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Genome-wide meta-analyses identifies 7 loci associated with platelet aggregation in response to agonists

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Platelet function mediates both beneficial and harmful effects on human health, but few genes are known to contribute to variability in the process. We tested association of 2.5 million SNPs with platelet aggregation responses to 3 agonists (ADP, epinephrine and collagen) in two European-ancestry cohorts ($N \leq 2{,}753$ in the Framingham Heart Study, $N \leq$

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Author contributions

A.D. Johnson, L.R.Y., C.J.O and L.C.B. led the study. A.D. Johnson took primary responsibility for drafting the manuscript with contributions and editing from L.R.Y., M-H.C., N.F., M.G.L., G.T., A.T.K., Q.Y., D.M.B., C.J.O and L.C.B. G. Tofler, M.G.L. and C.J.O. were involved in the original guidance, collection and analysis of Framingham platelet phenotype data. L.R. Yanek, N.F., D.M.B. and L.C.B. were involved in the guidance, collection and analysis for the Genetic Study of Atherosclerosis Risk (GeneStar) phenotype data. S.J. Lin, A.T.K. and M.A.P. designed the database and analysis system used in GeneStar analyses. A.D. Johnson, L.R.Y., and M-H.C conducted genome-wide association analyses. A.D. Johnson, L.R.Y., M-H. C. and A.T.K. conducted additional analyses on SNP replication and the percent of variance explained. M-H. Chen conducted the meta-analyses. All authors read and approved the final version of the manuscript.

1,238 in the Genetic Study of Atherosclerosis Risk), with replication ($P < 0.05$) in an African-American cohort ($N \leq 840$ in the Genetic Study of Atherosclerosis Risk). We identified associations of seven loci with platelet aggregation, near/in *GP6* ($P = 4.6\times10^{-13}$), *PEAR1* ($P = 3.4\times10^{-12}$), *ADRA2A* ($P = 3.3\times10^{-11}$), *PIK3CG* ($P = 3.1\times10^{-9}$), *JMJD1C* ($P = 1.6\times10^{-8}$), *MRVI1* ($P = 2.0\times10^{-8}$), and *SHH* ($P = 4.5\times10^{-8}$). Evidence of replication was found for all loci. In total these findings provide new functional insights into platelet aggregation pathways and may suggest novel anti-platelet therapeutic targets.

Aggregation of blood platelets, a critical physiological response to vessel injury, leads to platelet thrombus formation and preserves vascular integrity. Responses are triggered by shear-stress or receptor agonists including ADP, collagen, epinephrine, and thrombin. Aggregation responses are mediated by glycoprotein receptors, and intracellular signaling pathways that trigger receptor activation and release of granules and thromboxane, further mediating feedback signaling. While normal platelet responsiveness maintains homeostasis and promotes wound healing, platelet hyperactivity may promote abnormal thrombosis, and the activation of platelets by plaques is a well-known contributor to acute coronary syndrome or stroke. Thus, several platelet aggregation mechanisms are targets for anti-platelet therapies for the treatment and prevention of cardiovascular disease1. Inter-individual differences in anti-platelet responses suggest genetic variability, but studies to date, mainly focusing on candidate genes, have uncovered few consistent, replicated associations2,3.

The heritabilities of aggregation responses were previously established in the Framingham Heart Study (FHS)4 and the Genetic Study of Atherosclerosis Risk (GS)5. Rare platelet-related disorders are known6; however, with the exception of a limited scan in FHS7, no previous genome-wide scans have been reported for aggregation phenotypes. To investigate common genetic influences, we combined results from two cohorts' GWAS for platelet aggregation responses to three agonists: ADP, collagen, and epinephrine. Our meta-analysis includes European-ancestry (EA) study populations from FHS and GS, with participants free of symptoms of coronary artery disease and not taking anti-platelet medication. We conducted replication in an African-ancestry (AA) cohort that is also part of GS. The aim was to discover and replicate genome-wide significant loci associated with platelet aggregation and provide new insights into platelet aggregation mechanisms and its variability in humans.

The two GS cohorts were younger, and the GS AA sample had higher BMI and a higher prevalence of smoking, diabetes and hypertension than the two EA samples (Supplementary Table 1). There was no evidence for inflation of test statistics for the meta-analyses conducted, with all $\lambda \leq 1.01$. We observed 8 distinct platelet aggregation associations (Table 1) that met QC filters and surpassed a genome-wide significance threshold in meta-analysis ($P < 5.0 \times 10^{-8}$) with evidence for association in the same direction in both FHS and GS ($P < 0.05$ in both cohorts). Three regions were genome-wide significant for association with ADP-induced aggregation (Figure 1, Supplementary Figure 1a–c, Table 1): 1q23.1 (*PEAR1* rs12566888 $P = 3.4 \times 10^{-12}$), 11p15.4 (*MRVI1* rs7940646 $P = 2.0 \times 10^{-8}$), and 7q36.3 (*SHH* rs2363910 $P = 4.5 \times 10^{-8}$). The minor allele of the *PEAR1* SNP was associated with a decrease in aggregation response, whereas the minor alleles of the *MRVI1* and 7q36.3

variants were associated with increased responses (Table 2). All 3 regions showed evidence for replication ($P < 0.05$) in the African-ancestry sample based on genotyped SNPs that showed association with the same direction of effect (Table 3, *PEAR1* rs12041331 $P = 5.8 \times 10^{-9}$, *MRVI1* rs1874445 $P = 9.9 \times 10^{-3}$, *SHH* rs6943029 $P = 2.4 \times 10^{-3}$). Three additional loci were modestly associated with increased aggregation responses to ADP in EA and also showed evidence for association in AA at $P < 0.05$ (Supplementary Table 2).

