HHS Public Access

Author manuscript

Pac Symp Biocomput. Author manuscript; available in PMC 2023 January 01.

Published in final edited form as:

Pac Symp Biocomput. 2023 ; 28: 221–232.

The Role of Global and Local Ancestry on Clopidogrel Response in African Americans

Guang Yang[1], **Cristina Alarcon**[1], **Paula Friedman**[1], **Li Gong**[2], **Teri Klein**[3], **Travis O'Brien**[4], **Edith A. Nutescu**[5], **Matthew Tuck**[6], **David Meltzer**[7], **Minoli A Perera**[1]

[1.]Department of Pharmacology, Center for Pharmacogenomics, Feinherg School of Medicine, Northwestern University, Chicago, IL

[2.]Department of Biomedical Data Science, Stanford University, Stanford, CA

[3.]Department of Biomedical Data Science and Department of Medicine, Stanford University, Stanford, CA

[4.]Department of Pharmacology and Physiology, The George Washington University, School of Medicine and Health Sciences, Washington, DC

[5.]Department of Pharmacy Practice and Center for Pharmacoepidemiology and Pharmacoeconomic Research, University of Illinois Chicago, College of Pharmacy, Chicago, IL

[6.]Washington DC VA Medical Center, Washington, DC and The George Washington University, Washington, DC

[7.]Section of Hospital Medicine, Department of Medicine, University of Chicago, Chicago, IL

Abstract

Pharmacogenomics has long lacked dedicated studies in African Americans, resulting in a lack of in-depth data in this populations. The ACCOuNT consortium has collected a cohort of 167 African American patients on steady state clopidogrel with the goal of discovering population specific variation that may contribute to the response of this anti-platelet agent. Here we analyze the role of both global and local ancestry on the clinical phenotypes of P2Y12 reaction units (PRU) and high on-treatment platelet reactivity (HTPR) in this cohort. We found that local ancestry at the TSS of three genes, *IRS-1, ABCB1* and *KDR* were nominally associated with PRU, and local ancestry-adjusted SNP association identified variants in *ITGA2* associated to increased PRU. These finding help to explain the variability in drug response seen in African Americans, especially as few studies on genes outside of *CYP2C19* has been conducted in this population.

Keywords

African American; Pharmacogenomics; Clopidogrel; Ancestry

1. Introduction

Clopidogrel is an anti-platelet agent used in coronary artery disease (CAD), acute coronary syndrome (ACS) in patients undergoing percutaneous coronary intervention (PCI), peripheral vascular disease (PVD) and stroke. Wide inter-individual variation in response,

defined by either laboratory response (i.e., P_2Y_{12} reaction units [PRU]) or clinical response, has been documented.[1,2] High on therapy PRU (HTPR), defined as measures over 230, have been linked to greater risk of major cardiovascular events in clinical trials.[3] Variable response is, at least in part, heritable, with up to 70% of the variability observed in clopidogrel response attributed to genetic factors.[4,5]

Clopidogrel is an oral prodrug that requires the hepatic cytochrome P450 enzymes, *CYP2C19*, to be biologically active. Several pharmacogenomic studies have shown that both loss-of-function (LOF) and gain-of-function alleles in *CYP2C19* are associated with clopidogrel efficacy and risk of major adverse cardiac events. Specifically carriers of the *CYP2C19*2* and **3* alleles are at a significantly greater risk of myocardial infarction after stent placement than non-carriers.[6,7] Hence, the Food and Drug Administration (FDA) added a boxed warning recommending the reduction of clopidogrel in patient carrying LOF variants in *CYP2C19*.[8] Yet, African Americans (AAs) made up a small proportion of the initial pharmacogenomic discovery studies and clinical trials, and little is known about population-specific variation that may affect their response to clopidogrel.

Genetic admixture is the result of recent interbreeding between previously separated populations. In AAs, this has resulted in the addition of European DNA segments into the background of an African genome. The result is a genome which contains a mosaic of both populations. Genetic ancestry varies substantially between AAs, with the global proportion of African ancestry ranging from nearly 100% to as low as 20% in self-identified AAs.[9] However, at any specific loci the local genetic ancestry can vary drastically, even between individuals that have relatively similar global proportions of African ancestry. This more fine-scaled ancestry is dubbed local ancestry (LA). this may be especially important for gene regulation, which occurs in discrete nearby locations to the gene. We have already shown that LA is an important in eQTL mapping for admixed populations and that global ancestry proportions are significantly correlated to the expression of several hepatic gene.[10,11] Here we investigate the association of global or LA at candidate genes to clinical phenotypes related to clopidogrel response.

2. Methods

2.1. Cohort

One hundred and seventy AAs on clopidogrel were recruited from 5 hospital system in Chicago and Washington DC (University of Chicago Medical Center, University of Illinois and Northwestern Memorial Hospital, George Washington University Hospital and Medical Faculty Associates, and the Washington DC VA Medical Center) through the African American Cardiovascular Pharmacogenomics Consortium (ACCOuNT).[12] All subjects self-identified as AAs over the age of 18, were able to consent and provided at least two blood samples: one purple top tube for DNA extraction and one sodium citrate coagulation tube for PRU measurement. All subjects were on clopidogrel for at least 15 days at the time of recruitment and PRU measures. PRU measures were obtained from either the Northwestern Memorial Hospital or the VA medical Center clinical laboratories through the VerifyNow Assay (Accumetrics, San Diego, California). Clinical and demographic variables related to clopidogrel response were collected and included: age, sex, concomitant medications,

platelet counts, and indication for therapy. HTPR was prespecified as PRU greater than or equal to 230 on clopidogrel therapy as previously described.[13]

