SUPPLEMENTARY MATERIAL

SUPPLEMENTAL METHODS

Study Populations

Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study. The Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study (NCT0079936) recruited 565 apparently healthy Caucasian individuals 20 years old or older between August 2006 and January 2010. This population has been described in detail previously (11). Informative relative pairs for estimating heritability using this population consisted of 164 parent-offspring pairs, 286 sibling pairs, 87 avuncular pairs, and 8 first-cousin pairs. Participants were extensively phenotyped and physical examinations, medical and family histories, anthropometric measures, and blood samples after an overnight fast were obtained. Complete blood count with platelet numbers and levels of serum lipids (total cholesterol, high-density lipoprotein cholesterol, and triglycerides) were assayed by Quest Diagnostics (Horsham, Pennsylvania); levels of low-density lipoprotein cholesterol were calculated using the Friedewald equation.

All medications, vitamins and supplements were discontinued at least 1 week prior to baseline studies. Platelet-rich plasma (PRP) was isolated from blood samples of participants and baseline platelet function was assessed by optical aggregometry using a PAP8E Aggregometer (Bio/Data Corporation, Horsham, Pennsylvania) after stimulation with collagen (5 μg/ml) and was expressed as the maximal percentage change in light transmittance using platelet-poor plasma as a referent. After baseline platelet aggregation measurements, participants were given a 300 mg oral loading dose of clopidogrel followed by 75 mg per day for 6 days. One hour following the last dose of clopidogrel,

platelet aggregation measurements were repeated. A second follow-up platelet aggregation measurement was made later the same day, 1 hour after oral ingestion of 324 mg of chewable aspirin.

Sinai Hospital of Baltimore Study Patients. The Sinai Hospital of Baltimore Study (NCT00370045) enrolled 227 patients older than 18 years and undergoing nonemergent percutaneous coronary intervention (PCI) between January 2004 and May 2007. Detailed recruitment of these patients has been described previously (11). Of the recruited patients, 140 (61.7%) were Caucasian, 83 (36.6%) were African American, and 4 (1.8%) were of other race/ethnicity. Race/ethnicity information was obtained by self-report. Directly preceding the PCI, patients received either a 600 mg (n = 112) or a 300 mg (n = 25) loading dose of clopidogrel; 90 patients were already receiving maintenance therapy with a 75-mg daily dose at the time of PCI and received no loading dose. Patients also received 81 to 325 mg of aspirin daily for at least 1 week prior to PCI and 325 mg on the day of the procedure. There were no differences in baseline characteristic or in the long-term outcomes investigated in stratified analyses of acute clopidogrel dosing; thus these groups were combined for further analyses.

Anticoagulant therapy including bivalirudin or heparin, either with (n = 107) or without (n = 120) eptifibatide, was discontinued at the completion of the procedure in all patients. To minimize the effects of acute anticoagulant therapy during PCI, platelet function was measured on the day of hospital discharge in patients not treated with eptifibatide or 5 days or more post-discharge in patients treated with eptifibatide.

Platelet function measurements in response to collagen were available for 153 patients taking aspirin at the time of enrollment, and results of baseline and post-

clopidogrel platelet aggregation studies in response to ADP were available for 202 and 188 patients respectively. Platelet aggregation was assessed in PRP after stimulation with 2 μg/ml collagen using a Chronolog Lumi-Aggregometer (Model 490-4D; Chronolog, Havertown, Pennsylvania), and was expressed as the maximal percentage change in light transmittance using platelet-poor plasma as a referent as described previously (8). Aspirin (325 mg/d) and clopidogrel (75 mg/d) were prescribed for all patients at the time of hospital discharge, according to the American College of Cardiology/American Heart Association guidelines (27). We assessed medication adherence by self-report and by review of source documents from hospitalizations for ischemic events.

