rs342286 in *PIK3CG* had suggestive associations with RBC-related traits. The lack of associations of platelet reactivity implicated SNPs with other blood cell and hemostatic factors suggest divergent pathways for platelet reactivity from other blood cell and hemostatic factor traits.

The strongest associations with platelet aggregation in this study were with rs12566888 in *PEAR1*. In our previous GWAS, *PEAR1* influenced platelet aggregation to both ADP and epinephrine [3]. There, the T allele of intronic rs12566888 was associated with reduced reactivity to both ADP and epinephrine. Here, we replicate the association of the T allele with reduced aggregation to ADP and extend it to reduced aggregation to thrombin. Our studies in conjunction with others have now observed associations of *PEAR1* with platelet reactivity to epinephrine, ADP,

thrombin, and collagen [3, 19]. Its associations with multiple agonists indicate *PEAR1* functions in the intracellular propagation of general platelet activation as opposed to the recognition and downstream signaling of specific agonists. Platelet activation increases expression of PEAR1 and its phosphorylation at the cell surface. This phosphorylation of PEAR1 yields a signaling cascade that activates αIIbβ3 and allows it to bind to fibrinogen [5]. Despite a number of genetic studies implicating different *PEAR1* variants [3, 19–23], rs12566888 has been the strongest and most consistently associated variant. It also has been associated with aspirin, clopidogrel/prasugel, and dual anti-platelet responses, as well as sticky platelet syndrome and fetal loss [22, 24–26]. These findings indicate *PEAR1* plays an agonist independent role in platelet reactivity and suggest it as a valuable therapeutic target.

We also observed suggestive association of rs10761741 in *JMJD1C* with ADP-induced platelet reactivity. The G allele was associated with a higher ADP dose necessary to induce impedance changes, indicating reduced reactivity. The T allele of rs10761741, the alternative allele to G, was associated with increased reactivity to epinephrine but not ADP, at a genome-wide significant level, in our previous study [3]. In conjunction with these previous results, our study indicates that the G allele of rs10761741 in *JMJD1C* is associated with decreased platelet reactivity to ADP and epinephrine. However, our strongest associations of rs10761741 were with MPV and PLT as previously seen with this SNP and others in *JMJD1C* [17, 27, 28]. The G allele of rs10761741 was associated with increased MPV and decreased PLT, as well as decreased platelet reactivity. Associations with PLT/MPV and aggregation suggest that *JMJD1C* functions in pathways that influence the platelet development as well as their reactivity to various agonists. *JMJD1C* encodes a histone demethylase expressed and functional in pluripotent and multipotent cells including human bone marrow [29–31]. Its development functions, possibly through interactions with androgen receptor, are thought to modulate gene expression [32]. Identification of transcriptional targets and temporal relationships of *JMJD1C* within platelet development and activation will be necessary to disentangle causal pathways and to what extent they are shared between these processes.

Generally, the other SNPs did not associate with blood cell counts or hemostatic traits. rs12566888 in *PEAR1* notably did not show associations with any cell count or hemostatic trait ($p > 0.05$). The specificity of *PEAR1* associations with platelet aggregation indicates *PEAR1* functions primarily in a platelet's ability to respond to agonists and injury. The only other SNP to show association with MPV was rs342286 in *PIK3CG*. rs342286 was also marginally associated with various RBC traits, indicating *PIK3CG* influences function of multiple blood cell traits as suggested in animal models [33–35]. Also marginally associated with RBC traits was rs9996294 in *RAPGEF2*. Mouse knockouts of *Rapgef2* suggest the essentiality of the gene for embryonic RBC development but not adult hematopoiesis [36]. These developmental effects of *RAPGEF2* on hematopoiesis may have life-long effects on RBC-related traits that we marginally detected here. However, the general lack of associations of platelet reactivity SNPs with other hematological traits suggests that many platelet aggregation loci generally function in a platelet specific manner. This specificity implies that continued investigation of platelet aggregation genes should be examined in future cohort studies of larger sample size as well as animal and cellular models. To this end, we plan to perform a GWAS of platelet reactivity in CaPs to identify novel genetic elements that influence platelet function.

The findings of this study are subject to several limitations. First, although our aim was to further examine the strongest associations of our previous GWAS, the platelet aggregation measures of the two investigations were not identical. Both studies did have reactivity measures in response to ADP, although study doses and equipment varied. We could not, however, specifically query epinephrine-induced aggregation associations previously observed. Additionally, differences in agonist dosages and methods of measurements may explain why we are unable to replicate some previous findings. Second, our ability to detect common variant associations was limited by sample size, although CaPs is among the largest cohort studies with collected platelet aggregation. Increased sample size and meta-analysis with other cohort studies will further increase statistical power. Third, CaPs is comprised entirely of men from a local community in South Wales. These genetic associations should eventually be extended to more diverse cohorts and populations.

In this investigation, we confirm the association of rs12566888 in *PEAR1* with platelet aggregation to ADP and extend it to reactivity to thrombin. Our associations along with others indicate that *PEAR1*'s role in platelet reactivity is relatively agonist independent. Nine of the eleven SNPs examined showed at least suggestive association with platelet reactivity measures. We observed associations of rs10761741 in *JMJD1C* with PLT and MPV as well as provide marginal support for its association with ADP-induced platelet reactivity. These results further confirm the role of these genes in platelet aggregation. Our associations also indicate that there are shared and unique pathways governing platelet-related cell count and platelet reactivity. Further interrogation of these pathways by identifying more associated genetic factors through increased sample size and genomic coverage and by modeling these factors in animal and cellular models will help determine to what extent these pathways are shared.

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Compliance with ethical standards

Conflicts of interests The authors state that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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