2.2. Genotyping and Quality Control

The ACCOuNT clopidogrel cohort was genotyped with the Infinium Multi-Ethnic Genotyping Array (Illumina) at the University of Chicago Genomics Core. Quality control measures included: SNPs exclusion based on genotyping rate <95%, minor allele frequency (MAF) <5%, and failed Hardy-Weinberg equilibrium tests p <0.00001. SNPs were also excluded if they were: AT or C/G SNPs to eliminate flip-strand issues, SNPs on the X and Y chromosomes or mitochondrial SNPs. Genotype data was used to validate gender and identity-by-descent (IBD). Samples were also excluded due missingness >0.05, gender misspecification, or IBD >0.125. Additionally, principal components 1 and 2 were used to confirm ancestry of all individuals (Sup. Figure). Genotypes were phased using Eagle v2.4 and imputed using the TopMed Imputation server in NCBI build 38 (hg38) coordinates. Post imputation quality control involved exclusion of SNPs if the MAF was <0.05, imputation quality <0.8, and failed Hardy-Weinberg equilibrium tests p <0.00001. This resulted in 141 subjects retain in the analysis. Because of the small sample size of our cohort, we restricted the LA analysis (described below) to a set of candidate genes known to be associated with clopidogrel response, adverse events, or platelet function while on clopidogrel. Genes were chosen from a query of significant variants associated to clopidogrel phenotypes from PharmGKB (https://www.pharmgkb.org/chemical/PA449053/variantAnnotation). This resulted in 35 genes used the LA ancestry analyses (listed in Appendix A).

2.3. Global Ancestry Association Analysis

The genotypes of 141 subjects were merged with HapMap phase 3 reference data from four global populations: Yoruba in Ibadan, Nigeria (YRI); Utah residents with Northern and Western European ancestry (CEU); Han Chinese in Beijing, China (CHB); and Japanese in Tokyo, Japan (JPT). Population structure of the merged data was inferred by the Bayesian clustering algorithm STRUCTURE deployed within fastStructure v1.0 and performed without any prior population assignment.[14] We employed the admixture model, and the bum-in-period and number of Markov Chain Monte Carlo repetitions were set to 20,000 and 100,000, respectively. The number of parental populations (K) was set to 3. West African Ancestry (WAA) percentages of each subject were calculated and used for association to PRU and HTPR using a linear or logistic regression in R.

2.4. Local Ancestry Association Analysis

We estimated the local ancestry of each subject with RFMix version 2 using YRI and CEU samples from 1000 Genome phase 3 as the reference populations, using a window size of 0.2 Mb.[15] The LA at the gene transcriptional start site of each candidate gene was assigned as 2 African alleles (AFR/AFR), two European Alleles (EUR/EUR) of one of each (AFR/EUR) for association with mean PRU and HTPR. We used a general linear model (Gaussian method) in R for the association to PRU with LA and a general logistic binomial in R for the association of HTPR to LA. All analyses used age, sex, diabetes, hypertension and the first 2 genomics PCs as covariates. We prespecified a p<0.001 (0.05/35) as significant.

We conducted local ancestry-adjusted SNP association, restricted to SNPs within 1Kb of each candidate gene resulting in 10962 SNPs included in this analysis. We used the TRACTOR [16] deconvolved model to conduct the ancestry adjusted analysis in each ancestry separately. TRACTOR uses the following model:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5\ldots + b_kx_k$$

where x_1 is the number of haplotypes coming from the index ancestry, and x_2 and x_3 represent the risk alleles, and x_4-x_k are other covariates such as age, sex, genomics PCs. This analysis produces ancestry specific effect size estimates and p-values for each SNP in each ancestry. We prespecified a $p< 1x10^{-6}$ as significant. We then ran a meta-analysis on the deconvoluted AFR and EUR summary statistics using METAL.[17] All analyses used age, sex, diabetes, hypertension and the first 2 genomic PC as covariates. These results were compared to association without the inclusion of LA conducted in PLINK.

3. Results

We estimate WAA in our ACCOuNT clopidogrel cohort as well as in the 1000 genomes AFR and EUR superpopulations using fastSTUCTURE (Fig. 1). The average percentage of WAA in our cohort was 80.9% (range 53.9% - 95.8%).

We investigate the association of WAA on both PRU and HTPR. In this cohort of patients, the prevalence of high on-treatment platelet reactivity (HTPR), was 26%. There was no significant difference in any demographic or clinical covariates between cases with HTPR and controls (Table 1), though both Type 2 diabetes (T2D) and hypertension were more common in the cases (Percent difference 19.5%, p = 0.11, and 9.7%, p = 0.28 respectively) though not statistically different. Hypertension was associated to PRU ($p<0.05$) and was thus included in the downstream analysis. We included T2D as a covariate in all analyses as some of the candidate genes tested were specifically found in patient with diabetes while on clopidogrel. We then tested the association of both PRU and HTPR to WAA percentage. Neither of these phenotypes were associated with percentage of WAA (p = 0.09 and 0.14 respectively)

Next, we tested if LA at the TSS of candidate genes was associated to either PRU or HTPR. We found no significant associations in either analysis, though three genes, *IRS-1* (p= 0.02), *KDR* (p=02), and *ABCB1* (p= 0.03) reached nominal significance with PRU, and *IRS-1* reached nominal significance with HTPR (p= 0.05, lower HTPR in individuals with EUR ancestry). Additionally, *CYP2C19, CYP2C9*, and *ECS1* showed suggestive association (p= 0.09, 0.09 and 0.06 respectively) with PRU. Figure 2 shows that *IRS-1, KDR, and CYP2C19* had higher PRU in individuals with local AFR ancestry and *ABCB1* had lower PRU in individuals with local AFR ancestry.