Enrolled patients were contacted at the end of 1 and 12 months post-PCI to determine the occurrence of post-discharge cardiovascular ischemic events. A physician, blinded to the study results of the patient, adjudicated all end points through review of source documents obtained from medical records. Post-discharge ischemic events were defined as myocardial infarction (the occurrence of ischemic symptoms and a troponin I value greater than the upper limit of normal), ischemic stroke, stent thrombosis (definite stent thrombosis according to the Academic Research Consortium (28)), unplanned target vessel revascularization (revascularization of a vessel different from that treated at the time of enrollment), hospitalization for coronary ischemia without revascularization (hospitalization for chest pain with evidence of ischemia on electrocardiogram and no evidence of myocardial infarction as measured by troponin I value, and death secondary to any cardiovascular cause.

INternational VErapamil SR/trandolapril STudy GENEtic Substudy. The INternational VErapamil SR/trandolapril STudy (INVEST) GENEtic Substudy (INVEST-GENES) has been previously described (23). Briefly, genomic DNA from 5,979 INVEST patients with hypertension and stable coronary artery disease residing in the mainland United States and Puerto Rico were collected. Using these samples, a nested case-control group consisting of 361 patients who experienced the primary outcome (defined as first occurrence of death, nonfatal myocardial infarction, or nonfatal stroke) and 639 age-, sex-, and race-frequency-matched controls were evaluated. Individual components of the primary outcome were also assessed. All components of the primary outcome were adjudicated by an independent adjudication committee (24). In this sample, 570 (56.8%) participants were Caucasian, 154 (15.3%) were African American, and 276 (27.5%) were Hispanic. Information on race/ethnicity was obtained by self-report. Of these individuals, 499 were taking aspirin (dose unknown) and 501 were not.

All study protocols were approved by the respective institutional review boards at the University of Maryland, Sinai Hospital of Baltimore, and the University of Florida. Written informed consent was obtained from each participant; participants were compensated for their participation.

SUPPLEMENTARY TABLES

Supplementary Table 1. Multivariate Analysis of Dual Anti-Platelet Therapy Response as Measured by Collagen-Stimulated Platelet Aggregation in PAPI Study Participants (n = 565)

Characteristic	β ± SE	P-value[a]	Variance of Significant Predictors (%)
Age	0.31 ± 0.03	<0.001	12.1
Sex	3.02 ± 0.90	0.001	2.5
BMI	-0.18 ± 0.10	0.08	
Total cholesterol	0.01 ± 0.01	0.34	
HDL cholesterol	0.001 ± 0.03	0.96	
LDL cholesterol	0.01 ± 0.01	0.26	
Log triglycerides	-0.01 ± 0.01	0.27	
Systolic blood pressure	-0.05 ± 0.04	0.22	
Diastolic blood pressure	-0.10 ± 0.07	0.15	

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PAPI, Pharmacogenomics of Antiplatelet Intervention.

[a]All p-values adjusted for age, sex, and pre-clopidogrel/pre-aspirin collagen (5µg/ml)-stimulated platelet aggregation except age adjusted for sex and pre-clopidogrel/pre-aspirin platelet aggregation and sex adjusted for age and pre-clopidogrel/pre-aspirin platelet aggregation.

Supplementary Table 2. Summary GWAS Results from the Amish PAPI Study of All SNPs Associated with Collagen-Stimulated Platelet Aggregation after Aspirin and Clopidogrel Treatment with P-values < 1.0 x 10^{-5}

