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ORIGINAL ARTICLE
Thrombosis and antithrombotic therapy

Pharmacogenomic polygenic response score predicts ischaemic events and cardiovascular mortality in clopidogrel-treated patients

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Aims Clopidogrel is prescribed for the prevention of atherothrombotic events. While investigations have identified genetic determinants of inter-individual variability in on-treatment platelet inhibition (e.g. *CYP2C19**2), evidence that these variants have clinical utility to predict major adverse cardiovascular events (CVEs) remains controversial.

Methods and results We assessed the impact of 31 candidate gene polymorphisms on adenosine diphosphate (ADP)-stimulated platelet reactivity in 3391 clopidogrel-treated coronary artery disease patients of the International Clopidogrel Pharmacogenomics Consortium (ICPC). The influence of these polymorphisms on CVEs was tested in 2134 ICPC patients ($N = 129$ events) in whom clinical event data were available. Several variants were associated with on-treatment ADP-stimulated platelet reactivity (*CYP2C19**2, $P = 8.8 \times 10^{-54}$; *CES1* G143E, $P = 1.3 \times 10^{-16}$; *CYP2C19**17, $P = 9.5 \times 10^{-10}$; *CYP2B6* 1294 + 53 C > T, $P = 3.0 \times 10^{-4}$; *CYP2B6* 516 G > T, $P = 1.0 \times 10^{-3}$; *CYP2C9**2, $P = 1.2 \times 10^{-3}$; and *CYP2C9**3, $P = 1.5 \times 10^{-3}$). While no individual variant was associated with CVEs, generation of a pharmacogenomic polygenic response score (PgxRS) revealed that patients who carried a greater number of alleles that associated with increased on-treatment platelet reactivity were more likely to experience CVEs ($\beta = 0.17$, SE 0.06, $P = 0.01$) and cardiovascular-related death ($\beta = 0.43$, SE 0.16, $P = 0.007$). Patients who carried eight or more risk alleles were significantly more likely to experience CVEs [odds ratio (OR) = 1.78, 95% confidence interval (CI) 1.14–2.76, $P = 0.01$] and cardiovascular death (OR = 4.39, 95% CI 1.35–14.27, $P = 0.01$) compared to patients who carried six or fewer of these alleles.

Conclusion Several polymorphisms impact clopidogrel response and PgxRS is a predictor of cardiovascular outcomes. Additional investigations that identify novel determinants of clopidogrel response and validating polygenic models may facilitate future precision medicine strategies.

Keywords Clopidogrel • Pharmacogenetics • Platelet aggregation

Introduction

Clopidogrel is prescribed for the prevention of atherothrombotic events in patients with coronary artery disease.[1] Given its effectiveness and cost, clopidogrel continues to be used frequently compared to newer alternatives such as prasugrel and ticagrelor.[2,3] However, substantial inter-individual variability in clopidogrel response exists, and patients who experience sub-optimal therapy have high on-treatment platelet reactivity (HPR) and are at greater risk of experiencing a recurrent event.

Variability in clopidogrel efficacy is caused by multiple factors, including age, sex, smoking, existing comorbidities, and drug–drug interactions.[4] However, these factors explain a small proportion of the total phenotypic variation in clopidogrel response. Recent studies indicate that genetic variation substantially impacts clopidogrel efficacy, accounting for ~75% of the variability in response, as assessed by agonist-induced platelet aggregation.[5]

Multiple studies have been performed to identify genetic determinants of clopidogrel response. Though several candidate gene polymorphisms, most notably the loss-of-function *CYP2C19**2 variant, have been identified, relatively small sample sizes have hampered discovery efforts, and discrepant findings in replication efforts have made it difficult to draw firm conclusions. Here, we assessed the role of 31 candidate single nucleotide polymorphisms (SNPs) implicated in modulating clopidogrel transport (e.g. *ABCB1*), metabolism (e.g. CYP450 enzymes, *PON1*, *CES1*), and mechanism of action (e.g. *P2Y12*) in a large cohort of 3391 clopidogrel-treated patients of the International Clopidogrel Pharmacogenomics Consortium (ICPC). We also evaluated these polymorphisms on cardiovascular event (CVE) risk. Our results suggest that application of a pharmacogenomic polygenic response score (PgxRS), analogous to polygenic risk score for complex diseases, may be a superior predictive model with potential application to precision antiplatelet medicine.