We then investigated the ancestry-adjusted SNP association around candidate genes to PRU and HTPR. Given the high degree of African ancestry in our cohort, only the AFR-specific analysis is reported, as only a few AAs had adequate EUR ancestry at these SNP positions to be included in the analysis. However, we included the EUR-specific summary statistics in

the meta-analysis to adjust for both ancestries. We identify a new near significant association in Chr. 5 at *ITGA2* (lead SNP: rs7725246, $p = 4.75 \times 10^{-5}$, $\beta = -25.48$) in the meta-analysis. This SNP also showed a suggestive association in the AFR-specific analysis (lead SNP: rs7725246, $p = 1.36 \times 10^{-4}$, $\beta = -29.82$) (Figure 3). The most significant SNPs in the EUR analysis were also found on in Chr 5 (rs27618), but only reached a p-value at 0.003, as only 58 people were included in this analysis (Table 2). These SNPs are common across global populations. The most significant SNP at the *CYP2C19* locus (rs1200314) has a higher allele frequency in AFR populations, is associated with increased PRU and is not in LD with *CYP2C19*2* ($r^2 < 0.02$). Of note, the *CYP2C19*2* alleles were not significant ($p > 0.02$) in either the AFR-specific analysis or the meta-analysis. None of these SNPs were signficanat in in the standard association analysis using PC correction.

4. Discussion

Here we describe the association of both global ancestry and LA at candidate genes with clinically relevant association to clopidogrel response. Antiplatelet therapy with clopidogrel has been the mainstay for thromboprophylaxis of CVDs[18]. The American Heart Association and American College of Cardiology recommend clopidogrel as first-line antiplatelet therapy in patients suffering non-ST-elevation acute coronary syndrome.[19] Despite the clinical benefits, many patients have cardiovascular events after being prescribed clopidogrel and inter-individual variability in drug response affects both the efficacy and safety profile.[2] Little is known about the effect of population specific variants on clopidogrel response outside of East Asians and Europeans. Most GWAS studies of both PRU and adverse cardiovascular event after clopidogrel treatment have not included African Ancestry populations. Given the unique cohort of an admixed African population taking clopidogrel, we explored the association of both global and local ancestry on PRU (a clinical measure of clopidogrel efficacy) and HTPR (a clopidogrel outcomes measure).

We identified nominal associations with the candidate genes *IRS-1* (insulin receptor Substrate 1), *KDR* (Kinase Insert Domain Receptor, also called VEGFR2) and *ABCB1* (ATP Binding Cassette Subfamily B Member 1, also called *MDR1*) to PRU. *IRS-1*, a ligand of insulin receptor tyrosine kinase, is central to the insulin signal transduction pathway.[20] SNPs within this gene have previously been associated with HTPR (as defined as the 75th percentile of the ADP-induced platelet aggregation) and ADP and arachidonic acid induced platelet aggregation in diabetic patients on clopidogrel.[21,22] Notably, both studies were done in East Asian populations. Clinical trials have shown that diabetes mellites and high serum glucose are independently associated with clopidogrel nonresponse. In our study we did not see an association with HTPR and diabetes though we used T2D co-morbidity as a covariate in our analysis. As people with AFR ancestry at the locus had higher PRU, this suggests that *IRS-1* may be more highly expressed in those with local African ancestry at this locus and thus may result in great nonresponse to clopidogrel therapy. Further experimental validation is needed.

KDR has been associated to arthrosclerosis and coronary artery disease (CAD) as well as clopidogrel non-response.[23,24] KDR can bind to VEGF and cause angiogenesis. The dysregulation of this process is thought to contribute to a wide variety of diseases including

atherosclerosis and CAD.[25–27] Two SNPs, rs7667298 and rs2305948, in this gene have been associated with increased risk of angina pectoris when treated with clopidogrel in people with CAD.[28] Both of these risk alleles are more common in African ancestry populations. The effect of these SNPs on *KDR* expression has been limited, with rs2305948 thought to affect the binding efficacy of VEGF to KDR and KDR serum levels.[29,30]

ABCB1 encodes an intestinal efflux transporter protein, P-glycoprotein, which modulate the absorption of clopidogrel. A LOF allele, rs1045642, in this gene has been association with major adverse cardiovascular event and death in patient on clopidogrel.[31,32] *ABCB1* gene expression has been shown to be higher in European as opposed to African Americans in peripheral blood.[33] Between different ethnic groups in Brazil, the allele frequency of rs1045642 differs by group affiliation, with those identifying as African having the lowest frequency.[34] In our study AFR ancestry at *ABCB1* was associated with decreased PRU, suggesting this gene may play a smaller role in clopidogrel adverse events in African ancestry populations as compared to Europeans.

While the association of LA at neither *CYP2C19* nor *CYP2C9* reached nominal significance, we presented our findings as these genes have been the most widely studies in relation to clopidogrel response. The *CYP2C19*2* allele explains about 12% of the variability in PRU in Europeans and 7% of variability in PRU in admixed Puerto Ricans (mean EUR and AFR ancestry of 70% and 19% respectively).[35,36] Our result show that AFR ancestry at the TSS of both genes trend toward higher PRU. In our previous work in African American primary hepatocytes, we found that *CYP2C19* was significantly associated with proportion of WAA (global ancestry) with lower expression of *CYP2C19* with increase WAA. This agrees with previous findings that African Americans as a group have higher major adverse myocardial event while on clopidogrel than other populations.[37] Notably, *CYP2C19*2* was not significantly associated to PRU or HTPR, suggesting other variants may play a role in response variability. Taken together these findings suggest additional population specific variation in these genes may contribute to clopidogrel response.