Four regions had genome-wide significant associations for epinephrine-induced platelet aggregation (Figure 2, Supplementary Figure 1d–g, Table 1): 10q25.2 (*ADRA2A* rs4311994 $P = 3.3 \times 10^{-11}$), 1q23.1 (*PEAR1* rs12566888 $P = 7.3 \times 10^{-10}$), 7q22.3 (*PIK3CG* rs342286 $P = 3.1 \times 10^{-9}$), and 10q21.2 (*JMJD1C* rs10761741 $P = 1.6 \times 10^{-8}$). For the *ADRA2A*, *PEAR1*, and *PIK3CG* regions, the minor alleles were associated with reduced epinephrine-induced aggregation (Table 2), while the minor allele of the peak SNP nearest *JMJD1C* was associated with increased aggregation (Table 2). Three of the regions showed consistent results in the African-ancestry sample (Table 3, *PEAR1* rs12041331 $P = 8.3 \times 10^{-17}$, *ADRA2A* rs869244 $P = 2.2 \times 10^{-6}$, *JMJD1C* rs2893923 $P = 8.8 \times 10^{-3}$). Four SNPs at 7q22.3 did not meet replication criteria (best SNP rs342296, $P = 0.13$), though none was the peak SNP in EA meta-analyses. Eight additional regions showed moderate associations with epinephrine-induced aggregation in EA along with association in AA (Supplementary Table 2).

A single region, 19q13.42 (*GP6* rs1671152, EA $P = 4.6 \times 10^{-13}$, AA $P = 0.048$), was associated with $\log_{10}$ collagen lag time response at a genome-wide significant level in FHS with $P = 9.1 \times 10^{-14}$ (Supplementary Figure 1h, Supplementary Figure 2, Table 1). The peak associated SNP causes a Thr>Lys change at amino acid 323. The strong association of the Lys allele with decreased collagen response (increased lag time, Table 2) observed in FHS was weakly replicated in GS (EA, $P = 0.037$; AA, $P = 0.048$). Our first meta-analysis compared collagen doses of 190 *u*g/mL (FHS, calf-skin-derived collagen) with 2 *u*g/mL (GS, equine-tendon-derived collagen) since these provided the most similar lag time distributions (Supplementary Figure 3), consistent with several orders of magnitude higher efficacy of calf- vs. equine-derived collagen (pers. comm., BioData, Inc.). We additionally analyzed associations of the single FHS dose compared with results from three other doses in GS (1, 5, 10 *u*g/mL), but did not find any additional genome-wide significant loci or gain stronger replication evidence for the *GP6* locus. Three additional loci with evidence of moderate association in the main meta-analysis for collagen lag time in the EA sample showed similar association in the AA sample (Supplementary Table 2).

Given that the three platelet function agonists analyzed here target partially overlapping mechanisms of platelet aggregation, we inspected whether significantly associated loci overlapped across agonists. Four regions showed association with aggregation phenotypes in both the EA and AA samples and showed evidence for platelet responses to ≥2 different agonists (Supplementary Table 3).

While an understanding of rare disorders of platelet aggregation has emerged6, the discovery of common genetic variations contributing to platelet aggregation has been marginally successful even though aggregation traits are heritable4,5. Prior studies were

performed in modest sample sizes, utilized candidate gene approaches focusing on glycoprotein receptors, and often employed variable conditions in diseased populations. By adopting a GWAS approach in large cohorts of relatively healthy individuals and using similar platelet–rich plasma (PRP)-derived aggregation phenotypes, we discovered or replicated strong associations ($P = 5.0 \times 10^{-8}$) for 7 distinct loci with platelet aggregation, and found suggestive evidence for many additional loci (summarized in Table 4 and Supplementary Table 4). The findings for the *PEAR1*8,9, *ADRA2A*10,11 and *GP6*12,13 regions provide strong evidence in a much larger sample than past studies, while the associations in the regions of *MRVI1*, *SHH*, *JMJD1C*, and *PIK3CG* are novel.

Platelet endothelial aggregation receptor-1 (*PEAR1*) undergoes tyrosine phosphorylation after platelet-platelet contact14. A *PEAR1* promoter region variant (rs2768759) was associated with increased aggregation in PRP, most strongly in response to epinephrine, and in both pre- and post-aspirin treatment conditions8. Recently a candidate gene study found association of *PEAR1* SNPs with ADP and collagen responses in 500 whole blood-derived samples, and an increase in surface PEAR1 expression upon activation9. These candidate gene studies8,9 had limited coverage of the *PEAR1* region. In our study, the prior SNPs8,9 were not among the strongest associations; instead, the peak associations with ADP and epinephrine response lie within a relatively conserved region of intron 1 of *PEAR1*.

Variation in ADRA2A receptor numbers and polymorphisms in *ADRA2A* that influence epinephrine-induced aggregation in diverse populations were reported nearly 15 years ago10,11. The association of ADRA2A expression with epinephrine response is logical, given that ADRA2A serves as the primary receptor for epinephrine on platelets. Additional reports in small samples have reproduced *ADRA2A* associations15, including recognition of complex population patterns in the region and effects on RNA levels *in vitro*16. Notably, unlike prior studies focused on the immediate gene region, the peak SNP associations we observed are somewhat distant and 3’ from the gene (EA, rs4311994, 63kb, $P = 3.3 \times 10^{-11}$; AA, rs869244, 70kb, $P = 2.2 \times 10^{-6}$) suggesting partial LD with causal variants close to the gene or possible long range regulatory elements.