Methods

Study sites and participants

Participating ICPC sites and patient characteristics have been previously described.[6] Briefly, ~150 investigators who led clopidogrel-related clinical studies of 50 or more subjects and for which DNA samples, on-clopidogrel platelet reactivity data, and clinical outcome information available were invited to participate. In total, 17 centres from 13 countries contributed data. Data deposition into a central database was managed by the Pharmacogenomic Knowledge Base (PharmGKB) and data quality/accuracy was assessed by the Pharmacogenomics Research Network's Statistical Analysis Resource. This report includes 3391 Caucasian ICPC subjects in whom on-clopidogrel platelet reactivity data, covariate data, and DNA were available.

All protocols were approved by their respective institutional review boards and adhered to the Declaration of Helsinki. Informed Consent was obtained from each participant.

Study outcomes

Platelet reactivity

In ICPC sites, one or more of the following platelet reactivity assays were used to assess clopidogrel response: Vasodilator-Stimulated Phosphoprotein (VASP) assay, Light Transmittance Aggregometry (LTA), Multiplate, and the VerifyNow $P2Y_{12}$ assay. While all of these assays assess adenosine diphosphate (ADP)-stimulated platelet reactivity, differences in assay biochemistry and units to express each test result required the generation of a standardized platelet reactivity phenotype. The criteria to generate this phenotype were reported previously.[6] Briefly, for sites that used more than one platelet test, the following prioritization scheme was predefined and applied: VASP assay > VerifyNow $P2Y_{12}$ > LTA (higher > lower ADP concentration) > Multiplate. The standardized mean difference of the chosen platelet test within each site was calculated by subtracting mean platelet reactivity from the observed platelet reactivity and dividing by the standard deviation to generate a Z-score, thus harmonizing platelet reactivity data across sites.

Cardiovascular endpoints

Post-discharge CVEs were evaluated in a subset of 2134 patients in whom clinical event data were available and were defined as spontaneous myocardial infarction (troponin value greater than the upper-limits of normal with ischaemic symptoms or electrocardiogram changes), ischaemic stroke, stent thrombosis (probable or definite stent thrombosis according to the Academic Research Consortium criteria[7]), and cardiovascular-related death. A composite endpoint, consisting of all the aforementioned outcomes, was used for analysis. Individual components of the composite endpoint were also evaluated. Study site physicians locally adjudicated all endpoints through review of source documents obtained from medical records. More information regarding CVE determination and adjudication has been reported previously.[6]

Genotyping

Thirty-one candidate polymorphisms were chosen based on prior literature and unpublished data by consortium members. Genotyping was performed with the QuantStudio™ 12K Flex OpenArray® System (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Genotype calls were performed using QuantStudio™ 12K Flex Software v1.2.2. The mean SNP call rate was 98.6% and overall concordance rate in a subset of blind duplicates was 99.3%.

Statistical analyses

Summary statistics, frequencies, and measures of Hardy–Weinberg equilibrium were calculated using PLINK v1.07 (https://www.cog-genomics.org/plink2). Linkage disequilibrium (LD) metrics (|D'| and r^2) were calculated using Haploview v4.2.[8] To account for multiple testing, *P*-values < 0.0016 were considered statistically significant (0.05/31 SNPs) in single-SNP models. All statistical tests were two-sided.

Association analyses with platelet reactivity were performed using linear regression under an additive genetic model while adjusting for age, sex, site, body mass index (BMI), smoking, diabetes, and proton pump inhibitor (PPI) use (PLINK v1.07). For analyses of CVEs, we used logistic regression and adjusted for the same covariates. For variants that were significantly associated with on-clopidogrel platelet reactivity in single-SNP analyses ($N = 6$), a PgxRS was developed to assess the impact of multiple alleles (min = 0; max = 12) that resulted in increased platelet reactivity on the occurrence of CVEs. For this analysis, alleles that led to increased platelet reactivity were coded as 'risk' and logistic regression was used using identical covariates. The impact of carrying a high (eight or above) vs. low (six or fewer) number of alleles that were associated with increased platelet reactivity on CVEs was calculated. Weighted risk score analyses that accounted for genotype effect sizes were also performed. The proportion of variation in on-clopidogrel platelet reactivity explained by the PgxRS was calculated using SAS v9.4 (SAS Institute, Cary, NC, USA).

