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Mutational analysis clopidogrel resistance and platelet function in patients scheduled for coronary artery bypass grafting

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ABSTRACT

Clopidogrel is an oral antiplatelet pro-drug prescribed to 40 million patients worldwide who are at risk for thrombotic events or receiving percutaneous coronary intervention (PCI). However about a fifth of patients treated with clopidogrel do not respond adequately to the drug. From a cohort of 105 patients on whom we had functional data on clopidogrel response, we used ultra-high throughput sequencing to assay mutations in *CYP2C19* and *ABCB1*, the two genes genetically linked to respond. Testing for mutations in *CYP2C19*, as recommended by the FDA, only correctly predicted if a patient would respond to clopidogrel 52.4% of the time. Similarly, testing of the *ABCB1* gene only correctly foretold response in 51 (48.6%) patients. These results are clinically relevant and suggest that until additional genetic factors are discovered that predict response more completely, functional assays are more appropriate for clinical use.

1. Introduction

Clopidogrel is an oral antiplatelet pro-drug prescribed to 40 million patients worldwide who are at risk for thrombotic events or receiving percutaneous coronary intervention (PCI) [1]. Many studies have demonstrated the efficacy of its use in addition to aspirin to prevent death, myocardial infarction, and stroke in patients with coronary syndromes. The FDA currently recommends clopidogrel as a therapy administered once daily for 365 days for patients undergoing PCI [1,2]. Clopidogrel has an irreversible antagonistic effect on the adenosine diphosphate (ADP) P2Y12 receptor and has been shown to significantly improve clinical outcomes in patients who respond to the medication [2–5]. However, the response to clopidogrel varies. About a fifth of patients treated with clopidogrel do not respond adequately due to decreased absorption, inadequate conversion to its active metabolite, or other reasons such as increased body mass index, diabetes mellitus, and acute coronary syndrome [6,7]. Interactions with other medications, such as the proton pump inhibitor omeprazole, have been also identified as responsible for the lack of effectiveness of clopidogrel [1].

Clopidogrel is administered orally, absorbed through the intestine, and then metabolized to its active form in the liver by cytochrome P450 enzymes in a two-step process [6,7]. Two genes, *ABCB1* and *CYP2C19*, have been identified as important players in this process. *ABCB1* encodes the P-glycoprotein efflux transporter, which is responsible for absorbing clopidogrel from the intestine [6–8]. *CYP2C19* (Cytochrome P450 2C19) encodes a member of the cytochrome P450 superfamily of enzymes which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids.

The *CYP2C19*2* (G681A) and *ABCB1* (C3435T) variants are the most common loss-of-function mutations described in the literature [1,9,10]. Patients with these variants are classified as low- or non-responders to clopidogrel and tend to have higher platelet reactivity, which places them at risk for adverse cardiovascular events [6,8,10–12]. However, the Genotype Information and Functional Testing (GIFT) study has cited *CYP2C19*2*, not *ABCB1* or other variants, as the crucial gene variant that significantly influences the reactivity and pharmacodynamics of clopidogrel in patients who have undergone PCI [10]. Nevertheless, Price and colleague note that this piece of genetic data provides only limited utility in patients who exhibit the potential for high adverse reactivity to clopidogrel [10].

A patient's likely functional response to clopidogrel can be assessed by pre-administration genetic testing. There are two bedside genetic tests currently used, but they require long wait times and are tested only for specific allele variations; the *CYP2C19* genotype has been shown to account for just 12% of the variation of clopidogrel response [9]. For these reasons, a functional platelet inhibition test (PIT) has become a more practical choice in clinical practice. The VerifyNow P2Y12 assay (Accumetrics, San Diego, CA) uses whole blood to measure

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aggregation of platelets to fibrogen-coated beads in response to exposure to prostaglandin E1 and ADP [7,10,13]. Patients with VerifyNow scores >240 P2Y12 reaction units (PRU) have a higher risk for cardiovascular-related death, stent thrombosis, and non-fatal myocardial infarction [3–5,14]. This >240 PRU cut-off is comparable to the established thresholds for other PITs such as light transmittance aggregometry, Plateletworks, and Innovance PFA P2Y assays (Breet, van Werkum et al., 2010). However, there is only a modest correlation between these tests and cardiovascular (CV)-related death, stent thrombosis, or non-fatal MI [3,5].

In a previous study, we demonstrated the benefit of using a PIT (without genetic testing) to guide the timing of coronary surgery for patients receiving clopidogrel [15]. Since then, genetic testing has become widely available and endorsed by the FDA [16]. With this current study, our goal was to validate in our surgical series of patients a genetic screen with a functional PIT (VerifyNow) to ascertain the true yield of these new assays against a functional test.

2. Results

Of the 105 patients analyzed, 71 (67.62%) were deemed non-responders by the VerifyNow Assay. Non-responders were more likely to female, older, not current smokers, diabetics, with a history of renal failure and taking beta blockers and ADP inhibitors (Table 1).