The association of *GP6* variants with collagen lag time is biologically plausible, as GP6 is the primary glycoprotein receptor that mediates collagen responses in platelets. The peak *GP6* SNP in FHS, a nonsynonymous variant (Thr323Lys), was strongly associated with collagen lag time (rs1671152, $P = 9.1 \times 10^{-14}$). Notably rs1671152 is in LD with rs1613662 (Ser219Pro, HapMap CEU r^2=1.0). Both variants have been associated with diminished collagen expression or downstream responses (e.g.,13,17). Due to multiple GP6 protein isoforms formed by splicing and a frameshift, Thr323Lys is alternatively His322Aln in a shorter isoform. Five nSNPs are in LD, including Ser219Pro and Thr323Lys/His322Aln, making it difficult to determine which are functional13,17, although a recent study supports an effect on receptor binding of Thr323Lys/His322Aln within this haplotype17. GP6 plays a role in thrombus formation18. Interestingly, two studies recently replicated association of the 219Pro allele with reduced risk for deep vein thrombosis, indicating potential clinical relevance for genetic findings in *GP6*19,20. In our study, both Thr323Lys and Ser219Pro were similarly associated with collagen lag time (EA, $P = 4.6 \times 10^{-13}$ vs. $P = 4.7 \times 10^{-12}$, AA, $P = 0.048$ vs. $P = 0.08$).

MRVI1 (also known as *IRAG*), which showed both ADP- and epinephrine-induced associations (Table 3, Supplementary Table 3), has prior evidence of functions in platelet aggregation. MRVI1 is a member of a signaling complex which influences smooth muscle cell relaxation through negative regulation of INP3-induced calcium signaling21. In mice MRVI1 plays a direct role in the inhibition of platelet aggregation and *in vivo* thrombosis22. There is also prior evidence for platelet-related functions for some genes at other novel loci we report. In a human heterologous system SHH^+ microvesicles induce differentiation along a megakaryocyte lineage suggesting a link to platelet biology23. Polymorphisms near *PIK3CG* (rs342293) were recently associated with decreased mean platelet volumes24. The SNP rs342286, associated here with epinephrine-induced aggregation ($P < 3.1 \times 10^{-9}$), is in a strong LD (HapMap CEU, $r^2=0.87$) with rs342293. Our finding did not directly replicate in African-Americans ($P = 0.13$) although the direction of effect was similar. A direct function for PIK3CG in platelet signaling has been demonstrated25, making it a putative mediator of the effects observed in ours and the prior study24.

We note associations near two genes, *MRVI1* and *PIK3CG*, with prior known platelet functions which are related to intracellular signaling pathways in platelets21,22,24,25. When we attempted to replicate regions with modest evidence for association ($P < 1.0 \times 10^{-4}$ in EA) we found further evidence for regions that encode proteins with known involvement in platelet signaling pathways, including *RGS18*26–29, *RAP1B*30–33, and *RAPGEF2*34,35, as well as others with putative platelet functions including *ST3GAL4*36,37 and *PRNP*38,39 (Supplementary Table 2, Supplementary Table 4). Evaluation of prior candidate SNPs and gene regions from the literature indicated evidence consistent with prior studies in the regions of *P2RY12*, a receptor that mediates ADP responses, *TAOK1*, previously associated with mean platelet volume40, and *FCER1G*, previously associated with collagen response9 and here associated with collagen lag time (Supplementary Table 5).

Combining our novel findings with prior studies, 7 loci are strongly implicated for genetic roles in platelet aggregation, with several more loci having consistent evidence in ≥2 populations (Supplemental Table 4). These additional loci contain genes with compelling biological links to platelet function and warrant further investigation. Known functions of genes at the novel loci suggest that in addition to glycoprotein receptors, proteins involved in intracellular signal transduction pathways and platelet homeostasis are also critical to mediating aggregation responses. Some variants from our study (e.g., *GP6*) have already been associated with clinically apparent cardiovascular disease. It will be important to conduct further functional and clinical studies to examine the clinically relevant function of genetic variants in these loci and the potential of corresponding proteins as targets for drug treatment, given the central role of platelet function in multiple disease etiologies including thrombosis, myocardial infarction, stroke, wound healing and response to infection.

Platelet aggregation phenotype collection

FHS is a community-based, prospective, longitudinal study following 3 generations of participants. The Offspring cohort studied here represents the second generation, including spouses44. GS is a family-based, prospective study. Apparently healthy subjects free of current aspirin or anti-platelet use were included in phenotype collection for FHS4,45 and

GS8,46. FHS participants were excluded from analysis if they self-reported use of aspirin or anti-platelet medication, or in the absence of response to 5 mg/mL arachidonic acid which was presumed indicative of aspirin therapy4,45. GS families were identified from probands with documented premature (age <60) coronary artery disease (CAD) in one of ten Baltimore area hospitals; unaffected, apparently healthy siblings, offspring of the siblings and probands, and parents of the offspring were recruited from 2003–2006 for a study of platelet reactivity8,46. Eligible participants were free of coronary artery disease, had no history of any bleeding disorder or hemorrhagic event, and no serious comorbidities. Participants with a history of aspirin intolerance, abnormal platelet count, hematocrit, or white blood cell count, or current use of anticoagulants or antiplatelet agents were excluded. Use of aspirin and/or nonsteroidal anti-inflammatory drugs was prohibited for ten days before the study visit.

Both studies isolated platelet-rich plasma (PRP) from blood samples taken from participants after fasting, and measured platelet aggregation after addition of agonists using a four-channel aggregometer (BioData Corp., Horsham, PA). FHS samples were collected at exam cycle 5 from participants' antecubital vein while in the supine position between 8AM and 9AM (previously described)4,45. Blood was placed in a 3.8% sodium citrate solution and centrifuged at 160 × g for 5 min at room temperature to separate out platelet-rich plasma (PRP). In GS participants, after an 8–12 h fast, blood was drawn and collected in vacutainer tubes containing sodium citrate (3.2%), after discarding the first 4 ml. PRP was prepared by centrifugation of whole blood at 180 × g for 15 min, and platelet poor plasma (PPP) was prepared by centrifugation at 2000 × g for 10 min. PRP was diluted with PPP to adjust platelet counts to 200,000/*u*l. All GS platelet function studies were completed within 2 h after the blood draw.