Power calculations ($N = 3391$) indicated 80% power at $\acute{\alpha} = 0.002$ to detect SNPs with minor allele frequencies (MAF) ranging from 0.01 to 0.30 with effect sizes ranging from 0.48 to 0.11 standard deviation units, respectively. In CVE analyses, we had 80% power to detect odds ratios (ORs) ranging from 1.70 (MAF = 0.30) to 2.55 (MAF = 0.05) given a sample size of 2134 and 129 recorded events ($\acute{\alpha} = 0.002$). Power calculations were performed using Quanto v1.2.4 (http://biostats.usc.edu/Quanto.html).

Results

Characteristics of the participants are listed in *Table 1*. Most subjects were male (72.7%), middle-aged (mean age = 60.8 years), overweight (mean BMI = 27.4 kg/m^2), and had high prevalence of conventional risk factors [e.g. hypertension (81.6%), hypercholesterolaemia (86.4%), and diabetes (18.3%)]. Most subjects were taking aspirin (94.3%), 64.4% were prescribed statins, and 21.1% were on PPIs.

Associations with platelet reactivity

We performed single-SNP association tests between all polymorphisms and platelet reactivity (*Table 2*, Supplementary material online, *Figure S1*, and Supplementary material online, *Table S1*). Participants who carried the loss-of-function *CYP2C19**2 allele (rs4244285) had significantly higher platelet reactivity compared to non-carriers ($\beta = 0.51$, $P = 8.8 \times 10^{-54}$). Participants who carried the loss-of-function *CES1* G143E allele (rs71647871) had significantly lower on-treatment platelet reactivity compared to non-carrier patients ($\beta = -0.79$, $P = 1.3 \times 10^{-16}$). Similarly, those who carried the putative gain-of-function *CYP2C19**17 allele (rs12248560) had better clopidogrel response compared to non-carriers ($\beta = -0.18$, $P = 9.5 \times 10^{-10}$). Polymorphisms in *CYP2B6* (rs8192719 and rs3745274) and *CYP2C9* (rs1057910) were associated with increased platelet reactivity ($\beta = 0.11$, $P = 3.0 \times 10^{-4}$, $\beta = 0.10$, 1.0×10^{-3}, and $\beta = 0.15$, $P = 0.001$, respectively). In contrast, *CYP2C9**2 (rs1799853) was associated with lower on-treatment platelet reactivity ($\beta = -0.11$, $P = 0.001$). After correction for multiple testing, no other variant showed association with platelet reactivity (*Table 2*).

Given the strong association between *CYP2C19**2 and platelet reactivity, we sought to determine if association signals of other variants might be unmasked by adjusting for this variant (*Table 2*). After adjustment, association between variants in *CES1* and *PEAR1* and platelet reactivity were strengthened, while associations of *CYP2B6* SNPs rs8192719 and rs3745274 with platelet reactivity were essentially unchanged (*Table 2*). Other variants that were not associated with platelet reactivity in the *CYP2C19**2 unadjusted model remained without significant association after adjustment.

The *CYP2C* locus on chromosome 10q24 includes several variants tested in this investigation and, through LD, could be associated with platelet reactivity because it is physically linked to a causative variant without having an independent effect. Indeed, adjustment for *CYP2C19**2 ablated association of *CYP2C19**17 with clopidogrel response after correction for multiple testing ($P = 0.006$). Adjustment

Table 1 Characteristics of candidate gene International Clopidogrel Pharmacogenomics Consortium cohort

