CYP2C19 and nongenetic factors predict poor responsiveness to clopidogrel loading dose after coronary stent implantation

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Aims: To investigate an association of responsiveness to clopidogrel loading dose with genotypes of cytochrome P450 (CYP) 2C19, other CYP isozymes and nongenetic factors in patients with coronary artery disease. **Materials & methods:** Genotyping for *CYP2C19* (*2, *3 and *17), *CYP3A4*1B* and *CYP3A5*3* variants was performed in patients (n = 237) who underwent percutaneous coronary intervention. Adenosine diphosphate-induced platelet aggregation was determined after first administration of 600 mg clopidogrel. **Results:** *CYP2C19**2 carriers showed significantly increased residual platelet aggregation (RPA) (OR: 4.6; 95% CI: 2.5–8.7; p < 0.0001) compared with noncarriers. All other polymorphisms had no influence on RPA. For the development of a risk score for better prediction of RPA, *CYP2C19**2 genotype and previously identified nongenetic risk factors (age >65 years, Type 2 diabetes mellitus, decreased left ventricular function, renal failure and acute coronary syndrome) were analyzed. Multivariable logistic regression analysis showed a significant correlation of the nongenetic factors ($\chi^2 = 5.32$; p = 0.021) and *CYP2C19**2 ($\chi^2 = 21.31$; p < 0.0001) with high RPA, and an even higher association for the combination of both ($\chi^2 = 25.85$; p < 0.0001). **Conclusions:** Prediction of responsiveness after clopidogrel loading dose may substantially be improved by adding *CYP2C19**2 genotype to nongenetic risk factors.

Intracoronary stent implantation has gained substantial success in the past owing to technological and therapeutic advances. Pretreatment with a loading dose of clopidogrel, an inhibitor of platelet aggregation induced by ADP, was shown to significantly improve clinical outcome in patients undergoing percutaneous coronary intervention (PCI) [1,2]. However, marked interindividual variability in clopidogrel-induced inhibition of platelet aggregation is well-known and up to 30% of patients do not achieve an adequate antiplatelet effect after an initial clopidogrel loading dose [3–7]. In a large-scale study of 1092 consecutively enrolled patients, we recently identified nongenetic factors (age >65 years, Type 2 diabetes mellitus, decreased left ventricular function, renal failure and acute coronary syndrome) for better prediction of patients with persistent high residual platelet activity after coronary stenting and dual antiplatelet therapy with clopidogrel and aspirin [8]. Using a factor-weighted model, a score (termed residual platelet aggregation after deployment of intracoronary stent [PREDICT]-score) at least higher than 4 score levels significantly increased the risk for persistent residual platelet aggregation (RPA). Of note, preliminary data indicate that after short-term follow-up of 30 days, the incidence

of major adverse cardiovascular events (e.g., myocardial infarction, cardiovascular death and ischemic stroke) was significantly increased in patients with high RPA [8].

In addition to nongenetic factors, putative mechanistic explanations of elevated RPA include functional polymorphisms in enzymes involved in the metabolism of clopidogrel. In favor, major evidence came from a healthy volunteer study demonstrating that carriage of the cytochrome P450 2C19 loss of function variant *CYP2C19**2 has been associated with markedly decreased platelet responsiveness to clopidogrel [9]. Exertion of the antiplatelet effect of clopidogrel requires *in vivo* conversion to an active metabolite, an unstable thiol compound, inhibiting platelet aggregation by the formation of a disulfide bridge between the thiol group of the active compound and a cysteine residue of the P_2Y_{12} receptor of platelets [10]. In addition to *CYP2C19*, the cytochrome P450 enzyme family *CYP3A* substantially contributes to the bioactivation of clopidogrel. *In vitro* and *in vivo* data conclusively demonstrate that *CYP3A4* is also involved [11,12].

