Association of single nucleotide polymorphism in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Korean

Lee, Jung Myung

Department of Medicine

The Graduate School, Yonsei University

Association of single nucleotide polymorphism in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Korean

Lee, Jung Myung

Department of Medicine

The Graduate School, Yonsei University

Association of single nucleotide polymorphism in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Korean

Directed by Professor Yangsoo Jang

The Master's thesis submitted to the

Department of Medicine,

the Graduate School of Yonsei University

in partial fulfillment of the requirements for the

degree of Master of Medical Science

Lee, Jung Myung

June 2008

This certifies that the Master's Thesis of Jung Myung Lee is approved.

Thesis Supervisor : Yangsoo Jang

Sungha Park

Jin Sung Lee

The Graduate School

Yonsei University

June 2008

Acknowledgements

I would like to thank professor Yangsoo Jang who gave me the opportunity to participate in such a great project and always provided me with good and thorough ments with deep sincerity. I am in debt to professor Sungha Park and Jin Sung Lee who gave me many great advices during this study. In addition, I am especially thanks to Dong Jik Shin who was an excellent helper about the pharmacogenetics. I express my greatest love to my parents who always pray for me. Finally I dedicate this master's thesis to my shepherd, the LORD almighty.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Association of single nucleotide polymorphism in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Korean

Lee, Jung Myung

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Yangsoo Jang)

Introduction: Clopidogrel is a prodrug that inhibits adenosine-induced platelet aggregation by blocking the P2Y12 receptor. Clopidogrel requires metabolic activation by hepatic cytochrome P450 (CYP) isoenzymes. The mechanisms responsible for clopidogrel resistance are incompletely defined. Differences in hepatic conversion to the active metabolite are thought to be an important factor. Recent studies suggested that cytochrome P450 2C19*2

polymorphism has significant association with clopidogrel resistance. In addition, there are some reports that SNPs (single nucleotide polymorphisms) of CYP3A4, CYP3A5 showed significant association with clopidogrel resistance. Most of the studies were done in the western population. In this study, we sought to determine the association of polymorphisms of CYP gene with clopidogrel resistance in Korean.

Methods: From October 2006 to July 2007, 450 patients who underwent successful percutaneous coronary intervention with drug-eluting stent were randomly assigned to treatment with dual antiplatelet regimen (aspirin plus clopidogrel) or triple antiplatelet regimen (aspirin plus clopidogrel plus cilostazol). Clopidogrel resistance checking and genetic analysis were fulfilled in 383 patients. Clopidogrel resistance was defined as % inhibition of less than 20% by VeryfyNow P2Y12 assay. The genotyping of 10 SNPs including COX2 rs5277, CYP3A5*3 rs776747, CYP1A1 rs1048943, CYP2C19*2 rs4244285, CYP2C19*3 rs4986893, CYP1A2 rs2470890,

CYP3A4 rs2242480, CYP3A4 rs2246709, CYP2J2 rs2280274, and P2RY12 were screened and statistic analysis was done.

Results: Clopidogrel resistance was found in 111 patients (29.0%). No significant differences in age, sex, body mass index, history of diabetes mellitus, history of hypertension, and smoking status were seen between clopidogrel resistant group and clopidogrel responsive group. In clopidogrel responsive group, there was a significant higher proportion of cilostazol use. Because cilostazol influence clopidogrel resistance significantly, we examined the association of SNPs and clopidogrel resistance in dual antiplatelet therapy group and triple antiplatelet group, respectively. In both dual and triple antiplatelet subjects, CYP2C19*3 demonstrated a significantly higher proportion of minor allele in clopidogrel resistant group compared with clopidogrel responsive group. Multiple logistic regression analysis demonstrated that CYP2C19*3 rs4986893 SNP is an independent predictor of clopidogrel resistance.

Conclusion: CYP2C19*3 SNP is an independent risk factor of clopidogrel resistance in Korean subjects with coronary artery disease. The results from this study suggested that CYP2C19*3 may have a more important role than CYP2C19*2 in the metabolism of clopidogrel in Koreans. Further investigation is needed to clarify whether the increased clopidogrel resistance in subjects with CYP2C19*3 minor allele polymorphism translates into increased cardiovascular outcomes.