Characteristics (units)	Male	Female	All
Number (*N*)	2464	927	3391
Age (years), mean ± SD	60.8 ± 12.8	60.6 ± 15.7	60.8 ± 13.6
BMI (kg/m^2), mean ± SD	27.3 ± 4.0	27.7 ± 5.1	27.4 ± 4.3
Systolic blood pressure (mmHg), mean ± SD	134.3 ± 23.1	127.9 ± 23.0	131.9 ± 23.3
Diastolic blood pressure (mmHg), mean ± SD	77.7 ± 12.2	73.5 ± 10.5	76.1 ± 11.7
Hypertension,[a] *n* (%)	2142 (86.9)	625 (67.4)	2767 (81.6)
Total cholesterol (mg/dL), mean ± SD	181.6 ± 49.9	205.3 ± 52.9	189.0 ± 52.0
LDL-cholesterol (mg/dL), mean ± SD	115.9 ± 42.9	130.3 ± 47.4	120.4 ± 44.9
HDL-cholesterol (mg/dL), mean ± SD	48.2 ± 14.8	59.6 ± 16.1	51.7 ± 16.1
Triglycerides (mg/dL), mean ± SD	130.5 ± 112.8	101.0 ± 66.8	121.3 ± 101.7
Hypercholesterolaemia,[b] *n* (%)	2216 (89.9)	714 (77.0)	2930 (86.4)
Taking aspirin, *n* (%)	2309 (93.7)	889 (95.9)	3198 (94.3)
Taking statins, *n* (%)	1734 (70.4)	450 (48.5)	2184 (64.4)
Taking PPI, *n* (%)	538 (21.8)	177 (19.1)	715 (21.1)
Self-reported diabetes, *n* (%)	449 (18.2)	171 (18.3)	620 (18.3)
Haematocrit ± SD (%)	41.6 ± 4.0	37.9 ± 3.4	40.5 ± 4.2
White blood cell count (*n* × 1000), mean ± SD	7.2 ± 3.1	6.7 ± 2.5	7.1 ± 2.9
Platelet count (*n* × 100 000), mean ± SD	2.3 ± 0.7	2.5 ± 0.7	2.4 ± 0.7
Ever smoker,[c] *n* (%)	1444 (58.6)	242 (26.2)	1686 (49.7)
Current smoker,[c] *n* (%)	485 (19.7)	85 (9.2)	570 (16.8)

SI conversion factors: to convert HDL-cholesterol, LDL-cholesterol, and total cholesterol values to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PPI, proton pump inhibitor; SD, standard deviation.
[a]Defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, taking prescription blood pressure-lowering medication, and/or source code for hypertension.
[b]Defined as LDL-cholesterol >160 mm·mg/dL, taking prescription cholesterol-lowering medication, and/or source code for hypercholesterolaemia.
[c]Self-reported history of smoking.

for *CYP2C19**2 also influenced the magnitude of association between platelet reactivity and the two *CYP2C9* SNPs (*Table 2*).

Since adjustment for *CYP2C19**2 substantially altered the association between *CYP2C19**17 with platelet reactivity, we assessed LD patterns between these polymorphisms and performed genotype-stratified association analyses (Supplementary material online, *Table S2A*). The *CYP2C19**2 and *17 variants (minor alleles of rs4244285 and rs12248560, respectively) are located ~20 kbp from each other, and consistent with prior reports,[9] the rs12248560 minor allele occurs only on the rs4244285 major allele haplotype (i.e. the so-called *1 allele; |D'|=1.0). Stratified analyses showed that the minor allele at rs4244285 (*CYP2C19**2) remained strongly associated with platelet reactivity in patients who were homozygous for the major allele at rs12248560 ($N = 2068$, $P = 7.9 \times 10^{-37}$), while association of the minor allele of rs12248560 (*CYP2C19**17) with platelet aggregation was markedly attenuated in patients who were homozygous for major allele (N = 2386, $P = 0.01$) or heterozygotes ($N = 933$, $P = 0.09$) at rs4244285 (Supplementary material online, *Figure S2*). These analyses provide further evidence that *CYP2C19**17 is not an independent predictor of on-clopidogrel platelet reactivity, but rather dependent on rs4244285 genotype.