Therefore, the present study was conducted to investigate comprehensively the contribution of functional polymorphisms in the drug-metabolizing enzymes, *CYP2C19* and *CYP3A4/5*, on

clopidogrel responsiveness, in addition to nongenetic factors in patients undergoing coronary intervention for symptomatic coronary artery disease (CAD).

Methods

Study population & design

From July 2006 to March 2007, patients admitted to the Department of Cardiology, University Hospital Tuebingen, Germany, for coronary intervention for symptomatic CAD were consecutively enrolled in this pharmacogenetic trial. Only patients of Caucasian origin were included. Moreover, 186 healthy unrelated individuals of Caucasian (German) origin, randomly recruited as described previously [13], were used as an independent control population to compare CYP allele frequencies between patients and controls. The study protocol was approved by the ethics committee of the Medical Faculty of the University of Tuebingen, Germany, and all patients and healthy controls gave their written informed consent to participate.

The study protocol specified the following criteria:

- Only patients with symptomatic CAD (stable angina or acute coronary syndrome) were included;
- Patients with known disorders of platelet function or treatment with glycoprotein IIb/IIIa receptor inhibitors 1 week prior to inclusion were excluded;
- A loading dose of 600 mg clopidogrel was given prior to PCI followed by a daily dose of 75 mg clopidogrel;
- All patients received 500 mg aspirin intravenous before PCI, followed by oral aspirin 100 mg per day, unless aspirin was contraindicated owing to gastrointestinal bleeding or allergic reactions;
- Unfractionated heparin (70 units per kg body weight intravenously) was administered to all patients prior to PCI.

Acute coronary syndrome was diagnosed if at least one of the following criteria was met: unstable angina (clinical symptoms and ECG changes without evidence of myocardial necrosis), acute myocardial infarction (positive markers of myocardial necrosis, for example, elevation of serum troponin and creatine kinase-myoglobin binding [CK-MB]) including ST-segment elevation myocardial infarction (STEMI), and non-STEMI (NSTEMI).

On the basis of our previous work including 1092 patients, we defined poor response to clopidogrel loading dose if the level of the individual RPA measured at least 6 h after first administration belong to the upper tertile of RPA levels with a predefined cut-off value of 47% [8].

Blood sampling & platelet aggregation

Patient blood was collected in 3.8% citrate plasma at least 6 h after first administration of 600 mg clopidogrel, when maximum platelet inhibition is achieved [14]. In brief, samples were centrifuged at 1000 rpm for 10 min to obtain platelet-rich plasma (PRP) and additionally 10 min at 3500 rpm to recover platelet-poor plasma (PPP). The platelet concentration of PRP was adjusted to $2 \times 10^5/\mu\text{l}$ by adding homologous PPP. Percent platelet aggregation after stimulation with 20 $\mu\text{mol/l}$ ADP was assessed with the turbidimetric method using a Chronolog Lumi aggregometer with Aggro-Link Software as described previously [15]. RPA was defined as aggregation measured 5 min after addition of ADP 20 $\mu\text{mol/l}$. We decided to use final platelet aggregation because it reflects stabilization of aggregates mediated by the P_2Y_{12} ADP receptor, which is blocked by clopidogrel, whereas peak platelet aggregation rather reflects P_2Y_{12} -mediated platelet shape change independently from clopidogrel effects [16].

Genotyping of CYP450 variants

Genomic DNA was isolated from ethylenediaminetetraacetic acid (EDTA) blood samples using the QIAmp® DNA Blood Mini Kit System (Qiagen, Hilden, Germany). Candidate genes of cytochrome P450 enzymes were selected on the basis of metabolic pathways known to be involved in clopidogrel metabolism [9,11,12]. The criteria for selection of variants in these genes were a representative allele frequency in Caucasians and clear evidence for functional consequences based on *in vitro/in vivo* data [10]. Thus, the following polymorphisms of *CYP2C19* were analyzed: *CYP2C19**2 (681G>A), *3 (636G>A) and *17 (-806C>T), as well as the variants *CYP3A4**1B (-392A>G) and *CYP3A5* *3 (6986A>G) in other CYP enzymes. Genotyping for *CYP2C19* variants *2, *3 and *17, as well as *CYP3A4**1B and *CYP3A5**3, was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the MassARRAY® Compact system (Sequenom, CA, USA) as previously

described [17]. Details of primers and assays are available upon request. Approximately 10% of samples within each assay were retyped as a quality control. Laboratory staff were blinded to the case status of the study participants during the entire genotyping process.

