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CYP2C19*2 and CYP2C9*3 alleles are associated with stent thrombosis: a case–control study

Ankie M. Harmsze[1,2,3]*, Jochem W. van Werkum[2,4], Jurriën M. ten Berg[2,4], Bastiaan Zwart[2,4], Heleen J. Bouman[2,4], Nicoline J. Breet[2,4], Arnoud W.J. van 't Hof[5], Hendrik J.T. Ruven[2,6], Christian M. Hackeng[2,6], Olaf H. Klungel[3], Anthonius de Boer[3], and Vera H.M. Deneer[1,2]

[1]Department of Clinical Pharmacy, St Antonius Hospital, PO Box 2500, Nieuwegein 3430 EM, The Netherlands; [2]St Antonius Center for Platelet Function Research, St Antonius Hospital, Nieuwegein, The Netherlands; [3]Division of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands; [4]Department of Cardiology, St Antonius Hospital, Nieuwegein, The Netherlands; [5]Department of Cardiology, Isala Clinics, Zwolle, The Netherlands; and [6]Department of Clinical Chemistry, St Antonius Hospital, Nieuwegein, The Netherlands

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Aims Despite treatment with clopidogrel on top of aspirin, stent thrombosis (ST) still occurs being the most serious complication after percutaneous coronary interventions (PCIs). In this study, we aimed to determine the effect of variations in genes involved in the absorption (ABCB1 C1236T, G2677T/A, C3435T), metabolism (CYP2C19*2 and *3, CYP2C9*2 and *3, CYP3A4*1B and CYP3A5*3), and pharmacodynamics (P2Y1 A1622G) of clopidogrel on the occurrence of ST.

Methods and results The selected genetic variants were assessed in 176 subjects who developed ST while on dual antiplatelet therapy with aspirin and clopidogrel and in 420 control subjects who did not develop adverse cardiovascular events, including ST, within 1 year after stenting. The timing of the definite ST was acute in 66, subacute in 87, and late in 23 cases. The presence of the CYP2C19*2 and CYP2C9*3 variant alleles was significantly associated with ST (OR_{adj}: 1.7, 95% CI: 1.0–2.6, $P = 0.018$ and OR_{adj}: 2.4, 95% CI: 1.0–5.5, $P = 0.043$, respectively). The influence of CYP2C19*2 (OR_{adj}: 2.5, 95% CI: 1.1–5.5, $P = 0.026$) and CYP2C9*3 (OR_{adj}: 3.3, 95% CI: 1.1–9.9, $P = 0.031$) was most strongly associated with subacute ST. No significant associations of the other genetic variations and the occurrence of ST were found.

Conclusion Carriage of the loss-of-function alleles CYP2C19*2 and CYP2C9*3 increases the risk on ST after PCI.

Keywords Clopidogrel • Percutaneous coronary intervention • Genetic variants • Absorption • Metabolism • Stent thrombosis

Introduction

Clopidogrel plays an important role in the prevention of atherothrombotic events in patients undergoing percutaneous coronary interventions (PCIs) with stent implantation.[1] Despite this treatment, a substantial number of thrombotic events still occur. The most serious thrombotic complication is stent thrombosis (ST). This acute re-occlusion of the artery causes acute myocardial infarction (MI) and is associated with substantial morbidity and mortality. The reported incidence of ST varies from 0.2 to 4.6%.[2,3] The pathophysiology of ST involves complex and multifactorial mechanisms, and many issues are still unresolved.[4–7] Heightened platelet reactivity despite clopidogrel treatment has been associated with the occurrence of ST.[8,9] The magnitude of on-clopidogrel platelet reactivity is highly variable between subjects. Clinical, cellular, and genetic factors are thought to play an important role in this phenomenon.[10,11]

Clopidogrel is a thienopyridine that inhibits platelet activation through an irreversible blockage of the platelet adenosine diphosphate (ADP) P2Y12 receptor.[12,13] Clopidogrel is an inactive prodrug that requires several biotransformation steps to become active.[13] After intestinal absorption, which is mediated by P-glycoprotein, clopidogrel conversion to the active metabolite is mediated mainly by the hepatic cytochrome P450 system.[12,13]

* Corresponding author. Tel: +31 306092612, Fax: +31 306093080, Email: a.harmsze@antoniusziekenhuis.nl