FHS tested aggregation for periods 4 min post-ADP (0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, 15.0 *u*M), 5 min post-epinephrine (0.01, 0.03, 0.05, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0 *u*M), and lag time (s) to aggregation with 190 *u*g/mL calf-skin-derived Type-I collagen (BioData Corp., Horsham, PA). Threshold concentrations (EC50) were determined as the minimal concentration of agonist required to produce a >50% aggregation. Testing was not conducted at higher concentrations if >50% aggregation was observed. The maximal aggregation response (% aggregation) was also determined for each participant at each concentration tested. GS recorded maximal aggregation (% aggregation) for periods 5 min post-ADP (2.0, 10.0 *u*M) and post-epinephrine (2.0, 10.0 *u*M), and lag time (s) to aggregation with equine-tendon-derived Type-I collagen (1, 2, 5 and 10 *u*g/mL, Chronolog Corp., Havertown, PA).

Genotyping and imputation

DNA was extracted and genotyped for consenting FHS participants with the Affymetrix 500K array and an additional gene-focused 50K array as part of the SNP Health Association Resource (SHARe) project. DNA was extracted and genotyped for the GS samples with the Illumina 1M (duo) array at deCODE Genetics (Reykjavik, Iceland). FHS and GS both used MACH to impute ~2.54 million SNPs based on the HapMap CEU phased haplotypes (release 22). SNPs were excluded from imputation in FHS that had MAF < 1%, HWE *P* <

1.0×10^{-6}, SNP call rate < 97.0%, MISHAP test $P < 1.0 \times 10^{-9}$, Mendelian errors > 100, or were missing from the HapMap CEU population release 22. Two hundred unrelated individuals were selected from FHS who had low SNP missingness, low numbers of Mendelian errors and who did not show up as outliers in EIGENSTRAT 2.047 (default parameters). The 200 individuals were used to infer MACH model parameters first (MACH flags used: --rounds 100 –greedy), and subsequently applied on all 8,481 individuals (MACH flags used: --greedy --mle –crossovermap –errormap). FHS samples were excluded from GWAS analysis if they had genome-wide call rates < 97.0%, high Mendelian error rates or exhibited genome-wide heterozygosity > 5 s.d. away from the mean. In GS, participants with sex discrepancies or Mendelian errors > 2% were excluded from imputation. SNPs excluded from imputation had MAF < 1%, HWE $P < 1.0 \times 10^{-6}$, or call rate < 95.0%. GS selected 200 EA individuals (pre-screened to be unrelated) by prioritizing those individuals with low missingness, balanced in number of males and females; none of them identified as outliers by EIGENSTRAT 2.0. Similar to FHS, the 200 pre-selected individuals were used to infer model parameters first (MACH flags used: --rounds 100 --greedy), and subsequently that model was applied to all 1,991 EA individuals with genotypes (MACH flags used: --greedy --mle –crossovermap --errormap).

Genetic analyses in each cohort

Both cohorts evaluated age- and sex- adjusted models for aggregation phenotypes. FHS and GS included the principal components (PC) from EIGENSTRAT 2.047 (n=8 and n=2, respectively) as covariates to account for potential population admixture. Collagen lag times and EC50 concentrations were $\log_{10}$ transformed before analysis. Epinephrine maximal aggregations were BLOM-transformed due to non-Gaussian distributions. Linear mixed effects (LME) models were used in FHS and GS individually to test the association under an additive model between a SNP and specific phenotype adjusted for age, sex and PCs. The linear mixed effects (LME) model follows in a matrix form: $\boldsymbol{Y} = \boldsymbol{XB} + \boldsymbol{ZU} + \varepsilon$, where $\boldsymbol{Y}$ is an $m \times 1$ vector of responses; $\boldsymbol{X}$ is an $m \times p$ design matrix of the fixed effects; $\boldsymbol{B}$ is the parameter $p \times 1$ vector of fixed effects; $\boldsymbol{Z}$ is an $m \times q$ incidence matrix of random effects, and $\boldsymbol{U}$ is a $q \times 1$ vector of random effects with $E(\boldsymbol{U}) = \mathbf{0}$, and covariance matrix $\boldsymbol{G}$; $\boldsymbol{0}$ is an $m \times 1$ vector of random effects with $E(\boldsymbol{0}) = \mathbf{0}$ and covariance matrix $\boldsymbol{R}$. In the fixed effects we included SNP genotypes using an additive model (0 for one major allele, 1 for the heterozygote, and 2 for the minor allele homozygote genotype) for the original genotypes and dosage (probabilistic estimations) for the imputed genotypes. We tested whether the SNP additive effects differed from zero. FHS used the R kinship and GWAF packages48, accounting for familial relatedness, while GS used PROC MIXED in SAS (v. 9.1.3 for Linux OS) with the option for EMPIRICAL variance49 and including the family identification number in the random effects to account for relatedness.