Similarly, we assessed LD and performed stratified association analyses between *CYP2C19**2 and the 2 *CYP2C9* SNPs [*2 (minor allele at rs1799853) and *3 (minor allele at rs1057910)]. LD data and estimation of haplotype frequencies are shown in Supplementary material online, *Tables S2B* and *S2C*. While *CYP2C19**2 was strongly associated with platelet reactivity in patients who were major allele homozygotes of rs1799853 ($N = 2444$, $P = 6.8 \times 10^{-10}$), the *CYP2C9* minor allele at rs1799853 (*CYP2C9**2) was not associated with platelet reactivity in *CYP2C19**1 homozygotes ($N = 2375$, $P = 0.69$). In contrast, when we evaluated the impact of *CYP2C9* minor allele of rs1057910 (*CYP2C9**3) in *CYP2C19**1 homozygotes ($N = 2375$), we observed a significant association between this SNP and clopidogrel response ($P = 1.7 \times 10^{-4}$), suggesting that rs1057910 increases platelet reactivity independently of *CYP2C19**2.

Associations with clinical outcomes

We evaluated the impact of investigated polymorphisms on CVEs in a subset of 2134 patients for whom event data were available. Clinical characteristics of this subset of participants are shown in Supplementary material online, *Table S3*. In total, 129 of the 2134 patients experienced an event (event rate = 6.0% over a mean follow-up of ~13 months). No single variant was associated with CVEs before or after adjustment for *CYP2C19**2 (Supplementary material online, *Table S4*). Similarly, we observed no evidence of association between polymorphisms and the individual components of the composite events phenotype, although the number of events and statistical power for these sub-analyses was considerably diminished (Supplementary material online, *Table S5*).

Table 2 Association of candidate gene single nucleotide polymorphisms with adenosine diphosphate-stimulated platelet aggregation in International Clopidogrel Pharmacogenomics Consortium participants

Gene	Allele designation	rs number	Chromosome	MAF	HWE *P*-value	Beta	*P*-value	*CYP2C19**2 adjusted *P*-value
CYP2C19	*2	rs4244285	10q23.33	0.163	0.084	0.506	**8.80×10^{-54}**	NA
CES1	G143E	rs71647871	16q12.2	0.016	0.631	-0.787	**1.30×10^{-16}**	**4.27×10^{-18}**
CYP2C19	*17	rs12248560	10q23.33	0.219	0.755	-0.177	**9.48×10^{-10}**	0.006
CYP2B6	1294 + 53C > T	rs8192719	19q13.2	0.229	0.013	0.105	**2.99×10^{-4}**	**1.15×10^{-4}**
CYP2B6	Q172H	rs3745274	19q13.2	0.215	0.032	0.099	**9.98×10^{-4}**	**3.53×10^{-4}**
CYP2C9	*2	rs1799853	10q23.33	0.151	0.022	-0.113	**0.001**	0.489
CYP2C9	*3	rs1057910	10q23.33	0.072	0.908	0.153	**0.001**	**2.81×10^{-7}**
PEAR1	NA	rs12041331	1q23.1	0.123	0.005	-0.116	0.003	**4.15×10^{-4}**
P2RY12	T744C	rs2046934	3q25.1	0.175	0.003	0.088	0.004	0.003
CES1	NA	rs2244613	16q12.2	0.206	1.000	0.080	0.007	0.006
MED12L	NA	rs1472122	3q25.1	0.480	0.715	-0.063	0.009	0.002
ITGB3	L59P	rs5918	17q21.32	0.150	0.437	-0.081	0.016	0.005
CYP2C19	*8	rs41291556	10q23.33	0.002	0.016	0.560	0.020	0.008
CYP2B6	485-18C > T	rs4803419	19q13.2	0.310	0.414	0.058	0.026	0.023
CYP2B6	14593C > G	rs4803418	19q13.2	0.306	0.444	0.056	0.038	0.033
CYP2B6	*1B	rs7254579	19q13.2	0.322	0.972	0.051	0.050	0.045
CYP2C19	*4	rs28399504	10q23.33	0.003	1.000	-0.488	0.065	0.197
CYP2C19	*3	rs4986893	10q23.33	0.001	1.000	0.709	0.107	0.071
POR	IVS11 + 20G > A	rs2286823	7q11.23	0.293	0.071	0.040	0.125	0.086
CYP2C9	*11	rs28371685	10q23.33	0.004	1.000	0.307	0.167	0.772
CYP2C19	*6	rs72552267	10q23.33	0.002	0.024	0.335	0.175	0.198
POR	*28	rs1057868	7q11.23	0.288	0.249	-0.032	0.234	0.240
POR	NA	rs2302429	7q11.23	0.145	*6.41×10^{-5}*	-0.036	0.296	0.354
CYP2C19	*7	rs72558186	10q23.33	0.002	*4.03×10^{-8}*	-0.248	0.332	0.585
CYP1A2	*1F	rs762551	15q24.1	0.317	0.648	-0.024	0.343	0.521
ABCB1	C1236T	rs1128503	7q21.12	0.408	0.875	0.021	0.405	0.318
PON1	L55M	rs854560	7q21.3	0.371	0.359	-0.019	0.456	0.617
CYP2C19	*5	rs56337013	10q23.33	2.32×10^{-4}	1.000	-0.600	0.542	0.627
CYP3A5	*3F	rs28365085	7q22.1	2.31×10^{-4}	*1.16×10^{-4}*	0.163	0.741	0.621
PON1	Q192R	rs662	7q21.3	0.290	0.853	0.007	0.785	0.872
ABCB1	C3435T	rs1045642	7q21.12	0.496	0.107	0.007	0.786	0.614