Variations in genes involved in the absorption, metabolism, and pharmacodynamics of clopidogrel are thought to influence the response to the drug.[14–20]

In addition, recent studies have demonstrated a relationship between carriage of CYP2C19 loss-of-function alleles and adverse cardiovascular events, including ST, in patients on clopidogrel treatment.[19,21–26] However, all these studies had a limited amount of cases with ST, with the largest number of subjects being 24.[21] In the present study, 176 subjects with ST were included who were all on clopidogrel treatment at the time the event occurred. The aim of the present study was to investigate whether variations in genes involved in clopidogrel absorption (ABCB1 C1236T, G2677T/A, C3435T), metabolism (CYP2C19*2 and *3, CYP2C9*2 and *3, CYP3A4*1B and CYP3A5*3), and the P2Y1 receptor (P2Y1 A1622G) are associated with the occurrence of ST in patients undergoing coronary stent placement who were treated with clopidogrel and aspirin.

Methods

Study population

All consecutive patients with an angiographically confirmed ST presenting from January 2004 to February 2007 in three high-volume centres in the Netherlands were enrolled.[27] Stent thrombosis was defined according to the Academic Research Consortium (ARC) 'definite' definition.[28] Stent thrombosis was categorized according to the time of the event as acute (occurrence within the first 24 h after the index procedure), subacute (from 24 h to 30 days), and late (from 30 days to 1 year). Patients were only selected as cases when they were still on aspirin and clopidogrel at the time of ST.

Control subjects were consecutive patients who underwent PCI with stent implantation between December 2005 and December 2006 in one of the participating centres, with no adverse cardiovascular events, including ST, during a 1-year follow-up post-PCI. All control subjects were on clopidogrel maintenance therapy and aspirin (80–100 mg) during the entire follow-up period. Of all subjects, medication records of community pharmacies were used to verify the use of clopidogrel, aspirin, proton pump inhibitors (PPIs), and calcium channel blockers (CCBs) from the time of index PCI until 1-year post-PCI. The ethnicity of the population in and around the cities of the participating centres is primarily Caucasian (>85%).[29–31] The study complies with the Declaration of Helsinki, the study protocol was approved by the hospital's Medical Ethics Committee, and informed consent was obtained from each patient.

Genotyping

Genomic DNA of all control subjects and of 38 cases was isolated from EDTA blood (MagNA Pure LC DNA Isolation kit 1, MagNA Pure; Roche Diagnostics, Basel, Switzerland). Genomic DNA of the remaining 138 cases was manually extracted from saliva samples (Oragene kit, DNA Genotek, Inc., Ottawa, Ontario, Canada; Laboratory Protocol for Manual Purification of DNA from 4.0 mL of Oragene® DNA/saliva on www.dnagenotek.com).

CYP2C19*2 and *3, CYP2C9*2 and *3, CYP3A4*1B, and the ABCB1 G2677T/A and C3435T alleles were identified by real-time PCR. CYP3A5*3, ABCB1 C1236T, and the P2Y1 A1622G alleles were identified by using restriction fragment length polymorphism. Method validation was carried out by DNA sequence analyses.

Data analysis

The Kolmogorov–Smirnov test was used to check for normal distribution of continuous data. Continuous data, except for the time to ST, were normally distributed. Normally distributed continuous data were expressed as mean ± standard deviation (SD). Continuous data not meeting the criteria for normal distribution were expressed as median [interquartile range (IQR)]. Comparisons between groups were made with the chi-square test for categorical variables. For continuous variables, comparisons were made with the two-sided Student's *t*-test. Chi-square tables were used to compare the observed number of each genotype with those expected for a population in Hardy–Weinberg equilibrium ($P > 0.05$). The linkage disequilibrium (LD) correlation coefficient (r^2) between each pair of variant alleles that was associated with ST was calculated with the Cubic exact solutions for the estimation of pairwise haplotype frequencies.[32] We assumed a dominant model for our genetic analyses. Logistic regression was used to analyse the association between the presence of variant alleles and ST and to adjust for potential confounders. Variates that have been associated with an altered response to clopidogrel or with an increased risk of adverse cardiovascular events after PCI in previous publications were selected as potential confounders. The included confounders were: age, gender, body mass index (BMI), smoking, diabetes mellitus, prior MI, the use of PPIs, the use of CCBs, acute coronary syndrome (ACS) as the indication for PCI, peri-procedural variables being stent length, stent diameter, and stent type (bare metal or drug eluting), and the use of glycoprotein IIb/IIIa antagonists during the procedure. A *P*-value of <0.05 was considered statistically significant. All associations that were statistically significant were corrected for multiple testing by performing the false discovery rate test (*q*-value threshold 0.20).[33] Statistical analysis was performed using SPSS software (version 15.0.1 for Windows, SPSS Chicago, IL, USA).