The agonist conditions and number of subjects analyzed in GWAS were as follows: for ADP: in FHS, EC50 (n=2,372), 3*u*M (n=2,753), 5*u*M (n=1,803) and in GS, 2*u*M (n=1,110), 10*u*M (n=1,227); for epinephrine: in FHS, EC50 (n=2,364), 1*u*M (n=2,166), 3*u*M (n=1,220), and for GS, 2*u*M (n=1,238), 10*u*M (n=1,232); for collagen lag time: in FHS (n=2,310) and in GS, 1, 2, 5 and 10 *u*g/mL (n=931, 1,162, 1,222, 1,223, respectively). In GS, participants from 230 families with African-ancestry were used in replication analyses: collagen lag 2

*u*g/mL (n=763), ADP 2*u*M (n=788) and 10*u*M (n=836), epinephrine 2*u*M (n=837) and 10*u*M (n=840).

Meta-analysis

SNPs considered in the meta-analyses did not have missing information for either cohort, and had MAF >= 1.0% and an imputation observed to expected ratio >= 0.30 in both cohorts. After this QC filtering ~2.33 million SNPs were included in the meta-analysis for each trait. Sample-size weighted meta-analysis was conducted with the software METAL combining the GS and FHS. The phenotypes used in meta-analyses were for the same agonists at the concentrations with the best available overlap (see Supplementary Table 1). Additionally, when meta-analyzing FHS threshold response (EC50) associations for ADP and epinephrine and GS maximal aggregation, the sign of the beta in FHS was flipped, since threshold response and maximal aggregation are inversely related. Results presented are based on individual cohort age-, sex- and PC-adjusted analyses, and meta-analyses corrected for individual study genomic control inflation rates. Regional association plots (Supplementary Figure 1a–h) were generated with SNAP41.

Replication analysis

We conducted testing for replication in an independent, African-ancestry sample within GS. Since LD patterns in general for African-ancestry individuals at the genome level are more complex and diverse than in populations that are primarily of European-ancestry, relying on single sentinel SNPs from European-ancestry individuals or on imputed or proxy SNPs in African-ancestry individuals for replication comparisons could lead to spurious associations. Thus, we chose to focus replication efforts on all SNPs in regions with evidence for association in the EA meta-analyses ($P<1.0\times10^{-4}$) that were *directly genotyped* with the Illumina 1M (duo) array and had MAF ≥ 1.0% in the AA replication sample. We searched for evidence of age- and sex-adjusted association in the AA samples only for the same platelet aggregation phenotypes corresponding to those in the main scan. Replication evidence was defined by SNPs with effects in the same direction in AA samples as in EA samples at a $P<0.05$ threshold.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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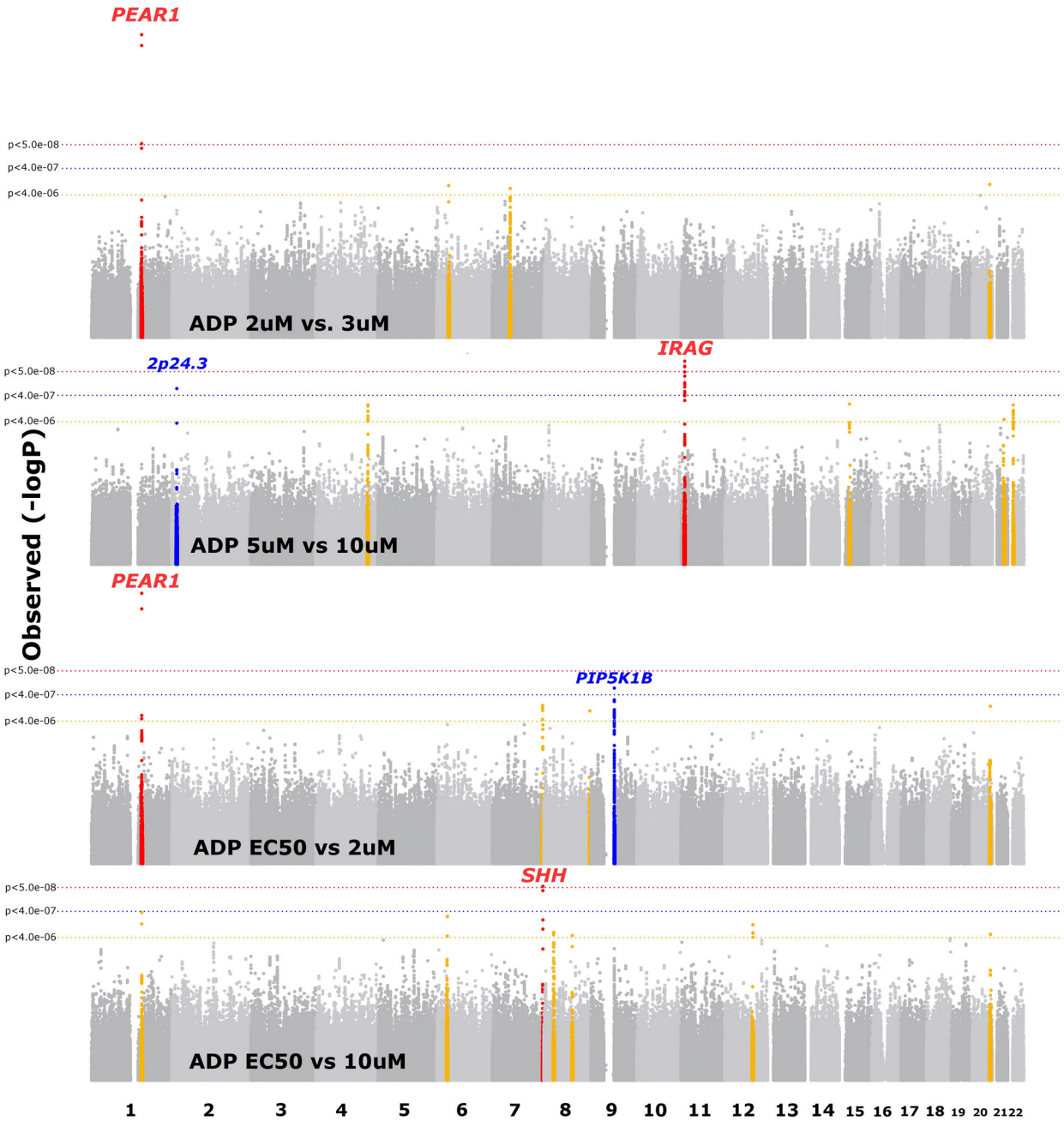

Figure 1.