rs Number	Chr.	Position	Major Allele	Minor Allele	Minor Allele Frequency	Mean Maximal Platelet Aggregation			Additive P-value
						Major Allele Homozygotes	Heterozygotes	Minor Allele Homozygotes	
rs2148135	1	156050109	T	C	0.43	13.1	18.6	25.2	4.84E-08
rs11264825	1	156054894	C	T	0.44	13.1	18.4	24.8	8.10E-08
rs6696137	1	156049205	C	T	0.42	13.1	18.6	25.4	1.11E-07
rs7534239	1	155325048	T	C	0.49	13.2	18.0	22.3	2.33E-07
rs16831160	3	175058110	G	A	0.06	18.5	11.2	6.7	7.07E-07
rs1176535	1	155328174	T	C	0.48	13.4	18.4	21.8	9.13E-07
rs7210295	17	51477739	G	A	0.15	19.6	13.3	9.9	1.03E-06
rs1300252	10	70172432	A	C	0.08	19.0	11.2	7.5	1.34E-06
rs8066419	17	51475956	C	G	0.15	19.6	13.5	9.9	1.38E-06
rs10740315	10	70351384	C	T	0.09	19.2	11.4	9.4	1.53E-06
rs9807083	17	51477132	C	T	0.17	19.9	13.9	11.4	1.58E-06
rs11239104	10	44330302	G	A	0.15	19.3	13.8	12.6	2.20E-06
rs1387657	10	55167590	G	A	0.26	20.0	15.5	11.7	2.34E-06
rs7068100	10	55158579	C	T	0.26	20.0	15.6	11.7	2.40E-06
rs9394755	6	41114658	T	C	0.16	19.3	14.2	14.2	2.52E-06
rs10825102	10	55166711	C	T	0.26	19.9	15.6	11.8	3.27E-06
rs538811	11	30383651	A	G	0.06	18.8	11.8	0.0	3.63E-06
rs7085943	10	55166893	G	C	0.26	20.0	15.5	11.8	3.67E-06
rs17720273	2	2369626	G	C	0.12	18.9	13.7	9.5	4.74E-06
rs11004071	10	55538491	G	A	0.16	19.3	14.7	8.6	4.83E-06
rs12408952	1	155996652	A	G	0.34	21.6	15.9	12.1	5.63E-06
rs9381024	6	41092240	C	A	0.16	19.3	14.2	13.5	6.25E-06
rs9369260	6	41118346	G	C	0.16	19.2	14.1	14.4	7.13E-06
rs823994	5	132895169	C	G	0.05	18.7	9.8	4.8	7.76E-06
rs7933061	11	98519105	A	G	0.07	18.6	12.2	16.2	8.06E-06
rs1883595	6	41043706	G	A	0.17	19.3	14.3	14.4	8.11E-06
rs1940504	11	98518431	A	G	0.07	18.5	12.4	16.4	8.27E-06
rs1578774	10	55158280	C	T	0.24	19.8	15.6	10.8	8.28E-06
rs1940505	11	98518377	G	T	0.07	18.6	12.2	16.2	8.74E-06
rs1940503	11	98518522	C	T	0.07	18.6	12.3	16.4	9.22E-06
rs7105720	11	98518290	G	C	0.07	18.6	12.2	16.2	9.46E-06

Supplementary Table 3. Association of *PEAR1* Variant rs12041331 with Cardiovascular and Metabolic Traits in Participants of the Amish PAPI Study

Trait	GG Homozygotes		GA Heterozygotes		AA Homozygotes		Additive P-value
	Mean	SE	Mean	SE	Mean	SE	
BMI	26.953	0.218	27.912	0.480	27.600	1.926	0.002
% Body Fat	27.839	0.466	27.074	1.032	23.226	6.647	0.047
Triglyceride Levels	70.496	1.139	78.348	2.446	72.876	7.733	0.345
Total Cholesterol	195.892	17.296	210.587	3.685	212.262	1.627	0.939
HDL-Cholesterol	59.134	0.498	56.219	1.071	54.327	4.204	0.625
LDL-Cholesterol	139.051	1.234	138.547	2.812	128.916	11.699	0.891
Systolic Blood Pressure	117.343	0.346	118.006	0.668	118.041	4.531	0.877
Diastolic Blood Pressure	70.606	0.210	70.461	0.389	69.510	2.267	0.858
Common Carotid IMT	0.639	0.006	0.635	0.013	0.573	0.079	0.946
CRP Levels	1.948	0.033	2.182	0.074	2.168	0.399	0.741

