

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009;360:363-75. DOI: 10.1056/NEJMoa0808227.

Genetic determinants of clopidogrel response and cardiovascular events

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Supplementary Appendix

Methods and reaction conditions used for genotyping

Allele	Nucleotide change	Genotyping method
P2RY12		
	(rs16846673)	SNPlex [£]
	(rs6809699)	SNPlex [£]
	(rs6785930)	SNPlex [£]
ITGB3	(rs5918)	Taq man [#]
ABCB1	3435 C>T (rs1045642)	SNPlex [£]
CYP3A5 *3	6986 G>A (rs776746)	SNPlex [£]
CYP2C19		
CYP2C19*2	681G>A (rs4244285)	SNPlex [£]
CYP2C19*3	636 G>A (rs4986893)	SNPlex [£]
CYP2C19*4	1 A>G (rs28399504)	Taq man [#]
CYP2C19*5	1297 C>T (-)	Taq man [#]
CYP2C19*17	-806C/T (rs12248560)	Taq man [#]

* Numbers in parentheses are dbSNP accession numbers.

[£] **Oligo-ligation assay** (SNPlex, Applied Biosystems, Foster City, CA): According to the manufacturer's guidelines, we used two primers, one carrying the SNP-base-specific 3' end and one common primer that starts with the next base 5' to the base-specific primer in the target sequence. The two allele-specific primers carry unique ZIP codes that distinguish each allele. Primers are annealed to the target sequence according to the manufacturer's recommendations. The ligation reaction will join the allele-specific primer with the common primer if the allele-specific 3' base is present. A short fluorescent dye-labelled probe, homologous to the ZIP code sequence, is then hybridized to the immobilized product (www.appliedbiosystems.com/).

[#] **Allelic-discrimination reaction** (TaqMan, Applied Biosystems, Foster City, CA): The PCR amplification protocol for the TaqMan assays included denaturation at 95°C for 10 min, followed by 40 cycles at 92°C for 15 sec, 60°C for 1 min, and 72°C for 45 sec, followed by elongation at 72°C for 5 min. The Taq-Man assays were then read on a 7900HT Fast Real-Time PCR System and alleles were called using SDS software (www.appliedbiosystems.com/).

Table 1: Characteristics of patients in the propensity-matched cohort

	Control (n=280)	2 variant alleles [¶] (n = 56)	p
Demographic and clinical characteristics			
Sex Male / Female, No (%)	208 (74%)/72 (26%)	44 (79%)/12 (21%)	0.61
Age, y *	64.4 ± 13.5	64.9 ± 13.6	0.80
Hypertension, No (%)	145 (52%)	29 (52%)	1.0
Hypercholesterolemia, No (%)	167 (60%)	32 (57%)	0.73
Diabetes mellitus, No (%)	84 (30%)	16 (29%)	0.83
Family history of CAD, No (%)	66 (24%)	13 (23%)	0.95
Current smokers, No (%)	108 (39%)	21 (38%)	0.88
Prior myocardial infarction, No (%)	44 (16%)	10 (18%)	0.69
Prior CABG, No (%)	23 (8%)	4 (7%)	1.0
Prior PTCA, No (%)	22 (8%)	5 (9%)	0.79
Prior stroke or transient ischemic attack, No (%)	6 (2%)	1 (2%)	1.0
Prior Heart Failure, No (%)	12 (4%)	4 (7%)	0.32
Chronic Renal Failure, No (%)	0 (0%)	1 (2%)	0.17
AMI = first Cardiovascular event, No (%)	203 (73%)	38 (68%)	0.52
NSTEMI / STEMI, No (%)	136 (47%)/144(53%)	28 (50%)/28 (50%)	0.94
Body mass index (kg/m ²) *	26.7 ± 4.6	26.9 ± 4.5	0.79
Systolic blood pressure at admission, *	139 ± 28	142 ± 31	0.53
Diastolic blood pressure at admission, *	80 ± 18	84 ± 19	0.15
Killip class = 2 or more, No (%)	72 (26%)	15 (27%)	0.87
GRACE Score *	161.7 ± 38.5	162.2 ± 37.1	0.93
Left ventricular ejection fraction (%) *	52.3 ± 12.6	50.2 ± 16.6	0.46
In-hospital Management			
Angioplasty, No (%)	182 (65%)	37 (66%)	0.88
Thrombolysis, No (%)	39 (14%)	8 (14%)	1.0
Coronary artery bypass surgery, No (%)	10 (4%)	1 (2%)	0.70
Statins, No (%)	257(92%)	54 (96%)	0.40
Beta-blockers, No (%)	243 (87%)	47 (84%)	0.53
Calcium Channel blockers, No (%)	65 (23%)	9 (16%)	0.30
ACEI or ARB, **No (%)	211 (75%)	47 (84%)	0.22
Nitrated Derivatives , No (%)	190 (68%)	38 (68%)	1.0
Aspirin, No (%)	278 (99%)	55 (98%)	0.42
Heparin, No (%)	274 (98%)	55 (98%)	0.83
Proton pump inhibitors (PPI), No (%)	202 (72%)	38 (68%)	0.52
Omeprazole, No (%)	149 (53%)	26 (46%)	0.38
Diuretics, No (%)	101 (36%)	20 (36%)	1.0
Aldosterone antagonists, No (%)	25 (9%)	9 (16%)	0.14
Glycoprotein IIb/IIIa inhibitor, No (%)	140 (50%)	30 (54%)	0.66
Digitalics glycosides, No (%)	7 (3%)	1 (2%)	1.0

* mean ± sd; ** ACEI or ARB: angiotensin-converting enzyme inhibitors or angiotensin receptor blockers; [¶] 2 variant CYP2C19 loss-of-function alleles.