Results

Characteristics of the study population and genotype

Of a total of 21 009 patients undergoing stent implantations in the participating hospitals, 437 patients presented with an angiographic confirmed ST during the inclusion period. In total, 210 patients were still on dual antiplatelet therapy at the time of ST. From these, DNA was obtained from 176 patients. In total, 176 cases and 420 control subjects were included in the study. The timing of the 'definite' ST was acute in 66 (37.5%), subacute in 87 (49.4%), and late in 23 (13.1%) subjects. The median time (IQR) for the occurrence of ST in relation to the index procedure was 3.0 (0–9) days. *Table 1* summarizes the characteristics of the cases and control subjects. There were no significant differences with regard to sex, age, diabetes mellitus, BMI, hypertension, and hypercholesterolaemia between the two groups. Cases were more frequently current smokers ($P < 0.001$) than control subjects. The control group consisted of significantly more patients who had suffered from a previous MI. No significant deviations from Hardy–Weinberg equilibrium were observed for any of the genetic variants (*Table 2*). Genotype and allele frequencies of control subjects were not different from previously reported frequencies in healthy Caucasian populations.[16,34] As we found only one subject carrying a CYP2C19*3 allele, we did not include this allele in our analysis.

Table 1 Baseline characteristics

Variable	Control subjects (n = 420)	Cases (n = 176)	P-value
Age (years)	62.1 ± 9.4	64.1 ± 10.5	0.14
Gender, male	334 (79.5)	137 (77.8)	0.66
BMI (kg/m^2)	27.4 ± 3.8	27.1 ± 2.2	0.76
Diabetes mellitus	69 (16.4)	31 (17.6)	0.55
Dyslipidaemia	212 (50.5)	92 (52.3)	0.72
Hypertension	208 (49.5)	82 (46.6)	0.53
Prior MI	178 (42.4)	42 (23.9)	<0.0001
Current smoking	51 (12.1)	39 (22.2)	<0.0001
Glycoprotein IIb/IIIa receptor antagonist use	39 (9.3)	61 (34.7)	<0.0001
Acute coronary syndromes as indication for PCI	103 (24.6)	136 (77.3)	<0.0001
Drug eluting stent	199 (47.4)	55 (31.3)	<0.0001
Stent length (mm)	29.6 ± 17.6	18.9 ± 5.7	<0.0001
Stent diameter (mm)	3.1 ± 0.6	3.1 ± 0.4	0.36
Proton pump inhibitors	95 (22.7)	51 (29.0)	0.12
CYP3A4-metabolized statins	297 (70.7)	128 (72.7)	0.44
Calcium channel blockers	120 (28.6)	53 (30.1)	0.77

Data presented are mean ± SD or number of patients (percentage). P-value: Student's t-test for continuous variables and chi-square test for categorical variables. ST, stent thrombosis; PCI, percutaneous coronary intervention; MI, myocardial infarction; BMI, body mass index.

Association between genotype and the occurrence of stent thrombosis

As shown in *Table 2*, 40.0% of the cases had at least one CYP2C19*2 allele, compared with 29.5% of the control subjects ($P = 0.013$). The CYP2C19*2 allele was associated with ST in univariate analysis, with an OR of 1.6 (95% CI: 1.1–2.3, $P = 0.013$, *Table 3*). This association remained significant after the adjustment for confounders (OR_{adj}: 1.7, 95% CI: 1.0–2.6, $P = 0.018$). When cases were divided according to the time of ST after PCI, carriers of CYP2C19*2 were at an approximately two-fold higher risk of developing a subacute ST (OR: 2.0, 95% CI: 1.3–3.3, $P = 0.003$), which remained significant after the adjustment for confounders (OR_{adj}: 2.5, 95% CI: 1.1–5.5, $P = 0.026$, *Table 4*). Subanalyses in cases with acute or late ST did not reveal any significant associations of genotypes with the occurrence of these types of ST (*Table 4*).