Statistical analysis

To assess the power of the study we note that the frequency of the *CYP2C19*2* allele is approximately 15% in healthy Caucasians, which amounts to observing approximately 28% persons carrying the allele. Due to the definition of high RPA, it is expected that approximately 30% of the patients not carrying the allele show high RPA. To detect an odds ratio (OR) of at least 2.5 describing the association of RPA and genotype, 230 patients have to be included in the study to achieve a power of 86%.

All values are presented as median and interquartile range. Univariate analyses were performed to identify genotypes of major influence on responsiveness to clopidogrel. χ^2 analysis or Fisher's exact test were used as appropriate.

The dichotomous logistic regression model was computed using the statistics software R, version 2.4.1 [102] and the Design library [18]. OR are presented with 95% confidence intervals (CIs) and two-sided p-values. Statistical significance was defined as $p < 0.05$.

Observed and expected allele and genotype frequencies within populations were compared by means of Hardy–Weinberg equilibrium calculations [103].

Results

Demographic & clinical data

Base line characteristics of the 237 consecutively enrolled study patients treated with clopidogrel after implantation of stents owing to symptomatic CAD are summarized in Table 1. A median RPA of 34% (interquartile range 33.8%) was found. According to our previous large-scale study [8], we used the predefined RPA cut-off value of 47% to categorize all 237 patients into subjects with a lower ($n = 175$, responder) or higher ($n = 62$, poor responder) RPA. In the majority of patients, RPA was determined at around 20 h (median: 21 h [5.1–285.0]) after loading dose of 600 mg. Time difference in the two groups with low (median: 21 h [5.1–285.0]) and high (median: 22 h [5.9–177.0]) RPA was not significantly different. A total of 106 (44.7%) patients were treated for acute coronary syndrome; 35% of patients suffered from Type 2

diabetes. In the present study, the frequency distribution of all clinical parameters (Table 1), except hyperlipidemia, was similar to our previous results, which included 1092 individuals [8]. In analogy to these data, we defined the individual PREDICT score based on the five nongenetic risk factors (age >65 years, acute or stable coronary syndrome, Type 2 diabetes, decreased left ventricular function and renal failure) for all 237 patients. As expected, the use of comedications was frequent and predominately statins (66%), angiotensin-converting enzyme (ACE) inhibitors (69%), AT1 receptor antagonists (12%), β -blockers (79%) and calcium antagonists (19%) were prescribed.

Association with CYP polymorphisms

Overall, no significant differences between allele and genotype frequencies for all tested CYP polymorphisms were found when patients and healthy volunteers were compared (Table 2). As expected, owing to the rare allele frequency, no carrier of *CYP2C19*3* was detected. All genotype frequencies of CYP polymorphisms in healthy controls were in Hardy–Weinberg equilibrium. Again this was the case in the patient group of responders as well as poor responders except *CYP2C19*17* (only in nonresponders), which may be explained by the low sample size. Carriers of the *CYP2C19*2* variant showed significantly increased RPA after clopidogrel loading dose compared with noncarriers (Figure 1) and the median RPA of heterozygous (46%) and homozygous carriers (54%) was considerably higher than in noncarriers (30%). RPA differed significantly between the three *CYP2C19*2* genotype groups when patients were categorized into responders (low RPA) and poor responders (high RPA) ($\chi^2 = 27$; $p < 0.001$). These data hold true when patients who were heterozygous or homozygous for variant *CYP2C19*2* were pooled together (e.g., *CYP2C19*1/*1* vs *CYP2C19*1/*2* plus **2/*2*, OR: 4.6; 95% CI: 2.5–8.7; $p < 0.0001$). Carriers of at least one *CYP2C19*2* allele had a 4.38-fold increased risk ($p < 0.0001$; 95% CI 2.3–8.33) for developing high RPA (>47%). All other polymorphisms tested (*CYP2C19*17*, *CYP3A4*1B* and *CYP3A5*3*) had no influence on RPA (Table 3), with or without pooling heterozygote and homozygote variant patients. Moreover, the median RPA of heterozygous (30%) and homozygous carriers (37%) of *CYP2C19*17* were not significantly different compared with noncarriers (36.0%).