In our AFR specific GWAS and meta-analysis we identified SNPs within *ITGA2* with near significant associated with PRU. SNPs in this gene have been associated with residual platelet activity in the plasma of patient on clopidogrel and increase platelet aggregation.[38,39] *ITGA2* also positively correlated to platelet aggregation with collagen.[40] Our previous work on population difference in the platelet transcriptome did not identify *ITGA2* as differentially expressed.[41]

There are several limitations to this study. Our cohort size is small, especially for the HTPR analyses in which only 38 subjects were defined as cases. Thus, we are underpower to detect small to medium effects. Our LA-adjusted method is able to identify those alleles with large difference in allele frequency between populations but may have reduced power to find allele that have more similar allele frequencies between populations as previously reported.[42] We were not able to replicate the previous associations found in *CYP2C19* in the ancestry-adjusted SNP associations. Others have reported that the association to *CYP2C19*2* to mortality and myocardial infarction risk in AAs was not significant though these associations were robust in European subjects.[43] The lack of AA cohorts on

clopidogrel with PRU data hampers our effort to replicate our findings. Even the most recent GWAS by the International Clopidogrel Pharmacogenomics Consortium, which included 2592 patients, was exclusively European.[44]

Our studies represent a unique analysis on an all AA clopidogrel cohort with PRU and HTPR phenotypes. This work highlights who variability in ancestry between African Americans may be useful in identifying potential genes and SNPs associated to pharmacogenomic phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by 1U54MD010723 and the participants in ACCOuNT studies along with the physicians, nurses and clinical recruiters that enabled the gathering of this data.

Appendix

6.

6. Appendix A: Candidate Gene List

Gene	Effect on Clopidogrel
ABCB1	Metabolism, Efficacy, ADR
AGAP3	Efficacy (Asian only)
ATP10A	Efficacy (Asian only)
B4GALT2	Platelet Aggregation, PRU
CDH13	Efficacy
CDH15	Efficacy
CES1	Metabolism, Platelet Aggregation, Efficacy,
CES1P1	PRU (East Asian only)
CYP1A2	Survival and ADR (African Americans only)
CYP2B6	PRU (European only)
CYP2C19	PRU, HTPR, Platelet aggregation, Efficacy, Metabolism, ADR
CYP2C9	PRU, HTPR, Platelet Aggregation, Metabolism, Efficacy, ADR
CYP3A4	Platelet Aggregation (European only)
CYP3A5	Efficacy, Platelet Aggregation, ADR, PRU, Metabolism
CYP4F2	Efficacy, Platelet Aggregation,
ECHS1	Efficacy (East Asian only)
EFR3A	Efficacy (East Asian only)
F2R	PRU (East Asian only)
FMO3	HTPR (East Asian only)

Gene	Effect on Clopidogrel
IRS-1	PRU (East Asian only)
ITGA2	Platelet Aggregation, PRU,
ITGA3	Efficacy
KDR	Efficacy
MED12L	Platelet Aggregation, PRU
MYOM2	Efficacy (East Asian only)
N6AMT1	Metabolism, Efficacy (East Asian only)
NECAB1	Efficacy (East Asian only)
NOS3	Efficacy
P2RY12	Efficacy, ADR, PRU, Platelet Aggregation
PEAR1	HTPR, PRU, Platelet Aggregation, Efficacy
PON1	Efficacy, PRU, Platelet Aggregation
PTGS1	Efficacy
SLC14A2	Efficacy (East Asian only)
WDR24	Efficacy (East Asian only)
ZDHHC3	Efficacy (East Asian only)

References

1. Nagareddy P, Smyth SS. Inflammation and thrombosis in cardiovascular disease. Curr Opin Hematol. 2013;20(5):457–463. [PubMed: 23892572]
2. Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: a review of the evidence. J Am Coll Cardiol. 2005;45(8):1157–1164. [PubMed: 15837243]
3. Sharifi H, Habibi V, Emami Zeydi A. Platelet reactivity unit (PRU) in patients undergoing elective PCI: Rethinking the optimal cut point. Anatol J Cardiol. 2017;18(2):164. [PubMed: 28766514]
4. Lewis JP, Shuldiner AR. Clopidogrel pharmacogenetics: Beyond candidate genes and genome-wide association studies. Clin Pharmacol Ther. 2017;101(3):323–325. [PubMed: 27649515]
5. Shuldiner AR, O'Connell JR, Bliden KP, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA. 2009;302(8):849–857. [PubMed: 19706858]
6. Carlquist JF, Knight S, Horne BD, et al. Cardiovascular risk among patients on clopidogrel anti-platelet therapy after placement of drug-eluting stents is modified by genetic variants in both the CYP2C19 and ABCB1 genes. Thromb Haemost. 2013;109(4):744–754. [PubMed: 23364775]
7. Cavallari LH, Lee CR, Beitelshees AL, et al. Multisite Investigation of Outcomes With Implementation of CYP2C19 Genotype-Guided Antiplatelet Therapy After Percutaneous Coronary Intervention. JACC Cardiovasc Interv. 2018;11(2):181–191. [PubMed: 29102571]
8. Administration USFD. FDA Drug Safety Communication: Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug. https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/fda-drug-safety-communication-reduced-effectiveness-plavix-clopidogrel-patients-who-are-poor. Published 08/03/2017. Updated 08/03/2017. Accessed February 26, 2021, 2021.
9. Bryc K, Durand EY, Macpherson JM, Reich D, Mountain JL. The genetic ancestry of African Americans, Latinos, and European Americans across the United States. Am J Hum Genet. 2015;96(1):37–53. [PubMed: 25529636]
10. Zhong Y, De T, Alarcon C, Park CS, Lee B, Perera MA. Discovery of novel hepatocyte eQTLs in African Americans. PLoS Genet. 2020;16(4):e1008662. [PubMed: 32310939]