For CYP2C9, the carriage of the *3 allele was associated with an increased risk of ST when compared with CYP2C9*3 non-carriers: OR: 1.8, 95% CI: 1.1–3.0, $P = 0.027$; OR_{adj}: 2.4, 95% CI: 1.0–5.5, $P = 0.043$. The influence of CYP2C9*3 was most prominent on the occurrence of subacute ST (OR: 2.2, 95% CI: 1.1–4.4, $P = 0.024$; OR_{adj}: 3.3, 95% CI: 1.1–9.9, $P = 0.031$), whereas the associations of this variant allele and the occurrence of acute and late ST were not statistically significant (*Table 4*).

In multivariate analysis, in which besides the non-genetic covariates, both CYP2C19*2 and CYP2C9*3 were included as covariates, the two genetic variants were found to be independent predictors of ST (for CYP2C19*2: OR_{adj}: 1.7, 95% CI: 1.0–3.1, $P = 0.040$; for CYP2C9*3: OR_{adj}: 2.5, 95% CI: 1.1–5.8, $P = 0.035$).

We found no evidence of LD for the pair CYP2C19*2–CYP2C9*3 ($r^2 = 0.01$). The distribution of CYP2C19 and CYP2C9 genotypes among cases and control subjects is shown in *Table 5*. In CYP2C19*2 non-carriers, CYP2C9*3 was associated with an almost two-fold increased risk of ST: OR: 1.9, 95% CI: 1.0–3.4, $P = 0.042$, which remained statistically significant after the adjustment for confounders: OR_{adj}: 3.0, 95% CI: 1.1–8.6, $P = 0.037$. Cases were more often carriers of both CYP2C19*2 and CYP2C9*3 alleles when compared with control subjects: 4.5 vs. 1.7% (OR: 1.9, 95% CI: 1.2–10.0, $P = 0.029$; OR_{adj}: 2.1, 95% CI: 1.3–3.5, $P = 0.003$; *Table 5*).

No interaction between the indication for PCI [ACS vs. stable angina pectoris (SAP)] and the carriage of CYP2C19*2 or CYP2C9*3 was found (P-values of 0.97 and 0.18, respectively). In addition, stratified analysis according to the indication of PCI was performed. In subjects with ACS as the indication for PCI (136 cases and 103 control subjects), CYP2C19*2 and CYP2C9*3 both increased the risk on the occurrence of ST (OR_{adj}: 2.0, 95% CI: 1.1–4.5, $P = 0.032$ and OR_{adj}: 2.9, 95% CI: 1.0–9.3, $P = 0.039$, respectively).

In the subgroup of subjects with SAP (40 cases and 317 control subjects), a trend towards an association for CYP2C19*2 was found (OR: 1.7, 95% CI: 0.9–4.1, $P = 0.076$), whereas for CYP2C9*3, no association with ST (OR: 1.2, 95% CI: 0.4–6.5, $P = 0.56$) was observed.

No significant associations of the other genetic variations and the occurrence of ST were found (*Table 3*). For all associations, the multiple testing parameter q was found to be <0.20.

Discussion

This case–control study aimed to determine the influence of genetic variations related to the pharmacokinetics and pharmacodynamics of clopidogrel on the occurrence of ST in patients who were on clopidogrel and aspirin treatment at the time of the

Table 2 Genotype frequencies

SNP (allele)/dbSNP/accession number	Genotype	Frequency control subjects (%)	HWE control subjects	Frequency cases (%)
CYP2C19/G681A (*1>*2)/rs4244285	*1/*1	70.5	0.12	60.0
	*1/*2	25.7		34.9
	*2/*2	3.8		5.1
	AF	17.0		22.6
CYP2C19/G636A (*1>*3)/rs4986893	*1/*1	99.8	0.98	100
	*1/*3	0.2		0
	*3/*3	0		0
	AF	0		0
CYP2C9/C430T (*1>*2)/rs1799853	*1/*1	77.2	0.66	77.7
	*1/*2	21.5		20.0
	*2/*2	1.2		2.3
	AF	12.0		12.3
CYP2C9/A1075C (*1>*3)/rs1057910	*1/*1	90.0	0.99	83.5
	*1/*3	9.8		15.3
	*3/*3	0.2		1.1
	AF	5.2		8.6
CYP3A4/A290G (*1>*1B)/rs2740574	*1/*1	91.7	0.28	92.6
	*1/*1B	6.7		7.4
	*1B/*1B	1.6		0
	AF	5.0		3.7
CYP3A5/A6986G (*1>*3)/rs776746	*1/*1	0	0.17	0.6
	*1/*3	12.7		11.0
	*3/*3	87.3		88.4
	AF	94.0		93.9
ABCB1/C1236T/rs1128503	CC	29.5	0.07	32.0
	CT	54.0		53.7
	TT	16.5		14.3
	AF	43.5		41.2
ABCB1/G2677T/A/rs2032582	GG	29.7	0.06	28.6
	GT+GA	53.8		56.0
	TT+TA+AA	16.5		15.4
	AF	43.0		43.4
ABCB1/C3435T/rs1045642	CC	16.8	0.12	21.6
	CT	56.6		54.0
	TT	26.6		24.4
	AF	54.9		51.4
P2Y1/A1622G/rs701265	AA	72.0	0.63	70.1
	AG	26.0		29.3
	GG	1.9		0.6
	AF	15.0		15.3