Table 1. Clinical characteristics of patients according to their individual residual platelet activity.			
Variables	Low RPA (n = 175)	High RPA (n = 62)	Total (n = 237)
Residual platelet activity,%*	26.0 (27.0)	54.00 (9.0)	34.00 (33.8)
Age (years)*	69.0 (13.0)	69.0 (11.3)	69.0 (13.0)
Sex, no. (%)			
Male	136 (77.7)	45 (72.6)	181 (76.4)
Female	39 (22.3)	17 (27.4)	56 (23.6)
Acute coronary syndrome, no. (%)			
STEMI	76 (43.4)	30 (48.4)	106 (44.7)
NSTEMI	21 (12.0)	9 (14.5)	30 (12.7)
	40 (22.9)	14 (22.6)	54 (22.8)
Left ventricular function (ejection fraction), no. (%)			
45–55%	45 (25.7)	17 (27.9)	62 (26.3)
35–45%	14 (8.0)	12 (19.7)	26 (11.0)
<35%	23 (13.1)	9 (14.8)	32 (13.6)
Diabetes, no. (%)	56 (32.0)	26 (41.9)	82 (34.6)
Hypertension, no. (%)	147 (84.0)	55 (88.7)	202 (85.2)
Renal failure, no. (%)	20 (11.5)	14 (22.6)	34 (14.4)
(serum creatinine >1.5 mg/dl)			
Smoking, no. (%)	67 (38.3)	22 (35.5)	89 (37.6)
Hyperlipidemia, no. (%)	129 (73.7)	46 (74.2)	175 (73.8)

*Median (interquartile range).
NSTEMI: Non-STEMI; STEMI: ST-segment elevation myocardial infarction; RPA: Residual platelet aggregation.

Multivariable logistic regression analysis
In addition to the PREDICT score exclusively based on nongenetic factors, we developed a risk score model including *CYP2C19**2 genotypes. The PREDICT score was defined as previously described [8], using a weighting factor of 1–3 for each variable to account for the unequal influence of nongenetic parameters. Thus, age and acute coronary syndrome were weighted by factor 1, diabetes and renal failure by factor 2, and left ventricular dysfunction by factor 3, thus giving scores ranging from 0 to 9. Of note, multivariable logistic regression analyses showed a significant correlation of the nongenetic risk factors ($\chi^2 = 5.32$; $p = 0.021$), as well as the

*CYP2C19**2 genotype ($\chi^2 = 21.31$; $p < 0.0001$) with high RPA, but interestingly an even higher association was found for the combination of both ($\chi^2 = 25.85$; $p < 0.0001$). The risk for developing high RPA increased by the presence of one *CYP2C19**2 allele (OR: 3.71; 95% CI: 1.87–7.35), and was even more pronounced by the presence of two variant alleles (OR: 10.72; 95% CI: 2.56–44.88).