The genome sequencing mutation detection results are presented in Table 2. Overall, 17 patients (16.2%) had no mutations in either *ABCB1* or *CYP2C19*. Of these, 7 responded to the drug and 10 were non-responders. Mutations were common in the *ABCB1* gene, with 76.2% of patients having the C3435T variation. About a third of patients (32.4%) had a loss-of-function mutation in *CYP2C19*, with a majority of these showing the **2* variant (G636A) (29.5%) compared to the 1.9% and 1.0% of **4* (C1297T) and **8* (T358C) variants, respectively. 23 patients had mutations in both genes. We were unable to determine frequency of **17* variants (T-808C) as the mutation occurs in an intron and our experimental design assayed only the exons.

Twenty-four of the 34 patients with a *CYP2C19* loss-of-function mutation were non-responders ($p = 0.414$). Similarly, of the 80 patients with a loss-of-function mutation in *ABCB1*, 55 were non-responders ($p = 0.483$). *CYP2C19* mutations were found in 10 (29.41%) of responders and in 24 (33.80%) of non-responders. The C3435T *ABCB1* variant was found in 25 (73.53%) responders and in 55 (77.46%) non-responders. A chi-square analysis showed non-significance ($p = 0.781$).

3. Discussion

There has been great interest in surveying genetic variants to predict potential response to clopidogrel. A number of commercial tests have been developed that focus on two genes, *CYP2C19* and *ABCB1*,

Table 1
Patient demographics.

	All (n = 105)		Responder (n = 34)		Non-responder (n = 71)		p-Value
Male	81	77.4%	33	97.1%	48	67.6%	0.001
Caucasian	100	95.2%	33	97.1%	67	94.4%	0.544
Age	68.3	11.3	62.7	8.8	71.0	11.3	0.0001
Weight (kg)	85.2	17.3	86.6	20.7	84.6	15.5	0.577
Height (cm)	172.4	9.9	176.3	8.8	170.6	10.0	0.006
Body surface area	2.01	0.24	2.05	0.28	2.00	0.22	0.323
Body mass index	28.62	4.95	27.77	5.81	29.03	4.47	0.224
History of Smoking	59	56.2%	22	64.7%	37	52.1%	0.224
Currently smoking	21	20.0%	13	38.2%	8	11.3%	0.001
Present comorbidity							
Diabetes	41	39.1%	9	26.5%	32	45.1%	0.052
Dyslipidemia	98	93.3%	33	97.1%	65	91.6%	0.290
Creatine	1.08	0.5	0.98	0.2	1.14	0.6	0.138
Renal failure	14	13.3%	1	2.9%	13	18.3%	0.024
Dialysis	2	1.9%	0	0.0%	2	2.8%	0.323
Hypertension	96	91.4%	32	94.1%	64	90.1%	0.496
Endocarditis	0	0.0%	0	0.0%	0	0.0%	1.000
Chronic lung disease		9.5%	3	8.8%	7	9.9%	0.785
Immuno-suppressed	2	1.9%	1	2.9%	1	1.4%	0.591
Peripheral vascular disease	11	10.5%	3	8.8%	8	11.3%	0.702
Pacemaker	2	1.9%	1	2.9%	1	1.4%	0.591
Cardiac History							
Myocardial infarction	55	52.4%	21	61.8%	34	47.9%	0.183
Cerebrovascular accident	3	2.9%	1	2.9%	2	2.8%	0.971
Cardiovascular disease	11	10.5%	3	8.8%	8	11.3%	0.702
Percutaneous coronary intervention	35	33.3%	14	41.2%	21	29.6%	0.238
Chronic heart failure	15	14.3%	2	5.9%	13	18.3%	0.089
Angina	88	83.8%	28	82.4%	60	84.5%	0.779
Arrhythmia	11	10.5%	3	8.8%	8	11.3%	0.702
Medications							
Beta blockers	98	93.3%	30	88.2%	68	95.8%	0.039
ACE inhibitors	53	50.5%	19	55.9%	34	47.9%	0.433
Statins	91	86.7%	29	85.3%	62	87.3%	0.229
Nitrates	6	5.7%	3	8.8%	3	4.2%	0.342
Heparin	44	41.9%	16	47.1%	28	39.4%	0.532
Steroids	1	1.0%	0	0.0%	1	1.4%	0.487
Aspirin	82	78.1%	26	76.5%	56	78.9%	0781
ADP inhibitors	21	20.0%	3	8.8%	18	25.4%	0.048
Glycoprotein IIbIIIa inhibitor	2	1.9%	1	2.9%	1	1.4%	0.591
# of diseased vessels	2.9	0.4	2.9	0.4	2.9	0.3	0.789
Left main disease	56	53.3%	16	47.1%	40	56.3%	0.372
Ejection fraction	52.7	11.4	53.5	10.5	52.3	11.9	0.624
30-day mortality	1	0.95%	0	0.00%	1	1.41%	0.487
Late mortality	8	8.6%	1	2.9%	7	11.3%	0.211
Total mortality	9	9.5%	1	2.9%	8	12.5%	0.154