Association *P*-values in bold represent those that are statistically significant after correction for multiple testing ($P = 0.05/31 = 0.0016$). Italicized Hardy–Weinberg *P*-values denote deviations from expected Hardy–Weinberg proportions (cut-off *P*-value $<1.0 \times 10^{-4}$).
HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; NA, not applicable.

Finally, we developed a PgxRS to assess the impact of carrying multiple alleles that impact platelet reactivity on the occurrence of CVEs. Specifically, we used *CYP2C19**2 and the five SNPs that were significantly associated with platelet reactivity after adjustment for *CYP2C19**2 and coded each allele that corresponded to increased platelet reactivity as the 'risk' allele (*Figure 1*). Collectively, these SNPs accounted for ~3.5% of the variation in platelet reactivity. We observed that patients who carry an increasing number of alleles that are associated with high on-clopidogrel platelet reactivity were more likely to experience CVEs and cardiovascular-related death compared to those who carry alleles that lead to better platelet inhibition ($\beta = 0.17$, $P = 0.01$ and $\beta = 0.43$, $P = 0.007$, respectively). Consistent with these findings, when comparing patients by number of risk alleles, patients who carried eight or more alleles associated with increased platelet reactivity were more likely to experience both CVEs [OR = 1.78, 95% confidence interval (CI) 1.14–2.76, $P = 0.01$] and cardiovascular death (OR = 4.39, 95% CI 1.35–14.27, $P = 0.01$) compared to patients who carried six or fewer of these alleles. Weighting these results based on genotype effect size did attenuate significant findings (CVE $P = 0.07$) but provided comparable OR estimates (*Table 3*).

Discussion

We assessed 31 candidate gene polymorphisms on platelet reactivity and CVEs in clopidogrel-treated Caucasian participants of the ICPC. Consistent with prior reports, we observed that the *CYP2C19**2 allele was a strong determinant of on-clopidogrel platelet reactivity.[5,10,11] We also observed that *CES1* G143E strongly influenced clopidogrel response with carriers of the 143E-allele having

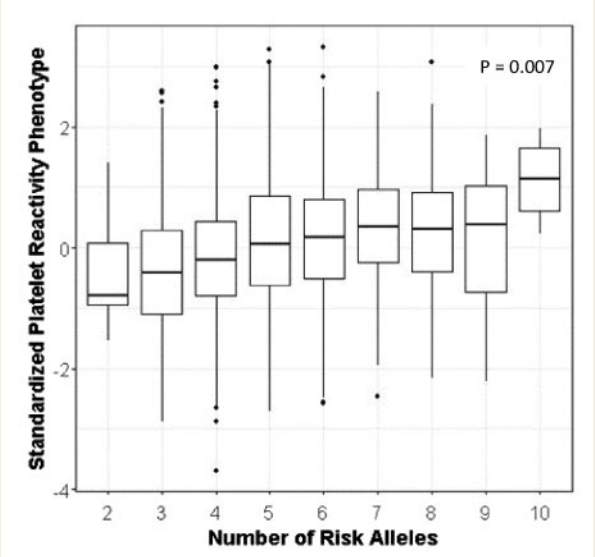

Figure 1 Change in standardized adenosine diphosphate-induced platelet reactivity based on increasing number of alleles used in the pharmacogenomic polygenic response score. For each box plot, the horizontal line within each box indicates the median; the top and bottom borders of each box indicate the interquartile range. The whiskers extending from each box indicate the 95% confidence interval and the points beyond the whiskers indicate outliers beyond ±2.5% CI. Platelet reactivity is expressed as Z-scores as described in the Methods section.