On the basis of the logistic regression model we developed a final proportional odds model (nomogram, Figure 2). It allows one to calculate the probability of experiencing a RPA higher than 47% for a given patient treated with a clopidogrel loading dose of 600 mg. In the first step (Figure 2A), for each factor on the left of panel A (i.e., weighted PREDICT score and *CYP2C19**2 genotype [0: wild-type, 1: heterozygous, 2: homozygous variant]) the appropriate number of points is determined on the upper scale and added up. In a second step (Figure 2B), the total sum of points is transformed into the corresponding estimated probability for high RPA (>47%), indicating poor response. Thus, the maximal number of points is 165, predicting a greater than 80% risk of high RPA. In contrast, the maximal number of points achievable in a patient with a wild-type *CYP2C19* is 65, predicting a risk of approximately 40%.

Table 2. Frequency distribution of variant CYP alleles in patients and healthy controls.		
	Patients,% (n = 237)	Healthy subjects,% (n = 186)
<i>CYP2C19</i> *2	15.2 [†]	15.2 [§]
<i>CYP2C19</i> *3	0	0
<i>CYP2C19</i> *17	25.5	25.7
<i>CYP3A4</i> *1B	3.4	4.6
<i>CYP3A5</i> *3	92.8	88.9

[†]*CYP2C19* genotypes: *1/*1, n = 175 (74%); *1/*2, n = 52 (22%); *2/*2, n = 10 (4%).
[§]*CYP2C19* genotypes: *1/*1, n = 135 (73%); *1/*2, n = 42 (23%); *2/*2, n = 7 (4%).

Figure 1. Genotype distribution of the *CYP2C192 polymorphism in patients with high and low residual platelet aggregation receiving a loading dose of 600 mg clopidogrel.**

An increasing incidence of carriers of at least one *CYP2C19**2 allele was found in patients with high RPA (>47%).
RPA: Residual platelet aggregation.

Discussion

There has been increasing evidence for a considerable interindividual variability of antiplatelet drug response to clopidogrel associated with an increased risk for thromboembolic complications after coronary intervention [7,19]. The present study describes a significant association between poor response to a 600-mg clopidogrel loading dose and patients with CAD carrying at least one allele of the *CYP2C19**2 loss-of-function polymorphism. According to our previous results [8], poor responsiveness after first administration of clopidogrel was defined as RPA of more than 47% despite standard dual antiplatelet therapy with clopidogrel and aspirin. In line with these data, it is well demonstrated by several clinical studies that elevated RPA after PCI for symptomatic CAD is an independent predictor for clinical outcome (e.g., adverse coronary events in patients with acute myocardial infarction).

Of note, Hulot *et al.* recently proposed a major impact of the bioactivating polymorphically expressed drug-metabolizing enzyme CYP2C19 on clopidogrel responsiveness in healthy subjects using the phosphorylation state

of the vasodilator phosphoprotein and platelet aggregation profile [9]. This study was later confirmed by the same authors [20], again indicating a significant reduction in ADP-induced platelet aggregation related to *CYP2C19**2 genotype ($p = 0.001$) in a larger cohort of 94 Caucasian medical students treated with a loading dose of 300 mg clopidogrel. Moreover, Brandt *et al.* [21] who had already claimed in 2006 such an association [22], investigated the contribution of *CYP2C19* polymorphisms in 74 healthy subjects predominantly with Caucasian ethnic backgrounds receiving a 300-mg loading dose of clopidogrel. These authors provided first evidence that the presence of *CYP2C19**2 was significantly associated with alteration of pharmacokinetic parameters of the active metabolite of clopidogrel, for example, decreased AUC_{0-24} and C_{max} . Very recently, the impact of *CYP2C19* genetics on clopidogrel response variability has been investigated in the clinical setting of high-risk vascular patients who underwent primary PCI and received dual antiplatelet therapy with a loading dose of 600 mg clopidogrel and of 500 mg of aspirin intravenously [23]. In fact, *CYP2C19**2 genotype distribution differed significantly between patients with and without residual platelet activity as evaluated by the use of 10 $\mu\text{mol/l}$ ADP-induced platelet aggregation ($p = 0.002$).