11. Park CS, De T, Xu Y, et al. Hepatocyte gene expression and DNA methylation as ancestry-dependent mechanisms in African Americans. NPJ Genom Med. 2019;4:29. [PubMed: 31798965]
12. Friedman PN, Shaazuddin M, Gong L, et al. The ACCOuNT Consortium: A Model for the Discovery, Translation, and Implementation of Precision Medicine in African Americans. Clin Transl Sci. 2019;12(3):209–217. [PubMed: 30592548]
13. Garabedian T, Alam S. High residual platelet reactivity on clopidogrel: its significance and therapeutic challenges overcoming clopidogrel resistance. Cardiovasc Diagn Ther. 2013;3(1):23–37. [PubMed: 24282742]
14. Raj A, Stephens M, Pritchard JK. fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics. 2014;197(2):573–589. [PubMed: 24700103]
15. Maples BK, Gravel S, Kenny EE, Bustamante CD. RFMix: a discriminative modeling approach for rapid and robust local-ancestry inference. Am J Hum Genet. 2013;93(2):278–288. [PubMed: 23910464]
16. Atkinson EG, Maihofer AX, Kanai M, et al. Tractor uses local ancestry to enable the inclusion of admixed individuals in GWAS and to boost power. Nat Genet. 2021;53(2):195–204. [PubMed: 33462486]
17. Wilier CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010;26(17):2190–2191. [PubMed: 20616382]
18. Ray S. Clopidogrel resistance: the way forward. Indian Heart J. 2014;66(5):530–534. [PubMed: 25443607]
19. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;130(25):2354–2394. [PubMed: 25249586]
20. Feng X, Tucker KL, Parnell LD, et al. Insulin receptor substrate 1 (IRS1) variants confer risk of diabetes in the Boston Puerto Rican Health Study. Asia Pac J Clin Nutr. 2013;22(1):150–159. [PubMed: 23353623]
21. Zhang D, Zhang X, Liu D, et al. Association between insulin receptor substrate-1 polymorphisms and high platelet reactivity with clopidogrel therapy in coronary artery disease patients with type 2 diabetes mellitus. Cardiovasc Diabetol. 2016;15:50. [PubMed: 27005817]
22. Angiolillo DJ, Bernardo E, Zanoni M, et al. Impact of insulin receptor substrate-1 genotypes on platelet reactivity and cardiovascular outcomes in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol. 2011;58(1):30–39. [PubMed: 21700086]
23. Liu D, Song J, Ji X, Liu Z, Cong M, Hu B. Association of Genetic Polymorphisms on VEGFA and VEGFR2 With Risk of Coronary Heart Disease. Medicine (Baltimore). 2016;95(19):e3413. [PubMed: 27175642]
24. Al Awaida W, Ahmed AA, Hamza AA, et al. Association of KDR rs1870377 genotype with clopidogrel resistance in patients with post percutaneous coronary intervention. Heliyon. 2021;7(2):e06251. [PubMed: 33665428]
25. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. J Biol Chem. 1994;269(43):26988–26995. [PubMed: 7929439]
26. Barger AC, Beeuwkes R 3rd, Lainey LL, Silverman KJ. Hypothesis: vasa vasorum and neovascularization of human coronary arteries. A possible role in the pathophysiology of atherosclerosis. N Engl J Med. 1984;310(3):175–177. [PubMed: 6197652]
27. Moulton KS. Plaque angiogenesis: its functions and regulation. Cold Spring Harb Symp Quant Biol. 2002;67:471–482. [PubMed: 12858573]
28. Zhang LJ, Zhang YQ, Han X, Zhang ZT, Zhang ZQ. Association of VEGFR-2 Gene Polymorphisms With Clopidogrel Resistance in Patients With Coronary Heart Disease. Am J Ther. 2016;23(6):e1663–e1670. [PubMed: 25738571]
29. Paradowska-Gorycka A, Stypinska B, Pawlik A, et al. KDR (VEGFR2) Genetic Variants and Serum Levels in Patients with Rheumatoid Arthritis. Biomolecules. 2019;9(8).
30. Wang Y, Zheng Y, Zhang W, et al. Polymorphisms of KDR gene are associated with coronary heart disease. J Am Coll Cardiol. 2007;50(8):760–767. [PubMed: 17707181]