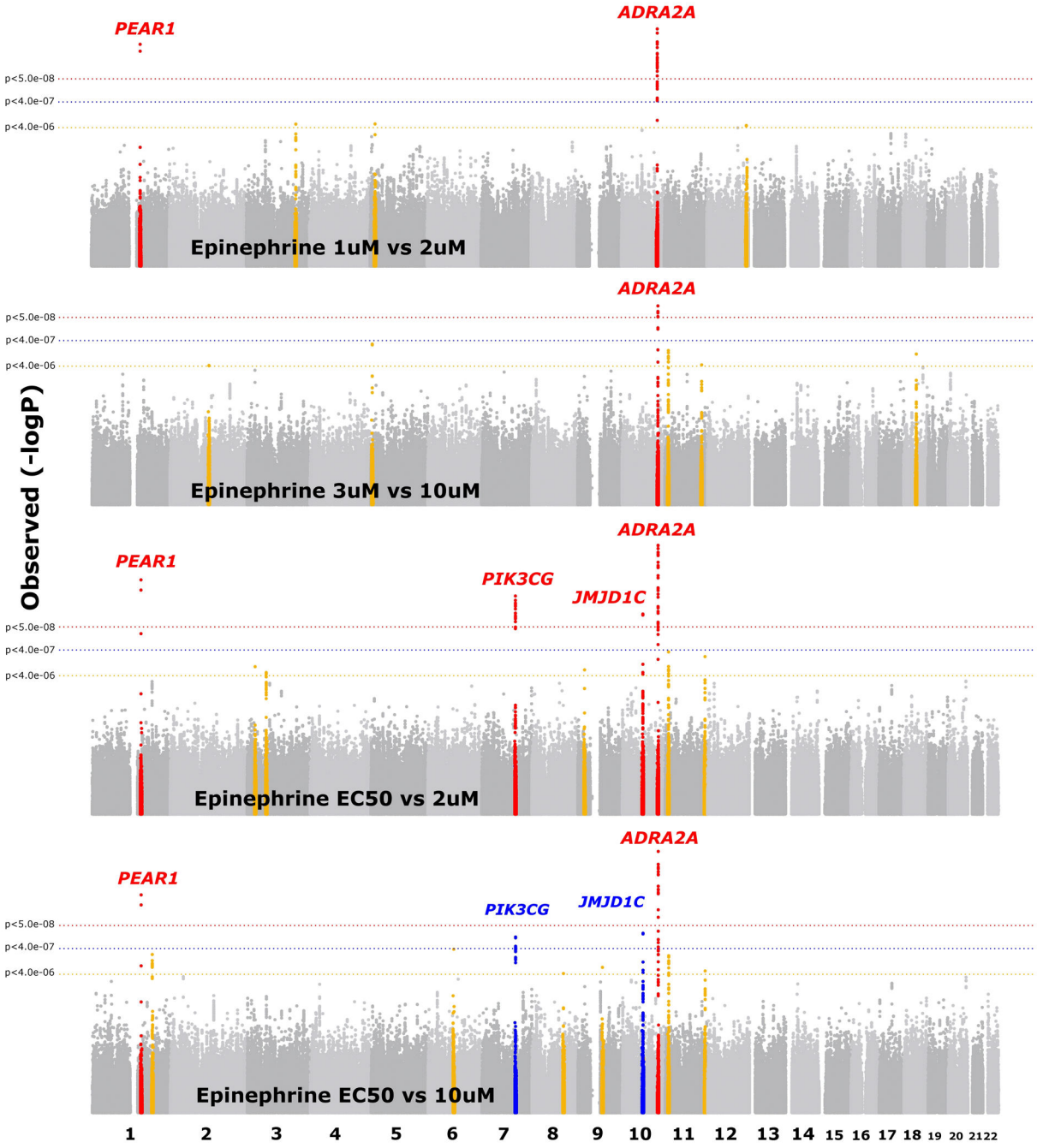

Figure 2.

Table 1

Associations of top SNPs in loci with $p<5.0\times10^{-8}$ in European-ancestry only meta-analyses.