SNP, single nucleotide polymorphism; HWE, Hardy–Weinberg Equilibrium; AF, allele frequency. All frequencies are expressed as percentages.

event. We found that carriers of the CYP2C19*2 and CYP2C9*3 loss-of-function alleles were at a 1.7- and 2.4-fold increased risk of developing ST, respectively. The influence of these genetic variants was most profound on the risk of subacute ST. We found no significant associations between the other investigated genetic variants and the occurrence of ST.

Of all genotypes included in this study, CYP2C19 has been by far the most extensively investigated. After absorption, 85% of clopidogrel is metabolized into an inactive compound. The remaining 15% of clopidogrel is metabolized into 2-oxo-clopidogrel. This intermediate metabolite is then hydrolysed and generates a highly unstable active thiol (R-130964) metabolite.[12,13] CYP2C19 contributes in both of the two sequential metabolic steps of clopidogrel activation. Data from several studies report that carriage of the CYP2C19*2 allele is associated with an impaired pharmacodynamic response to different dosing regimens of clopidogrel, as measured with various platelet function assays.[14,15,20] In two studies in healthy subjects, the carriers of CYP2C19*2 exhibited

Table 3 Associations of genetic variants and risk on stent thrombosis

Carriers ≥1 variant allele	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[a]	*P*-value
CYP2C19/G681A (*1>*2)	1.6 (1.1–2.3)	0.013	1.7 (1.0–2.6)	0.018
CYP2C19/G636A (*1>*3)	ND	ND	ND	ND
CYP2C9/A1075C (*1>*3)	1.8 (1.1–3.0)	0.027	2.4 (1.0–5.5)	0.043
CYP2C9/C430T (*1>*2)	1.0 (0.6–1.5)	0.90	0.6 (0.2–1.7)	0.12
CYP3A4/A290G (*1>*1B)	0.8 (0.5–1.8)	0.76	0.6 (0.3–2.0)	0.45
CYP3A5/A6986G (*1>*3)	0.2 (0.1–1.2)	0.99	0.2 (0.1–1.3)	0.99
ABCB1/C1236T	0.9 (0.6–1.4)	0.74	0.7 (0.4–1.2)	0.48
ABCB1/G2677T/A	1.0 (0.7–1.6)	0.79	0.9 (0.5–1.6)	0.89
ABCB1/C3435T	0.8 (0.5–1.2)	0.30	0.6 (0.3–1.2)	0.18
P2Y1/A1622G	1.1 (0.7–1.6)	0.64	1.2 (0.6–2.2)	0.28

OR, odds ratio; CI, confidence interval; ND, not determined.
[a]Adjusted for age, gender, body mass index, smoking, diabetes mellitus, prior MI, use of PPIs, use of CCBs, use of glycoprotein IIb/IIIa receptor antagonists, stent length, type, and diameter, and ACS as indication for PCI.