The findings of our study investigating *CYP2C19* polymorphism in patients with coronary intervention for symptomatic CAD corroborate the assumption that patients carrying the *CYP2C19**2 allele are at risk for poor response to clopidogrel loading dose. Patients carrying at least one *CYP2C19**2 allele had a fourfold increased risk ($p < 0.0001$) for developing high RPA. Moreover, the fact that the risk for developing high RPA increased by the presence of one *CYP2C19**2 allele (OR: 3.71) and was even more pronounced by two variant alleles (OR: 10.72) clearly reflects a gene–dose effect of clopidogrel action on ADP-mediated platelet aggregation. Why a recent study including a small number of cardiovascular outpatients did not show an impact of *CYP2C19* on clopidogrel responsiveness remains unclear [24].

Since clopidogrel requires bioactivation to its active metabolite 2-oxoclopidogrel via CYP2C19 to irreversibly block the platelet ADP receptor P_2Y_{12} [25], thereby resulting in an approximately 50% reduction in ADP-mediated platelet aggregation, the *CYP2C19**2 allele provides a plausible explanation for interindividual

Table 3. Summary of genotyping results according to residual platelet aggregation categorized in two groups (RPA cut-off value 47%).						
CYP polymorphism	Patients		Statistics		Low vs high RPA	
	Genotype	Low RPA (%)	High RPA (%)		χ ² or odds ratio (95% CI)	p-value
<i>CYP2C19</i> *2 (n = 237)	*1/*1	144 (82.3)	31 (50)	*1/*1 vs *1/*2 + *2/*2	4.6 (2.5–8.7)	<0.0001
	*1/*2	28 (16)	24 (38.7)	*1/*1 vs *1/*2 vs *2/*2	27.17	<0.001
	*2/*2	3 (1.7)	7 (11.3)	–	–	–
<i>CYP2C19</i> *17 (n = 237)	*1/*1	96 (54.9)	41 (66.1)	*1/*1 vs *1/*17 + *17/*17	0.62 (0.34–1.14)	0.14
	*1/*17	65 (37.1)	14 (22.6)	*1/*1 vs *1/*17 vs *17/*17	4.48	0.11
	*17/*17	14 (8.0)	7 (11.3)	–	–	–
<i>CYP3A4</i> *1B (n = 234)	*1/*1	162 (94.2)	57 (91.9)	*1/*1 vs *1/*1B + *1B/*1B	1.42 (0.46–4.33)	0.55
	*1/*1B	10 (5.8)	4 (6.5)	*1/*1 vs *1/*1B vs *1B/*1B	2.83	0.24
	*1B/*1B	0 (0)	1 (1.6)	–	–	–
<i>CYP3A5</i> *3 (n = 235)	*3/*3	149 (86.1)	53 (85.5)	*3/*3 vs *1/*3 + *1/*1	1.05 (0.46–2.41)	1.00
	*1/*3	24 (13.9)	8 (12.9)	*3/*3 vs *1/*3 vs *1/*1	2.82	0.24
	*1/*1	0 (0)	1 (1.6)	–	–	–

CI: Confidence interval; RPA: Residual platelet aggregation.

variability of drug response. *CYP2C19**2 results in a truncated protein unable to bind to the heme moiety [26], consequently leading to a nonfunctional enzyme. In addition, the *CYP2C19**17 allele was recently reported, resulting in a putatively increased enzyme function of *CYP2C19* [17,27], and possibly affects clopidogrel response in the opposite direction as reflected by low RPA. In the present study, we firstly investigated the contribution of the *CYP2C19**17 variant on RPA, but found no significant association (Table 3).