Key words : cytochrome P450, cytochrome 2C19*3, single nucleotide polymorphism, clopidogrel resistance, drug-eluting stent

Association of single nucleotide polymorphism in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Korean

Lee, Jung Myung

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Yangsoo Jang)

I. INTRODUCTION

Clopidogrel is a thienopyridine prodrug used clinically to inhibit adenosine diphosphate(ADP)-induced platelet aggregation by irreversibly blocking the P2Y12 receptor[1]. Clopidogrel requires oxidation by hepatic cytochrome P450 (CYP) to generate a metabolite that is an active inhibitor of ADP-induced platelet aggregation[2]. CYP catalyzes the oxidation of the thiophene ring of

clopidogrel to 2-oxoclopidogrel. The 2-oxo-intermediate is then oxidized further by CYP. The second oxidation results in opening of the thiophene ring to form both a carboxyl and a thiol group[3]. When the thiol group forms a disulfide bond with the $P2Y_{12}$ ADP-receptor on platelets, the ADP cannot bind to the covalently modified receptor, which normally activates the glycoprotein GPIIb/IIIa complex that binds fibrinogen and initiate blood clot formation[3]. Since clopidogrel and aspirin inhibit platelet aggregation through different pathways, combined antiplatelet therapy provides additive benefits compared to either agent alone[4]. Dual antiplatelet therapy with clopidogrel and aspirin is considered the "gold standard" for attenuation of platelet activation and aggregation in patients undergoing stenting[5].

Response variability and nonresponsiveness to clopidogrel have been demonstrated in patients following coronary stenting[6]. The prevalence of clopidogrel nonresponsiveness has been reported at 5–44%[7-15]. This variation in prevalence is due to various dosing, definitions, laboratory methods, and

the time at which blood samples were drawn [4, 11, 16-18]. There are some reports that link clopidogrel resistance to the occurrence of thrombotic events. Matetzky et al demonstrated that patients who exhibited the highest quartile of ADP-induced aggregation had a 40% probability for a recurrent cardiovascular event within 6 months in patients undergoing stenting for acute ST-elevation myocardial infarction[11]. Other investigators also reported that high platelet reactivity despite currently recommended antiplatelet therapy is a risk factor for ischemia in patients undergoing percutaneous coronary intervention[19-22].

The mechanisms responsible for clopidogrel resistance are incompletely defined. Differences in intestinal absorption, hepatic conversion to the active metabolite through CYP activity, and platelet receptor polymorphisms have been suggested[23-31]. Lau et al demonstrated that pharmacologic stimulation of CYP3A4 activity enhances the effect of clopidogrel, whereas competitive inhibitor of CYP 3A4 attenuate the effect of clopidogrel[29, 32]. Hulot et al,

Giusti et al, and Frere et al demonstrated that CYP 2C19*2 polymorphism is responsible for clopidogrel resistance[23, 25, 30]. All of the above data suggest the contribution of hepatic CYP metabolic activity to clopidogrel non-responsiveness. However, most of the studies have been done in the western population with paucity of data in the oriental population at the present. In this study, we sought to determine the association of polymorphisms of CYP gene with clopidogrel resistance in subjects undergoing coronary angioplasty and stent insertion.

II. MATERIALS AND METHODS

A. Patient population

From October 2006 to July 2007, 450 consecutive patients who underwent successful percutaneous coronary intervention (PCI) with drug-eluting stents (DES) were randomly assigned to treatment with dual anti-platelet regimens (aspirin plus clopidogrel, n = 225) or triple antiplatelet regimens (aspirin plus clopidogrel plus cilostazol, n = 225) after successful coronary stenting.

Inclusion criteria were: symptomatic coronary artery disease or documented myocardial ischemia (by treadmill exercise testing or sestamibi scan); angiographic evidence of ≥ 50% diameter stenosis and post procedure Thromobolysis In Myocardial Infarction (TIMI) flow grade 3. Exclusion criteria were: contraindication to antiplatelet agents; previous allergy or intolerance of aspirin or clopidogrel; treatment with warfarin; active bleeding; known platelet dysfunction; abnormal platelet count (< 100,000/mm3). Patients received a 300mg loading dose of clopidogrel at least 12 hours before the stenting. Stents were deployed according to standard techniques. The maintenance dose for each antiplatelet agent was 100 mg once a day for aspirin, 75 mg once a day for clopidogrel, and 100 mg twice a day for cilostazol. Among the enrolled patients, 383 patients (Group I, n = 180; Group II, n = 203) could be checked for clopidogrel resistance by VeryfyNow-P2Y12, and blood sampling for genetic analysis was done in these patients.

B. Laboratory evaluation of clopidogrel resistance

VerifyNow P2Y12 (Accumetrics, San Diego, California) is a rapid platelet-function, cartridge based assay designed to directly measure the effects of clopidogrel on the P2Y12 receptor. VerifyNow P2Y12 is more sensitive than ADP-induced platelet aggregometry and enhances specificity for the P2Y12

receptor by using prostaglandin E1 to attenuate P2Y1 activation[33].