Supplementary Table 4. Baseline Characteristics[*] for INVEST-GENES participants

Characteristics	Caucasians (n =570)		African Americans (n=154)		Hispanics (n=276)	
	Cases (n=168)	Controls (n=402)	Cases (n=40)	Controls (n=114)	Cases (n=71)	Controls (n=205)
Age, mean (SD), years	71.2 (9.5)	66.8 (9.6)	68.3 (11.2)	62.6 (9.1)	73.2 (8.9)	66.3 (9.6)
Women	73 (43.5)	158 (39.3)	24 (60.0)	72 (63.2)	36 (50.7)	130 (63.4)
Systolic BP, mean (SD), mmHg	150.8 (18.6)	150.0 (18.0)	156.1 (16.5)	152.4 (18.2)	146.8 (20.3)	146.8 (16.8)
Diastolic BP, mean (SD), mmHg	81.7 (10.6)	84.4 (10.8)	90.1 (10.4)	91.3 (12.1)	83.6 (11.2)	86.9 (10.1)
BMI, mean (SD), kg/m2	27.4 (5.0)	29.5 (5.8)	28.4 (4.6)	32.7 (7.2)	27.5 (4.3)	28.4 (4.5)
rs12041331 minor allele frequency	12.2	9.7	43.8	42.5	19.7	22.2
Myocardial infarction	64 (38.1)	176 (43.8)	16 (40.0)	35 (30.7)	17 (23.9)	17 (8.3)
Angina pectoris	91 (54.2)	194 (48.3)	22 (55.0)	92 (80.7)	57 (80.3)	191 (93.2)
Revascularization >1 month ago	84 (50.0)	165 (41.0)	10 (25.0)	23 (20.2)	11 (15.5)	9 (4.4)
Stroke/TIA	28 (16.7)	37 (9.2)	7 (17.5)	7 (6.1)	7 (9.9)	6 (2.9)
Left ventricular hypertrophy	24 (14.3)	45 (11.2)	14 (35.0)	28 (24.6)	12 (16.9)	22 (10.7)
Heart failure (class I–III)	19 (11.3)	13 (3.23)	5 (12.5)	5 (4.4)	7 (9.9)	0 (0)
Peripheral vascular disease	29 (17.3)	41 (10.2)	8 (20.0)	7 (6.1)	15 (21.1)	10 (4.9)
Smoking (ever)	93 (55.4)	194 (48.3)	21 (52.5)	43 (37.7)	31 (43.7)	61 (29.8)
Diabetes[†]	49 (29.2)	79 (19.7)	23 (57.5)	33 (28.9)	32 (45.1)	47 (22.9)
Hypercholesterolemia	115 (68.5)	280 (69.7)	22 (55.0)	45 (39.5)	34 (47.9)	79 (38.5)
Renal impairment[‡]	13 (7.7)	8 (2.0)	1 (2.5)	1 (0.9)	0 (0)	2 (1.0)
Aspirin use	119 (70.8)	242 (60.2)	25 (62.5)	43 (37.7)	29 (40.8)	41 (20.0)
Clopidogrel use	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Antidiabetic medication	42 (25.0)	72 (17.9)	20 (50.0)	29 (25.4)	29 (40.8)	38 (18.5)
Any lipid-lowering agent	82 (48.8)	208 (51.7)	10 (25.0)	29 (25.4)	22 (31.0)	57 (27.8)
Nitrates	45 (26.8)	76 (18.9)	14 (35.0)	29 (25.4)	34 (47.9)	47 (22.9)

Abbreviations: BP, blood pressure; BMI, body mass index; TIA, transient ischemic attack.

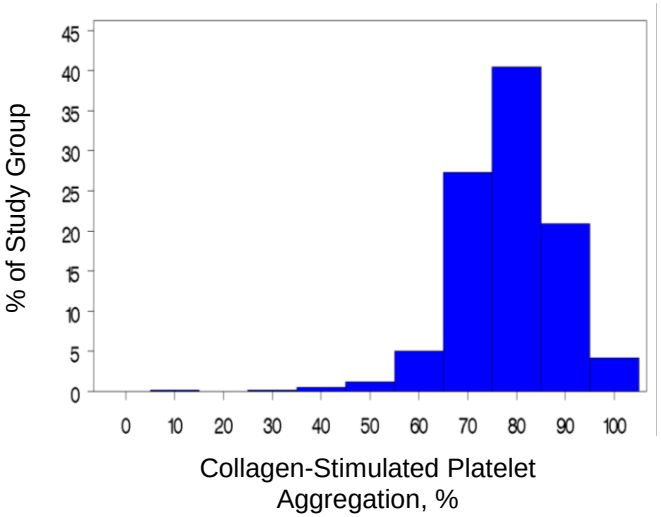
[*]Values expressed as number (percentage) unless otherwise indicated. Percentages may not equal exactly 100 due to rounding.