31. Su J, Xu J, Li X, et al. ABCB1 C3435T polymorphism and response to clopidogrel treatment in coronary artery disease (CAD) patients: a meta-analysis. PLoS One. 2012;7(10):e46366. [PubMed: 23056288]
32. Mega JL, Close SL, Wiviott SD, et al. Genetic variants in ABCB1 and CYP2C19 and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. Lancet. 2010;376(9749):1312–1319. [PubMed: 20801494]
33. Tornatore KM, Brazeau D, Dole K, et al. Sex differences in cyclosporine pharmacokinetics and ABCB1 gene expression in mononuclear blood cells in African American and Caucasian renal transplant recipients. J Clin Pharmacol. 2013;53(10):1039–1047. [PubMed: 23908147]
34. Santos PC, Soares RA, Santos DB, et al. CYP2C19 and ABCB1 gene polymorphisms are differently distributed according to ethnicity in the Brazilian general population. BMC Med Genet. 2011;12:13. [PubMed: 21247447]
35. Duconge J, Santiago E, Hernandez-Suarez DF, et al. Pharmacogenomic polygenic risk score for clopidogrel responsiveness among Caribbean Hispanics: A candidate gene approach. Clin Transl Sci. 2021;14(6):2254–2266. [PubMed: 34415683]
36. Peprah E, Xu H, Tekola-Ayele F, Royal CD. Genome-wide association studies in Africans and African Americans: expanding the framework of the genomics of human traits and disease. Public Health Genomics. 2015;18(1):40–51. [PubMed: 25427668]
37. Pendyala LK, Torguson R, Loh JP, et al. Racial disparity with on-treatment platelet reactivity in patients undergoing percutaneous coronary intervention. Am Heart J. 2013;166(2):266–272. [PubMed: 23895809]
38. Giusti B, Gori AM, Marcucci R, et al. Role of glycoprotein Ia gene polymorphisms in determining platelet function in myocardial infarction patients undergoing percutaneous coronary intervention on dual antiplatelet treatment. Atherosclerosis. 2008;196(1):341–348. [PubMed: 17157856]
39. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. 807 C/T Polymorphism of the glycoprotein la gene and pharmacogenetic modulation of platelet response to dual antiplatelet treatment. Blood Coagul Fibrinolysis. 2004;15(5):427–433. [PubMed: 15205592]
40. Eicher JD, Wakabayashi Y, Vitseva O, et al. Characterization of the platelet transcriptome by RNA sequencing in patients with acute myocardial infarction. Platelets. 2016;27(3):230–239. [PubMed: 26367242]
41. Garofano K, Park CS, Alarcon C, et al. Differences in the Platelet mRNA Landscape Portend Racial Disparities in Platelet Function and Suggest Novel Therapeutic Targets. Clin Pharmacol Ther. 2021;110(3):702–713. [PubMed: 34255863]
42. Hou K, Bhattacharya A, Mester R, Burch KS, Pasaniuc B. On powerful GWAS in admixed populations. Nat Genet. 2021;53(12):1631–1633. [PubMed: 34824480]
43. Cresci S, Depta JP, Lenzini PA, et al. Cytochrome p450 gene variants, race, and mortality among clopidogrel-treated patients after acute myocardial infarction. Circ Cardiovasc Genet. 2014;7(3):277–286. [PubMed: 24762860]
44. Verma SS, Bergmeijer TO, Gong L, et al. Genomewide Association Study of Platelet Reactivity and Cardiovascular Response in Patients Treated With Clopidogrel: A Study by the International Clopidogrel Pharmacogenomics Consortium. Clin Pharmacol Ther. 2020;108(5):1067–1077. [PubMed: 32472697]

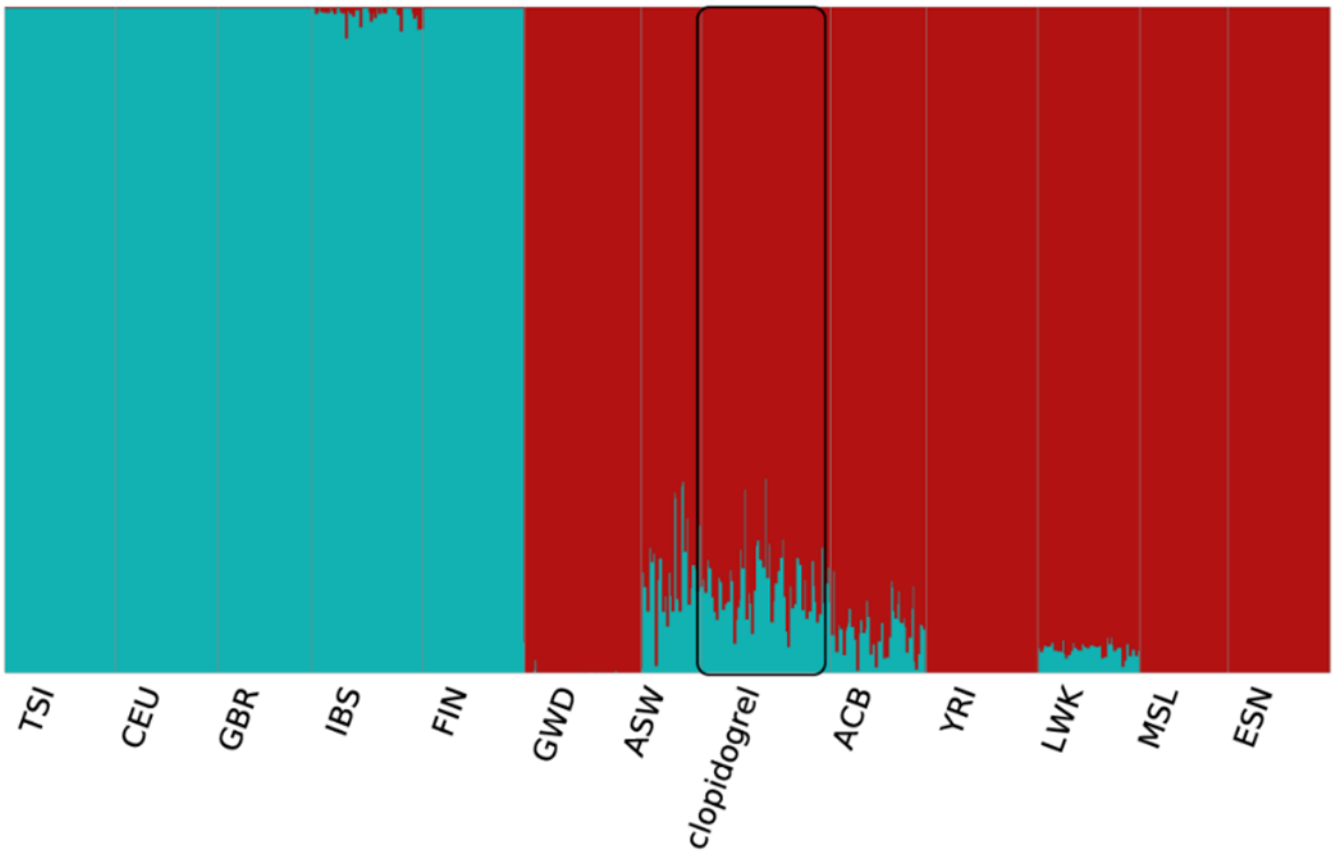

Figure 1: Ancestry proportions continental and admixed populations.