Table 4 Associations of genetic variants and risk on stent thrombosis, stratified by the timing of stent thrombosis

Genetic variants	Acute ST (*n* = 66)		Subacute ST (*n* = 87)		Late ST (*n* = 23)	
	OR (95% CI), OR_{adj} (95% CI)[a]	*P*-value	OR (95% CI), OR_{adj} (95% CI)[a]	*P*-value	OR (95% CI), OR_{adj} (95% CI)[a]	*P*-value
CYP2C19, G681A (*1>*2)	1.3 (0.8–2.3), 1.7 (0.8–3.5)	0.34, 0.11	2.0 (1.3–3.3), 2.5 (1.1–5.5)	0.003, 0.026	1.0 (0.4–2.6), 1.4 (0.6–9.5)	0.92, 0.54
CYP2C9, A1075C (*1>*3)	1.5 (0.7–3.1), 2.2 (0.9–6.8)	0.15, 0.10	2.2 (1.1–4.4), 3.3 (1.1–9.9)	0.024, 0.031	0.4 (0.06–3.1), 1.1 (0.1–12.5)	0.39, 0.73

OR, odds ratio; CI, confidence interval; ST, stent thrombosis.
[a]Adjusted for age, gender, body mass index, smoking, diabetes mellitus, prior MI, use of PPIs, use of CCBs, use of glycoprotein IIb/IIIa receptor antagonists, type, length, and diameter of the stent, and ACS as indication for PCI.

Table 5 Distribution of CYP2C19 and CYP2C9 variant alleles in control subjects and cases

Genotype groups	Control subjects [*n* (%)]	Cases [*n* (%)]	OR (95% CI), OR_{adj} (95% CI)*	*P*-value
Subjects carrying neither CYP2C19*2 nor CYP2C9*3	262 (62.4)	85 (48.3)	0.6 (0.4–0.8), 0.4 (0.2–0.7)	0.002, 0.003
Subjects carrying CYP2C19*2 but not CYP2C9*3	116 (27.6)	62 (35.2)	1.6 (1.1–2.4), 2.1 (1.1–3.9)	0.013, 0.018
Subjects carrying CYP2C9*3 but not CYP2C19*2	35 (8.3)	21 (11.9)	1.9 (1.0–3.4), 3.0 (1.1–8.6)	0.042, 0.037
Subjects carrying both CYP2C19*2 and CYP2C9*3	7 (1.7)	8 (4.5)	1.9 (1.1–3.2), 2.4 (1.3–4.3)	0.018, 0.004

Cases and control subjects are divided into four subgroups: (i) subjects carrying neither CYP2C19*2 nor CYP2C9*3, (ii) subjects carrying CYP2C19*2 but not CYP2C9*3, (iii) subjects carrying CYP2C9*3 but not CYP2C19*2, and (iv) subjects carrying both CYP2C19*2 and CYP2C9*3. Data expressed as number (%). OR, odds ratio; CI, confidence interval.
[a]Adjusted for age, gender, body mass index, smoking, diabetes mellitus, prior MI, use of PPIs, use of CCBs, use of glycoprotein IIb/IIIa receptor antagonists, type, length, and diameter of the stent, and ACS as indication for PCI.

significantly lower area under the plasma concentration time curves (AUCs) and lower maximal plasma concentrations of clopidogrel's metabolites than subjects homozygous for the CYP2C19 wildtype.[25,35] The results of our study regarding CYP2C19*2 are consistent with recent studies investigating the effect of CYP2C19*2 on clinical endpoints, including ST.[21–23,26]

To our knowledge, this is the first study showing that the carriage of CYP2C9*3 is associated with an increased risk of ST. The association of CYP2C9 genetic variants and ST is only explored in the study reported by Mega *et al.*[25] in which no associations of CYP2C9*3 and ST were found. However, the number of subjects with ST was rather small (*n* = 18). Together with the low

allele frequency of CYP2C9*3 (7.0–9.0% in Caucasians,[34] 4% in Asians, and not present in African populations[36]), this study was underpowered to detect the association. Our observation regarding CYP2C9*3 is supported by the results of two studies. In patients undergoing elective PCI, CYP2C9*3 carriers had a mean relative increase of 10% in on-clopidogrel platelet reactivity as measured with ADP-induced light transmittance aggregometry and the VerifyNow P2Y12 assay, compared with CYP2C9*3 non-carriers.[20] The carriage of CYP2C9*3 was associated with a four-fold increased risk on high on-clopidogrel platelet reactivity (HCPR). In the same study, the carriage of CYP2C19*2 was also associated with a more than 10% mean relative increase in on-clopidogrel platelet reactivity. CYP2C19*2 carriers had an ~3.5-fold increased risk of HCPR.[20] Brandt *et al.* found healthy subjects carrying the CYP2C9*3 loss-of-function allele to have a significantly lower AUC and lower maximal plasma concentrations of clopidogrel's active metabolite when compared with non-carriers. Furthermore, they also found CYP2C9*3 to be associated with an impaired pharmacodynamic response to a 300-mg clopidogrel loading dose.[35] CYP2C9 is thought to play a role in only clopidogrel's secondary metabolic step of activation.[25]