[†]History of or currently taking antidiabetic or lipid-lowering medications.

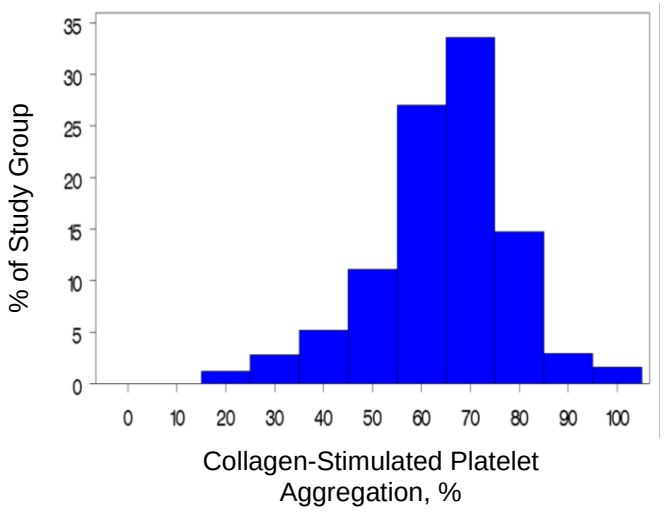
[‡]History of or currently have elevated serum creatinine level but less than 4 mg/dl (< 354 mmol/l).