SNPid	Chr	Nearest Genes	Coded allele	FHS phenotype	n	p-value	beta (sem)	MAF	GS phenotype	n	p-value	beta (sem)	MAF	Combined EA meta-analysis p-value
Loci associated with ADP aggregation														
rs12566888 (G>T)	1	*PEAR1*	T	ADP 3*u*M	2753	6.7×10^{-8}	−0.06 (0.01)	9.6%	ADP 2*u*M	1110	5.6×10^{-6}	−8.62 (1.9)	9.2%	3.4×10^{-12}
rs7940646 (C>T)	11	*MRVI1*	T	ADP 5*u*M	1803	1.6×10^{-5}	0.03 (0.007)	31.2%	ADP 10*u*M	1227	2.5×10^{-4}	2.14 (0.58)	31.8%	2.0×10^{-8}
rs2363910 (G>T)	7	*SHH*	T	ADP EC50	2372	6.1×10^{-4}	−0.04 (0.01) †	7.1%	ADP 2*u*M	1110	2.6×10^{-6}	4.52 (0.95)	9.2%	4.5×10^{-8}
Loci associated with epinephrine aggregation														
rs4311994 (C>T)	10	*ADRA2A*	T	Epi EC50	2364	1.7×10^{-8}	0.14 (0.02) †	14.0%	Epi 2*u*M	1238	2.8×10^{-4}	−7.24 (2.0)	15.7%	3.3×10^{-11}
rs12566888 (G>T)	1	*PEAR1*	T	Epi EC50	2364	8.8×10^{-7}	0.16 (0.03) †	9.2%	Epi 2*u*M	1238	1.3×10^{-4}	−9.77 (2.5)	9.2%	7.3×10^{-10}
rs342286 (A>G)	7	*FLJ36031, PIK3CG*	G	Epi EC50	2364	4.2×10^{-7}	0.09 (0.02) †	44.1%	Epi 2*u*M	1238	1.3×10^{-3}	−4.47 (1.4)	42.2%	3.1×10^{-9}
rs10761741 (G>T)	10	*JMJD1C*	T	Epi EC50	2364	1.5×10^{-6}	−0.08 (0.02) †	41.5%	Epi 2*u*M	1238	2.0×10^{-3}	4.05 (1.3)	42.2%	1.6×10^{-8}
Loci associated with collagen lag time														
rs1671152 (G>T)	19	*GP6*	T	Lag 190 *u*g/mL	2310	9.1×10^{-14}	0.03 (0.004)	14.2%	Lag 2 *u*g/mL	1162	0.037	0.017 (0.008)	15.9%	4.6×10^{-13}

† The betas presented in the Table relate directly to the phenotypes presented. Because threshold concentrations (EC50 traits) are inversely related with maximal aggregation response, the signs of the betas for EC50 traits were flipped before meta-analysis.

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Table 2

Phenotype means by genotype estimates for each cohort.

				Phenotype means (s.d.) by genotype							
SNP id	**Gene**	**GWAS *P* value**	**Genotypes**	**FHS**	**n†**	**FHS phenotype**	**%var‡**	**GS EA**	**n†**	**GS phenotype**	**%var‡**
ADP SNPs with p<5.0×10^{-8}											
rs12566888	*PEAR1*	3.4×10^{-12}	GG	68.6% (24)	2238	ADP 3*u*M	1.14%	21.3% (25)	912	ADP 2*u*M	1.94%
			GT	61.8% (27)	485			12.7% (23)	188		
			TT	58.3% (23)	30			0.44% (15)	10		
rs7940646	*MRVI1*	2.0×10^{-8}	CC	75.5% (20)	840	ADP 5*u*M	1.10%	70.3% (15)	572	ADP 10*u*M	1.06%
			CT	78.1% (18)	799			72.2% (13)	525		
			TT	82.6% (16)	164			75.0% (11)	130		
rs2363910	*SHH*	4.5×10^{-8}	GG	3.32 *u*M (1.5)	2047	ADP EC50*	0.40%	18.8% (25)	919	ADP 2*u*M	0.88%
			GT	3.22 *u*M (1.7)	312			23.4% (26)	184		
			TT	2.50 *u*M (1.3)	13			34.2% (25)	7		
Epinephrine SNPs with p<5.0×10^{-8}											
rs4311994	*ADRA2A*	3.3×10^{-11}	CC	1.74 *u*M (2.6)	1749	Epi EC50*	1.44%	35.5% (32)	893	Epi 2*u*M	1.33%
			CT	2.46 *u*M (3.4)	567			28.6% (34)	305		
			TT	3.15 *u*M (4.1)	48			17.5% (36)	40		
rs12566888	*PEAR1*	7.3×10^{-10}	GG	1.85 *u*M (2.8)	1939	Epi EC50*	0.99%	34.9% (32)	1023	Epi 2*u*M	1.45%
			GT	2.30 *u*M (3.1)	402			25.8% (34)	205		
			TT	3.07 *u*M (3.5)	23			11.5% (38)	10		
rs342286	*PIK3CG*	3.1×10^{-9}	AA	1.65 *u*M (2.5)	746	Epi EC50*	1.10%	35.8% (33)	398	Epi 2*u*M	0.91%
			AG	2.06 *u*M (3.0)	1135			34.3% (33)	597		
			GG	2.13 *u*M (3.0)	483			26.5% (33)	243		
rs10761741	*JMJD1C*	1.6×10^{-8}	GG	2.13 *u*M (2.9)	815	Epi EC50*	0.99%	30.5% (33)	387	Epi 2*u*M	0.71%
			GT	1.94 *u*M (2.9)	1136			33.2% (33)	637		
			TT	1.57 *u*M (2.6)	413			38.3% (32)	214		
Collagen lag SNPs with p<5.0×10^{-8}											

				Phenotype means (s.d.) by genotype							
SNP id	**Gene**	**GWAS *P* value**	**Genotypes**	**FHS**	**n†**	**FHS phenotype**	**%var‡**	**GS EA**	**n†**	**GS phenotype**	**%var‡**
rs1671152	*GP6*	4.6×10^{-13}	GG	80.45 s (19.2)	1692	Coll. lag	2.31%	104.76 s (32.5)	821	Coll. lag	0.31%
			GT	85.95 s (21.8)	580			108.12 s (33.8)	317		
			TT	99.21 s (21.0)	38			112.75 s (38.0)	24		

† genotype numbers and categorization are based on rounded imputed dosages from MACH

‡ the portion of phenotypic variation explained by the SNP was calculated by comparing models with and without the SNP

* since threshold concentrations (EC50 traits) are inversely correlated with maximal aggregation responses, the trend of the phenotype means is expected to be opposite

Table 3

Loci with replication evidence for platelet aggregation phenotypes based on genotyping in the GS African-ancestry (AA) cohort.