Admixture percentages inferred from fastSTRUCTURE in the EUR (TSI- Toscana in Italy, CEU – Utah residents with European ancestry, GBR – British, IBS – Iberian in Spain, FIN – Finns) and AFR (GWD – Gambians, ASW – African Americans, ACB, African Caribbeans, YRI - Yoruban, LWK – Luhya in Kenya, MSL - Mende in Sierra Leone, ESN – Esan in Nigeria) superpopulations in 1000 genomes and the ACCOuNT clopidogrel cohort (black box). fastSTRUCTURE was run with K= 3. Each column represents an individual within each population with the proportion of each population shown as the colored bars. The African proportion (shown as in red) was use in the global association analysis.

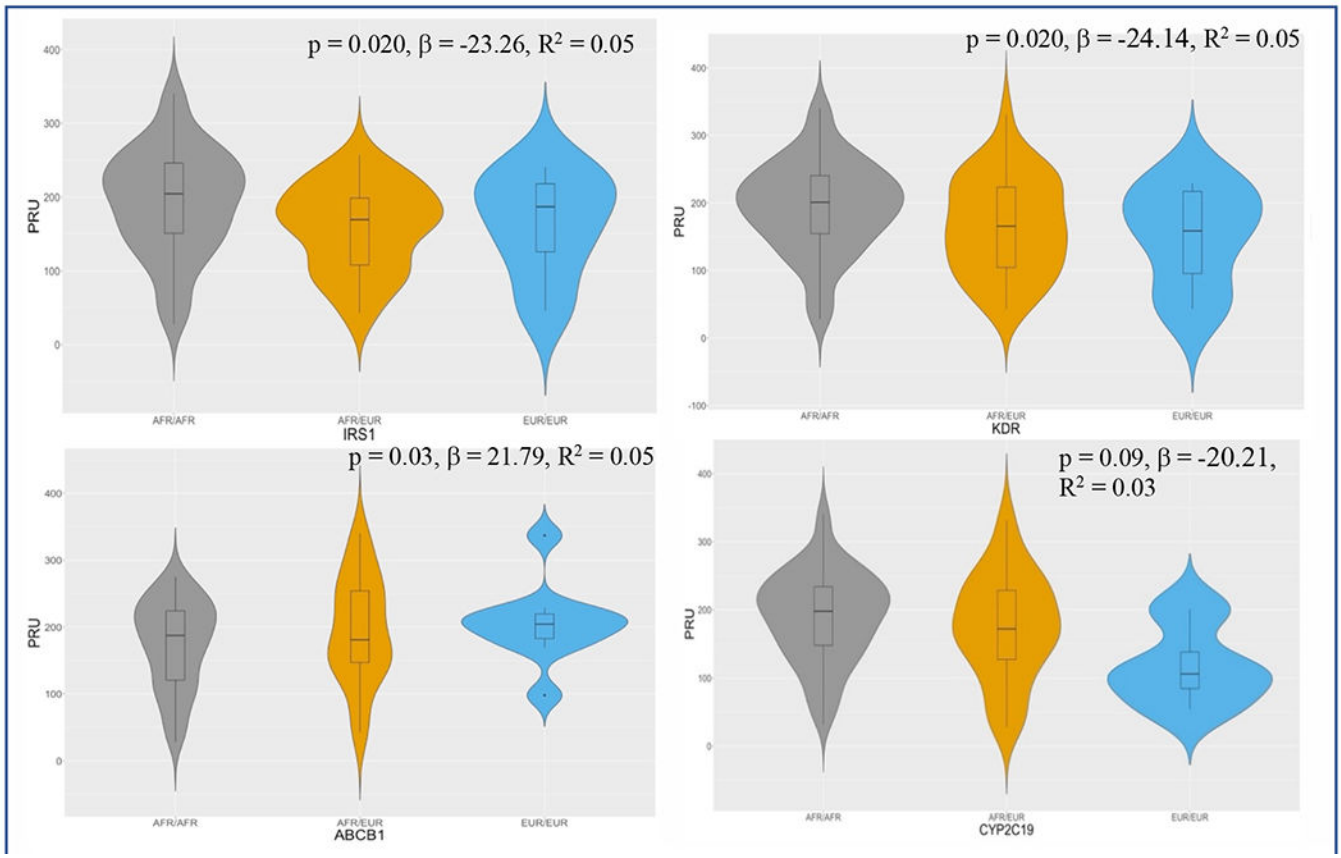

Figure 2:
Violin plots showing the association of LA at the gene TSS of *IRS-1, KDR, ABCB1* and *CYP2C19*