decreased on-treatment platelet reactivity. CES1 is responsible for converting clopidogrel into an inactive carboxylic acid derivative.[12] In fact, ~85% of administered clopidogrel is metabolized into inactive derivatives by CES1.[13] The 143E-allele results in an approximate 83% reduction in CES1 activity compared to the wild type 143G-allele, and is among the strongest determinant of clopidogrel response using physiology-directed pharmacokinetic/pharmacodynamic modelling.[14,15] These results suggest that comprehensive characterization of *CES1* G143E in clopidogrel-treated patients with regard to potential for pathological bleeding is needed.

We also assessed the well-described putative gain-of-function *CYP2C19**17 variant on platelet reactivity. *CYP2C19**17 is believed to lead to better inhibition of platelet aggregation by increasing transcription of the *CYP2C19* gene.[16] We initially observed that *CYP2C19**17 resulted in reduced platelet reactivity. However, in conditional analyses adjusting for *CYP2C19**2, no association was evident. These results are consistent with prior data showing that *CYP2C19**17 association results are highly influenced by *CYP2C19**2 through LD.[9] In stratified analyses, markedly attenuated association between *CYP2C19**17 and platelet reactivity was observed in patients who did not carry the *CYP2C19**2 allele. These results suggest that *CYP2C19**17 has little to no independent effect on clopidogrel response. This is particularly noteworthy given that multiple investigations have reported *CYP2C19**17 association results without taking into account the effects of *CYP2C19**2 and the fact that current pharmacogenetic prescribing algorithms may be incorrectly classifying patients who carry the *CYP2C19**17 allele as ultra-rapid clopidogrel metabolizers.[17]

Association between platelet reactivity and SNPs in *CYP2C9* was significantly influenced by *CYP2C19**2 adjustment. Rs1799853, which encodes *CYP2C9**2, was no longer associated with clopidogrel response while rs1057910, which encodes *CYP2C9**3, was more strongly associated with platelet reactivity. Evaluation of LD between *CYP2C19**2 and *CYP2C9* SNPs showed that while *CYP2C9* rs1799853 does not independently influence clopidogrel efficacy, rs1057910 increases platelet reactivity independently of *CYP2C19**2.

Two SNPs in *CYP2B6* (rs8192719 and rs3745274) and one SNP in *PEAR1* (rs12041331) were associated with platelet reactivity after adjustment for *CYP2C19**2. Previous investigations of *CYP2B6* on clopidogrel response have shown mixed results.[18,19] Of note, many of these investigations were conducted in a relatively small sample and likely had limited statistical power. *PEAR1* is a transmembrane receptor that is important in platelet aggregation-induced secondary signalling.[20] Rs12041331, which is an intronic variant proposed to modify *PEAR1* expression through differences in allele-specific DNA methylation,[21] has been implicated in baseline[22] and on-treatment aspirin,[23] ticagrelor,[24] and dual antiplatelet therapy with aspirin and clopidogrel[25] platelet aggregation and myocardial infarction in stable coronary artery disease patients.[25] Additional studies in large, well-powered cohorts will be needed to further define the role of *CYP2B6* and *PEAR1* in clopidogrel response.