It is well established that *CYP2C19* pharmacogenetics is of clinical relevance. For instance, carriers of two nonfunctional *CYP2C19**2 alleles have a decrease in oral clearance of proton pump inhibitors, such as omeprazole and lansoprazole, by approximately 85%, and several studies demonstrated that cure rates of *Helicobacter pylori* infection were lower in *CYP2C19* wild-type individuals compared with homozygous variant or heterozygous patients [28,29]. Thus, *CYP2C19* pharmacogenetically-guided dose adjustment has been suggested to improve the clinical efficacy of proton pump inhibitors. Interestingly the frequency of *CYP2C19* variant individuals varies among different racial groups [26]. By contrast to white and black subjects, among whom homozygous carriers occur at frequencies of 2–4%, in Asian subjects the frequency of mutant subjects is substantially higher (~14.0%), particularly in Japanese (20%).

Therefore, the impact of the *CYP2C19**2 polymorphism on clopidogrel responsiveness is expected to be of particular interest in patients of Asian origin.

In addition to *CYP2C19*, genetic variants in *CYP3A* enzymes (*CYP3A4* and *CYP3A5*) may contribute to poor response rates of clopidogrel. Both *CYP3A4* and *CYP3A5* are polymorphic, but unlike *CYP3A5* (*CYP3A5**3) there are no *CYP3A4* variant alleles described so far that appear at high allele frequencies with marked differences between populations and that may alter *CYP3A4* function to a clinically relevant extent [30,31]. Only the *CYP3A4**1B variant was suggested to contribute to small changes in *CYP3A4* protein function [32]. However, by far the best-characterized genetic polymorphism in *CYP3A* genes is the *CYP3A5**3 variant, which results in alternative splicing of an mRNA species harboring a premature termination codon and subsequent nonsense-mediated decay of the mRNA [33]. Consequently, homozygous carriers of *CYP3A5**3, who represent the vast majority of Caucasians, express up to 30-fold lower levels of *CYP3A5* protein compared with subjects, who carry at least one *CYP3A5**1 wild-type allele [33,34]. Alteration of drug disposition for several agents (e.g., tacrolimus), which are *CYP3A* substrates has been described in the presence of *CYP3A5* polymorphisms [35]. Interestingly, we did not find any contribution of both the *CYP3A4**1B and *CYP3A5**3 polymorphism on RPA in our

Figure 2. Nomogram for estimating individual risk for high residual platelet aggregation under a loading dose of 600 mg clopidogrel.

The nomogram was developed on the basis of the final logistic regression model (see statistical analysis). It allows calculating the probability for high RPA (>47%) based on nongenetic factors (weighted PREDICT score) and *CYP2C19*2* genotype (0: *CYP2C19* wild-type, 1: *CYP2C19*1*2*, 2: *CYP2C19*2*2*). For each factor on the left of panel (A) the appropriate number of points is determined on the upper scale. The total sum of points is transformed into the estimated probability panel (B) for a high RPA. RPA: Residual platelet aggregation.

patient population. However, since both *CYP3A* polymorphisms occur with low allele frequencies, and sample size calculation of our study was predominately based on *CYP2C19*2* allele frequency, a larger patient population needs to be screened to definitively determine the impact of these polymorphisms on RPA. Of note, recently an intronic variant in the *CYP3A4* gene (IVS10 + 12G>A; termed *CYP3A4*1G*), which is in strong linkage disequilibrium with *CYP3A4*1B*, was shown to be associated with a significantly reduced platelet glycoprotein IIb/IIIa receptor activation in patients with stable CAD during the steady-state phase, but also in the acute phase of clopidogrel treatment, thereby resulting in improved responsiveness. However, this variant did not influence the platelet inhibition profile [36]. By contrast, no association between the *CYP3A4*1G* genotype and ADP-mediated aggregation after clopidogrel treatment was found in healthy subjects [20] and in patients with acute coronary syndrome and PCI [21]. With respect to *CYP3A5* there are at least two studies available investigating systematically the contribution on ADP-induced aggregation in healthy volunteers [21] and patients undergoing

PCI [37]. In concordance with our data, *CYP3A5* did not explain variability in clopidogrel response as found by those authors.