The other investigated genetic variant in the CYP2C9 gene, CYP2C9*2, was not associated with the risk of ST. This is in concordance with other pharmacogenetic studies of CYP2C9-metabolized drugs, e.g. coumarins. The presence of the CYP2C9*2 allele also has less impact on the anticoagulation effect of acenocoumarol than CYP2C9*3.[37]

The influence of genetic variations is most prominent on subacute ST. However, it should be noted that subanalyses in the different groups of ST had less power due to lower number of cases in each of the ST subgroups.

No significant associations were found in patients presenting with acute ST. This observation is in line with previous findings that indicate that mechanical and procedural factors are the predominant cause of acute ST.[27,38] We found no associations of genetic variations on the occurrence of late ST. This phenomenon might partly be caused by the fact that only 23 patients with late ST were included in our study. Furthermore, when the time interval after the index PCI increases, it is likely that other mechanisms (e.g. late stent malapposition) might play a more prominent role. Our findings confirm recently published data from Geisler *et al.* showing no predictive value of residual platelet aggregation for the incidence of late ST. The authors concluded that other mechanisms might be involved in the development of late ST.[39]

Drug eluting stents (DES) are considered to be associated with the occurrence of particularly late ST. The lower percentage of cases who received DES might be caused by the fact that we observed mainly acute and subacute ST (in total 87% of the cases). These types of ST are more common with the use of bare metal stents.[40]

There are some limitations in this study. First, in this observational case–control study, we cannot completely exclude the possible bias by various risk factors and patients' characteristics. Nonetheless, the multivariable adjustment models confirmed the primary analyses. Second, our cases had more often ACS as the indication for PCI. Acute coronary syndrome is a known risk factor for the development of ST.[6] However, adjustment for this confounder and including interaction terms did not change findings. In addition, stratified analyses showed that the genetic variants CYP2C19*2 and CYP2C9*3 were associated with ST in the subgroup of patients with ACS. In the subgroup consisting of patients with SAP as the indication for PCI, a trend towards a significant association for CYP2C19*2 but no association for CYP2C9*3 was found. As only 40 cases had SAP as the indication for PCI and the fact that CYP2C9*3 has a low allele frequency, this subgroup was too small to detect significant associations. Finally, the cases more often received glycoprotein IIb/IIIa antagonists than the control subjects. Both in patients with ACS and SAP as the indication for PCI, the use of glycoprotein IIb/IIIa antagonists was limited to the provisional (bail-out) use at the discretion of the operator, after PCI. Nevertheless, we observed that ACS patients more often received glycoprotein IIb/IIIa antagonists than patients with SAP (29% of the patients with ACS and 10% of the patients with SAP). However, adjustment for the use of glycoprotein IIb/IIIa antagonists did not change the associations between the two genetic variants and ST. Also, in stratified analysis according to the indication of PCI, the adjustment for glycoprotein IIb/IIIa antagonists did not change findings.

Given the devastating consequences of ST, great efforts should be made to identify those patients at highest risk, who would benefit most from an alternative strategy. Specifically, the frequent presence of the CYP2C19*2 allele, seen in ~30% of the Caucasian and 60% of the Asian population, may require an alternative strategy in the prevention of atherothrombotic complications after stent implantation.[41] A randomized trial in 60 patients undergoing elective PCI, reported that CYP2C19*2 carriers had a greater platelet inhibition after a split 1200-mg clopidogrel loading dose or 150-mg clopidogrel maintenance dose than after a 600-mg loading dose and 75-mg maintenance dose, respectively. Interestingly, in patients with the CYP2C19*1/*1 genotype, no dose-dependent response was observed. This might indicate that subjects with a poor-response genotype may specifically benefit from a higher dose of clopidogrel.[42] However, large clinical trials are needed to confirm these observations.

In conclusion, we have shown that the carriage of the loss-of-function alleles CYP2C19*2 and CYP2C9*3 increases the risk on ST. Personalized therapy targeting patients who carry these genetic variants might help to improve the clinical outcome after stent implantation.

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