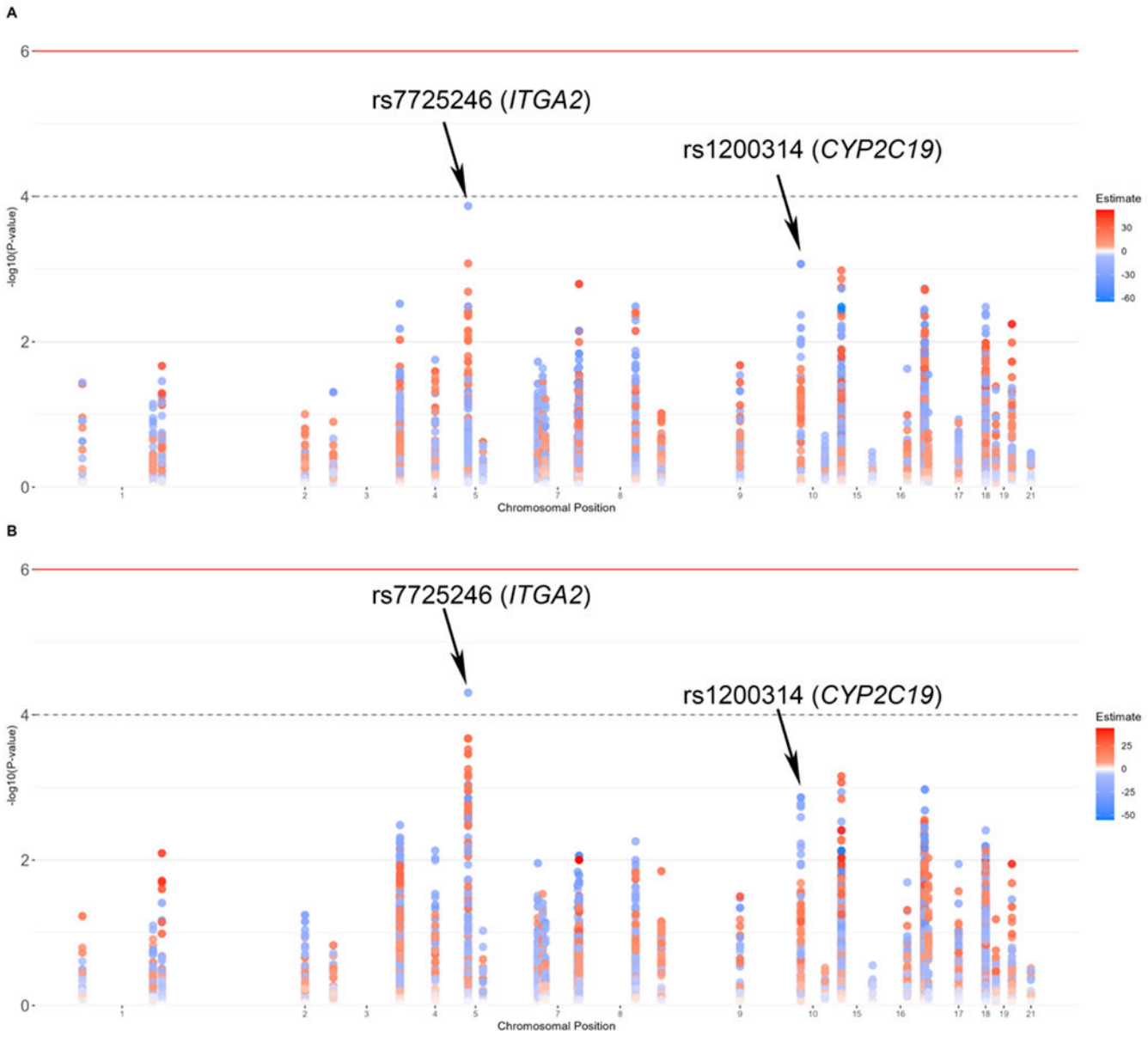

Figure 3: Ancestry-specific GWAS results

Manhattan plots of (A) AFR-specific and (B) Meta-analysis LA-adjusted SNP associations. Both analyses were corrected for age, sex, hypertension, diabetes and the first 2 PCs. The x-axis represents chromosomal location while the y-axis represents $-\log_{10}$(p value). Each dot is a SNP tested for an association with PRU and the color of each dot represents the effect size of the association where blue and red colors are negative and positive effects, respectively. A significant threshold line is drawn at $1x10^{-6}$. A suggestive threshold line is drawn at $1x10^{-4}$

Table 1:

Demographics of the ACCOuNT cohort.

Variable	Cases (PRU≥230) N=38	Controls (PRU<230) N = 103	P-value
Age (mean ± SD)	67.3 ± 11.4	64.6 ± 14.5	0.27
Sex (% Female)	14 (38.9%)	37 (41.6%)	0.94
BMI (mean ± SD)	28.96 ± 4.56	29.8 ± 6.7	0.41
Type 2 Diabetes (T2D)	24 (66.7%)	42 (47.2%)	0.11
Hypertension	33 (91.7%)	73 (82.0%)	0.28
Platelet count (mean ± SD)	237 ± 68.38	254.6 ± 81.78	0.15

Table 2:

Tod SNPs from LA inferred (LAI) SNP association.

AFR-specific results					
Chr	**SNP**	**EAF**	**Effect**	**P**	
5	rs7725246	0.70	−29.82	1.36E-04	
10	rs1200314	0.87	33.54	8.51E-04	
15	rs7182019	0.47	28.82	1.05E-03	
Meta-analysis results					
Chr	**SNP**	**EAF**	**Effect**	**P (LAI)**	**P (Standard analysis)**
5	rs7725246	0.70	−25.48	4.75E-05	0.89
10	rs1200314	0.87	30.58	1.37E-03	0.03
15	rs4271565	0.21	29.61	7.01E-04	0.67