While several polymorphisms in this investigation influenced platelet reactivity, none were individually associated with CVEs. This was relatively surprising, particularly for *CYP2C19**2, as there is an increasing amount of evidence that suggests this variant increases risk of cardiovascular outcomes, particularly in acute coronary syndrome patients undergoing percutaneous coronary intervention. Indeed, several high-profile investigations including the TRITON-TIMI 38 trial,[26] FAST-MI,[27] PLATO Genetic Substudy,[28] and IGNITE network[29] have shown that clopidogrel-treated patients who carry this allele have worse clinical outcomes compared to those who do not. Interestingly, however, when we combined the impact of multiple SNPs through generation of a PgxRS, it revealed that patients who carried a greater number of alleles that lead to increased platelet reactivity were more likely to experience CVEs. While significance was diminished in weighted analyses, OR point estimates do suggest enhanced risk of CVEs in these patients. These findings may have important implications. Specifically, for complex phenotypes such as CVEs, multiple genetic and non-genetic factors are critical in the development (or prevention) of the clinical outcome. Given this complexity, it is often difficult to reproducibly identify any one factor of small to moderate effect, and clinical utility of any single factor to predict risk and act to prevent adverse clinical outcomes is poor.[30,31] Our findings underscore the importance of considering multiple sources of genetic variability. Therefore, we highly encourage strategies such as those used in the current investigation to construct robust genetic response scores of platelet reactivity to evaluate potential impact on clinical outcomes. As shown here, these response scores can be built-in cohorts that utilize several platelet function tests through use of statistical approaches that implement Z-scores as well as cohorts that have limited genetic and platelet function data. The use of such strategies by the scientific community will

Table 3 Polygenic response score of alleles associated with platelet reactivity on the occurrence of cardiovascular endpoints

	Raw analysis			Weighted analysis		
Trait	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Composite CVE phenotype	1.78	1.14–2.76	0.01	1.55	0.97–2.48	0.07
Myocardial infarction	1.70	0.99–2.91	0.05	1.55	0.87–2.76	0.13
Cardiovascular death	4.39	1.35–14.27	0.01	2.49	0.81–7.56	0.11
Stent thrombosis	1.42	0.62–3.27	0.41	1.17	0.48–2.85	0.73
Stroke	1.23	0.57–2.66	0.90	1.04	0.45–2.39	0.93

Polygenic response score was derived from variants that were associated with ADP-based platelet reactivity in single-SNP analyses (*CYP2C19**2, *CES1 G143E*, *CYP2B6* 1294 + 53 C > T, *CYP2B6 Q172H*, *CYP2C9**3, and *PEAR1 rs12041331*) and alleles that led to increased ADP-based platelet reactivity were coded as 'risk'. Odds ratios were calculated comparing patients who carried eight or more alleles associated with increased platelet reactivity with patients who carried six or fewer of these alleles. Analyses were conducted in the 2134 ICPC participants in which clinical event data were available.
CI, confidence interval; CVE, cardiovascular event; OR, odds ratio.

be required to determine whether a clopidogrel PgxRS has high enough predictive value to identify those at high risk of recurrent CVEs in whom an alternative anti-platelet agent might be indicated.

This investigation has limitations. All participants evaluated were of European descent. Therefore, caution should be used in extrapolating these findings to populations of different ethnic or racial origins. This may be particularly important for polymorphisms that vary considerably in frequency across populations. For example, *CYP2C19**3, which is more common in Asian populations, and other reported loss-of-function variants in *CYP2C19* (i.e. *CYP2C19**4–8) were extremely rare in this population, consistent with other European-derived populations; therefore, caution should be used when interpreting the negative association results reported here as statistical power of analyses related to these variants is low. In addition, the prognostic value of the HPR phenotype is not established in patients at low cardiovascular risk even if they previously had a myocardial infarction.[32] While *CYP2C19**2 and the PgxRS used are strong determinants of platelet reactivity in these patients, the association with clinical outcomes will require further investigation. Finally, this was a retrospective study. Prospective studies will be required to determine whether alternative antiplatelet therapy can ameliorate increased risk of CVEs in high PgxRS patients.

In summary, we have performed one of the largest studies of clopidogrel pharmacogenetics conducted to date. Our findings suggest that several polymorphisms independently influence on-clopidogrel platelet reactivity and that accumulation of these alleles may increase CVE risk. The availability of alternative treatment options for patients with clinical indications for clopidogrel underscores the need to better understand the genetic architecture of variable drug response which is critical for optimizing anti-platelet pharmacotherapy, reducing therapeutic failure and adverse side effects, and ultimately improving patient outcomes.

Supplementary material

Supplementary material is available at *European Heart Journal – Cardiovascular Pharmacotherapy* online.

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