SUPPLEMENTARY FIGURES

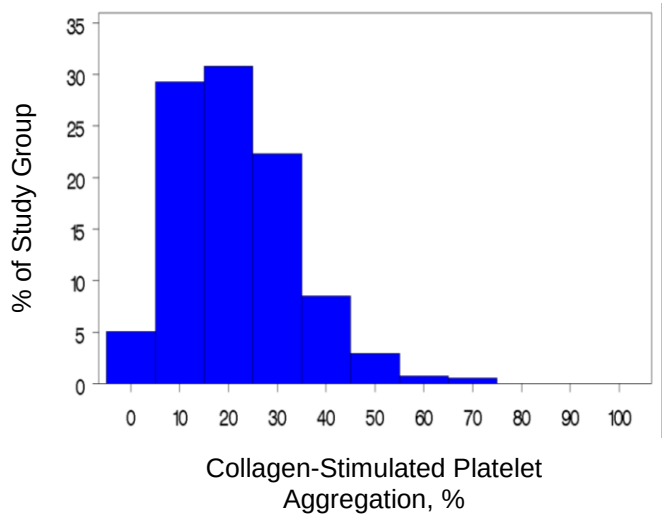
Baseline

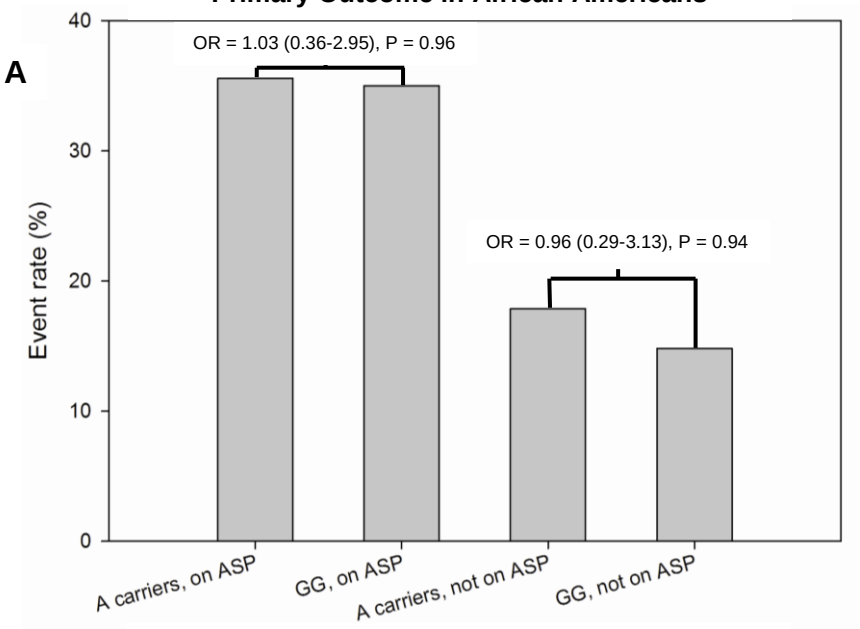


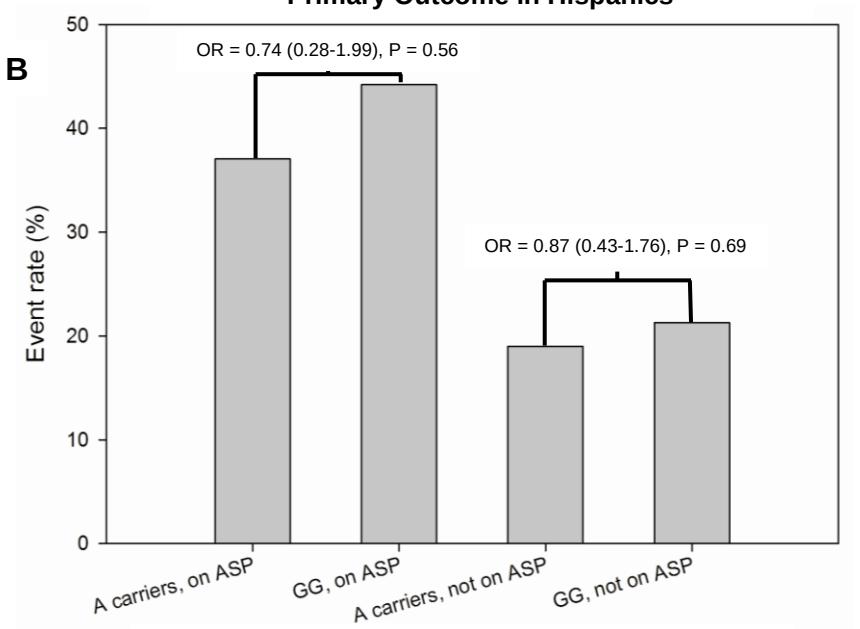
Post-Clopidogrel

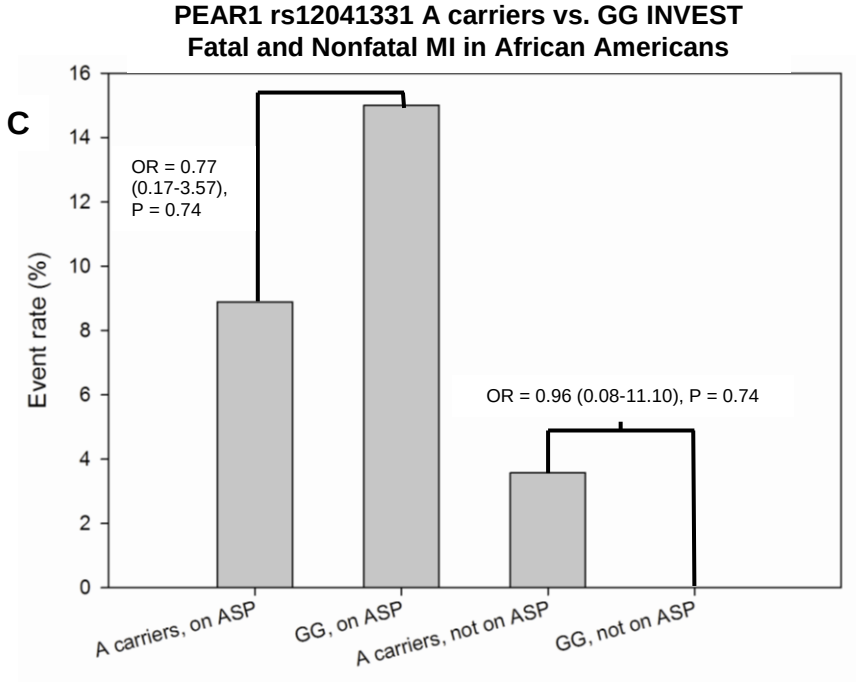


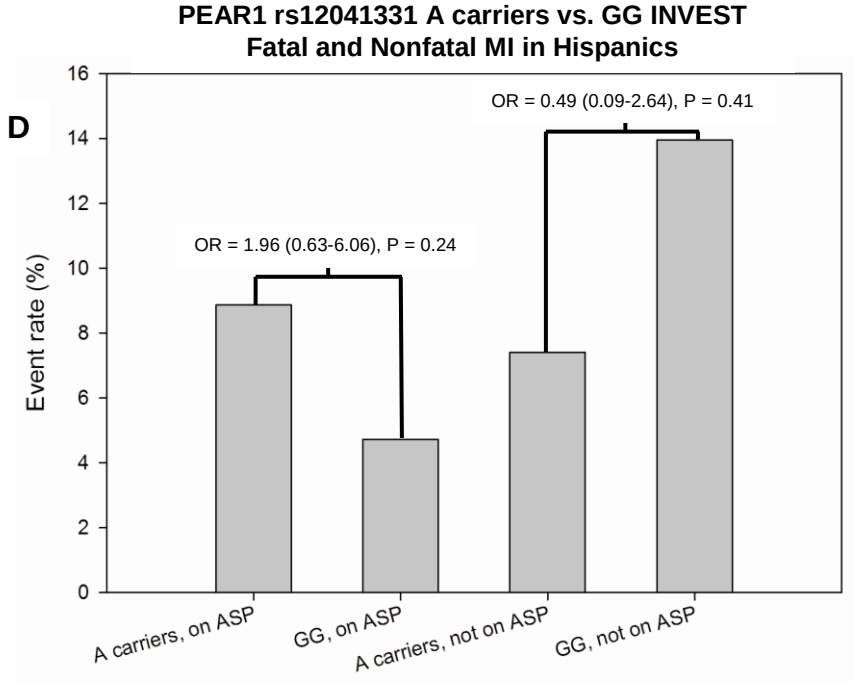
Post-Clopidogrel and Aspirin




PEAR1 rs12041331 A carriers vs. GG INVEST
Primary Outcome in African Americans
A
OR = 1.03 (0.36-2.95), P = 0.96
OR = 0.96 (0.29-3.13), P = 0.94
Event rate (%)
0
10
20
30
40
A carriers, on ASP
GG, on ASP
A carriers, not on ASP
GG, not on ASP


PEAR1 rs12041331 A carriers vs. GG INVEST
Primary Outcome in Hispanics
B
OR = 0.74 (0.28-1.99), P = 0.56
OR = 0.87 (0.43-1.76), P = 0.69
Event rate (%)
0
10
20
30
40
50
A carriers, on ASP
GG, on ASP
A carriers, not on ASP
GG, not on ASP


PEAR1 rs12041331 A carriers vs. GG INVEST
Fatal and Nonfatal MI in African Americans
C
OR = 0.77
(0.17-3.57),
P = 0.74
OR = 0.96 (0.08-11.10), P = 0.74
Event rate (%)
0
2
4
6
8
10
12
14
16
A carriers, on ASP
GG, on ASP
A carriers, not on ASP
GG, not on ASP


PEAR1 rs12041331 A carriers vs. GG INVEST
Fatal and Nonfatal MI in Hispanics
D
OR = 0.49 (0.09-2.64), P = 0.41
OR = 1.96 (0.63-6.06), P = 0.24
Event rate (%)
0
2
4
6
8
10
12
14
16
A carriers, on ASP
GG, on ASP
A carriers, not on ASP
GG, not on ASP

SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1. Distribution of collagen-stimulated platelet aggregation at baseline, after clopidogrel, and after clopidogrel and aspirin administration in 565 members of the Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study. Class intervals contain data greater than the lower limit and equal to the upper limit of each interval.

Supplementary Figure 2. Association between *PEAR1* SNP rs12041331 A-allele carrier status and the primary outcome (A-B) or fatal and non-fatal myocardial infarction (C-D) in aspirin-treated and non-treated African American (A, C), and Hispanic (B, D) patients of INVEST-GENES. All analyses were adjusted for age, sex, history of heart failure, and diabetes.

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