Taken together, whereas the evidence is conclusive that the *CYP2C19*2* polymorphism is significantly associated with reduction of ADP-induced platelet aggregation (present study, [9,20,21,23]), the contribution of *CYP3A4/5* to clopidogrel response remains open. Interestingly, it was shown that inhibition of *CYP3A4/5* by ketoconazole, the most potent inhibitor of *CYP3A*, significantly affects formation of the active metabolite of clopidogrel in healthy volunteers [38]. Since ketoconazole also inhibits *CYP2C19* even if to a smaller extent, additional data are required.

The concept that inheritance can have an important role in individual variation of drug response is complemented by the fact that, additionally, nongenetic factors such as age, disease status, organ function, comedication and so on, may influence drug efficacy [39,40]. Recently, we demonstrated that five nongenetic weighted variables (PREDICT-score: age >65 years, Type 2 diabetes mellitus, decreased left ventricular function, renal failure and acute coronary syndrome) render patients more susceptible to high RPA, consequently resulting in an increased risk for poor response to clopidogrel treatment [8]. Thus, an important finding of the present study is the fact that our multivariable logistic regression analysis showed an even higher association with high RPA for the combination of nongenetic risk factors (PREDICT-score) and the *CYP2C19*2* genotype ($\chi^2 = 25.85$; $p < 0.0001$). These data indicate that clopidogrel nonresponsiveness depends on both genetic and nongenetic (i.e., physiological/pathophysiological) factors. Finally, we developed a nomogram (Figure 2) based on our dichotomous logistic regression model, which includes both genetic and nongenetic factors to individually estimate the probability that a given patient will be susceptible to high RPA after first treatment with a 600-mg clopidogrel loading dose. For each factor the appropriate number of points is determined and the total sum of points is transformed into the estimated probability (see results section). The nomogram underscores one of the important findings of our study, namely that genetic and nongenetic factors may contribute to poor responsiveness of clopidogrel loading dose. However, before translation of our nomogram into practical clinical use

prospective clinical trials, including representative large patient cohorts, are warranted with particular interest in antithrombotic outcome under long-term clopidogrel therapy.

Conclusion

In summary, antithrombotic therapy in patients after coronary stenting is well established, but long-term major cardiovascular events occurred in up to 10% of patients, despite dual therapy with aspirin and clopidogrel (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance [CHARISMA] trial) [41]. There is substantial need, particularly in high-risk patients with vascular disease or multiple risk factors, to optimize antithrombotic therapy and to identify patients with poor responsiveness before initiating clopidogrel therapy. Thus, clopidogrel therapy tailored on the basis of pharmacogenetic knowledge, including the individual *CYP2C19*2* genotype, but also considering nongenetic factors such as age

over 65 years, Type 2 diabetes mellitus, decreased left ventricular function, renal failure and acute coronary syndrome, offers a novel dimension in antithrombotic therapy.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Clinical outcome in patients undergoing percutaneous coronary intervention is significantly improved if patients are pretreated with a loading dose of clopidogrel, an inhibitor of platelet aggregation.
- Due to a marked interindividual variability in clopidogrel-induced inhibition of platelet aggregation, up to 30% of all patients do not achieve a sufficient antiplatelet effect. In a recent large-scale study, nongenetic factors were identified for better prediction of patients with high residual platelet activity after coronary stenting and clopidogrel therapy.
- In the present study, we investigated the impact of functional polymorphisms in the drug-metabolizing enzymes CYP2C19 and CYP3A4/5 on clopidogrel responsiveness after a loading dose of 600 mg. Carriers of the *CYP2C19*2* variant demonstrated significantly increased residual-platelet activation (RPA) compared with noncarriers.
- Multivariable logistic regression analyses showed that *CYP2C19*2* genotype contributes predominately to high RPA in comparison with nongenetic risk factors. For the combination of both, an even higher association was found.
- These findings indicate that poor responsiveness after clopidogrel loading dose depends on both genetic and nongenetic factors and may be improved by genotyping for *CYP2C19*2*.

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