SNPid	Gene	Top EA SNP[1]	p-value	r^2 to top EA SNP	FHS n[†]	p-value	beta (s.e.m.)	MAF	GS EA n[†]	p-value	beta (s.e.m.)	MAF	GS AA n†	p-value	beta (s.e.m)	MAF	EA meta-analysis p-value	EA + AA meta-analysis p-value
Loci associated with ADP aggregation																		
rs12041331 (G>A)[*]	*PEAR1*	rs12566888	3.4×10^{-12}	0.85	2372	3.6×10^{-6}	0.06 (0.01) ‡	10.2%	1110	6.1×10^{-6}	−8.52 (1.9)	9.3%	788	5.8×10^{-9}	−9.21 (1.6)	35.8%	2.2×10^{-10}	3.8×10^{-16}
rs6943029 (G>A)[*]	*SHH*	rs2363910	4.5×10^{-8}	0.70	2372	3.7×10^{-4}	−0.05 (0.01) ‡	6.7%	1110	3.9×10^{-3}	5.62 (1.9)	9.8%	788	2.4×10^{-3}	5.35 (1.8)	26.6%	5.5×10^{-6}	8.2×10^{-8}
rs1874445 (C>T)[*]	*MRVI1*	rs7940646	2.0×10^{-8}	0.58	1803	4.5×10^{-3}	0.02 (0.007)	38.1%	1227	1.1×10^{-3}	1.84 (0.56)	40.2%	836	9.9×10^{-3}	2.29 (0.89)	39.3%	2.4×10^{-5}	9.9×10^{-7}
Loci associated with ADP aggregation																		
rs12041331 (G>A)[*]	*PEAR1*	rs12566888	7.3×10^{-10}	0.85	2364	2.1×10^{-6}	0.15 (0.03) ‡	10.3%	1232	1.4×10^{-4}	−9.66 (2.5)	9.3%	837	8.3×10^{-17}	−17.9 (2.1)	35.8%	1.8×10^{-9}	4.9×10^{-19}
rs869244 (G>A)[*]	*ADRA2A*	rs431194	3.3×10^{-11}	0.28	2364	1.5×10^{-5}	0.08 (0.02) ‡	35.3%	1232	4.0×10^{-4}	−5.00 (1.4)	35.3%	837	2.2×10^{-6}	−9.03 (1.9)	38.6%	3.3×10^{-8}	3.2×10^{-12}
rs2893923 (C>T)[*]	*JMJD1C*	rs10761741	1.6×10^{-8}	0.69	2364	8.8×10^{-6}	−0.08 (0.02) ‡	33.6%	1232	0.031	2.90 (1.3)	31.2%	837	8.8×10^{-3}	6.36 (2.4)	19.8%	1.4×10^{-6}	5.3×10^{-8}
Loci associated with collagen lag time																		
rs1671152 (G>T)[*]	*GP6*	rs1671152	4.6×10^{-13}	n.a.	2310	9.1×10^{-14}	0.03 (0.004)	14.2%	1157	0.037	0.017 (0.008)	15.9%	763	0.048	4.82 (0.02)	30.9%	4.6×10^{-13}	8.4×10^{-14}

[1] The top meta-analysis SNP and p-value in EA for the same trait, and LD with the selected replication SNP based on HapMap CEU using SNAP41.

[†] The analyzed traits indicated by sample size are for FHS: ADP EC50 (n=2,372), ADP 5*u*M (n=1,803), Epi EC50 (n=2,364), collagen lag to 190 *u*g/mL (n=2,310), for GS: ADP 2*u*M (n=1,110 or 788), ADP 10*u*M (n=1,227 or 836), Epi 2*u*M (n=1,238 or 837), collagen lag to 2*u*g/mL (n=1,162 or 763)

‡ The betas presented in the Table relate directly to the phenotypes presented. Because threshold concentrations (EC50 traits) are inversely related with maximal aggregation response, the signs of the betas for EC50 traits were flipped before meta-analysis.

[*] The coded allele is the second allele listed. Multiple SNPs in the gene region indicate replication (p<0.05 with effect in the same direction) but only the most significant SNP in combined analysis is given

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Table 4

Summary of loci associated with platelet aggregation and function.

Locus	Representative gene	Effects of minor alleles on platelet aggregation to agonists	Expression in platelets	Expression in megakaryocytes†	Other genes ± 60kb of peak SNP
Loci with $p<5.0\times10^{-8}$					
1q23.1	*PEAR1* (platelet endothelial aggregation receptor 1)	↓ ADP, ↓ epinephrine	Yes9,14	not measured	*NTRK1, C1orf92, ARHGEF11, INSRR*
11p15.4	*MRVI1* (murine retrovirus integration site 1 homolog)	↑ ADP, ↑ epinephrine	Yes22	↑ 1.1 fold42	
7q36.3	*SHH* (sonic hedgehog homolog)	↑ ADP	Unknown	↓ 0.7 fold	
10q25.2	*ADRA2A* (adrenergic, alpha-2A-, receptor)	↓ epinephrine	Yes15,16	↑ 2.3 fold	
7q22.3	*PIK3CG* (phosphoinositide-3-kinase, catalytic, gamma	↓ epinephrine	Yes24	↑ 3.7 fold	*FLJ36031*
10q21.2	*JMJD1C* (jumonji domain containing 1C)	↑ epinephrine	Unknown	↑ 4.0 fold	
19q13.42	*GP6* (glycoprotein VI (platelet))	↑ collagen lag	Yes13,17,18	↑ 7.0 fold	*NLRP2, RDH13*

† indicates the average fold intensity of a representative gene transcript relative to the mean background intensity across stem-cell derived megakaryocytes measured with the Illumina Human WG-6 v2 (n=4 individuals), further described in Watkins *et al.*43