Results are expressed as P2Y12 reaction units (PRU) and percentage inhibition. PRU reports the amount of P2Y12 receptor mediated aggregation. Percentage inhibition ((1-PRU/baseline PRU)x100) is the percent change from baseline aggregation and is calculated from the PRU result and the "estimated baseline" result, which is an independent measurement based on the rate and extent of platelet aggregation in the TRAP (Thrombin Receptor Activating Peptide) channel[34]. VeryfyNow P2Y12 assay's usefulness in evaluating clopidogrel responsiveness is demonstrated in various studies[33-36]. The percent inhibition (%) of < 20% indicates the absence of clopidogrel-induced platelet dysfunction and was defined as clopidogrel resistance.

C. Genotyping

The genotyping of 7 SNPs including cycloxygenase2 (COX2) rs5277, CYP3A5*3 rs776747, CYP1A1*3 rs1048943, CYP2C19*2 rs4244285, CYP2C19*3 rs4986893, CYP1A2 rs2470890, CYP3A4 rs2242480 were screened using single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommendation. Briefly, the genomic deoxyribonucleic acid (DNA) flanking the SNPs were amplified with polymerase chain reaction (PCR) with forward

and reverse primer pairs and standard PCR reagents in 10 micro liter reaction volume, containing 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1 micro liter of 10X PCR Gold buffer, 250μM dNTP, 3mM $MgCl_2$ and 0.25 unit i-StarTaq DNA Polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). The PCR reactions were carried out as follows: 10 min at 95℃ for 1 cycle, and 30 cycles on 95℃ for 30s, 55℃ (COX2 rs5277, CYP1A1 rs1048943), 60℃ (CYP2C19*2, CYP2C19*3), 65℃ (CYP3A5*3) for 1min, respectively, and 72℃ for 1min followed by 1 cycle of 72℃ for 7mins. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche) and exonuclease I (USB Corporation) at 37℃ for 60 minutes and 72℃ for 15 minutes to purify the amplified products. One micro liter of the purified amplification products were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15pmols of genotyping primer for primer extension reaction. The primer extension reaction was carried out for 25cycles of 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30 seconds. The reaction products were treated with 1 unit of SAP at 37℃ for 1 hour and 72℃ 15 minutes to remove excess fluorescent dye terminators. One micro liter of the final reaction samples containing the extension products were added to 9 micro liter of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95℃ for 5 min, followed by 5min on ice and then analyzed by electrophoresis in

ABI Prism 3730xl DNA analyzer. Results were analyzed using GeneScan analysis software (ABI, Foster City, CA). Primer sequences used in this study are shown in Table 1

Table 1. Oligonucleotide primers for the genotyping of CYP polymorphisms

Gene	SNP name	Rs number	Primer sequence	
CYP1A1	I462V	rs1048943	Forward primer	GTGATTATCTTTGGCATGG
			Reverse primer	TTGCAGCAGGATAGCCAG
			Genotyping primer	AAAGACCTCCCAGCGGGCAA
CYP1A2	N516N	rs2470890	Forward primer	CGACCTGACCCCCATCTAC
			Reverse primer	GGAAGAGAAACAAGGGCTGA
			Genotyping primer	CCTCAGAATGGTGGTGTCTTCTTCA
*CYP2C19*2*	681G/A	rs4244285	Forward primer	GGCATATTGTATCTATACCTTTATTAAATG
			Reverse primer	GAGGGTTGTTGATGTCCATC
			Genotyping primer	TTTTAAGTAATTTGTTATGGGTTCC
*CYP2C19*3*	W212X	rs4986893	Forward primer	AGCAATTTCTTAACTTGATGGAAAAA
			Reverse primer	GGATTTCCCAGAAAAAAAGACTG
			Genotyping primer	GCAAAAAACTTGGCCTTACCTGGAT
CYP3A4	IVS10+1G/A	rs2242480	Forward primer	CCAGCAGAAACTGCAGG
			Reverse primer	GAGTCAGTGAAAGAATCAGTGATT
			Genotyping primer	TACCCAATAAGGTGAGTGGATG
*CYP3A5*3*	A6986G(22893A/G)	rs776746	Forward primer	CGTTCTGTGTGGGGACAAC
			Reverse primer	GCCCATACAGGCAACATGA
			Genotyping primer	GAGCTCTTTTGTCTTTCA
COX2	V102V	rs5277	Forward primer	GCGATTGTACCCGGACAG
			Reverse primer	TTGGCGATTAAGATGGAAGG
			Genotyping primer	TTCGAAATGCAATTATGAGTTATGT

The genotyping of CYP3A4 rs2246709, CYP2J2 rs2280274, P2RY12 were screened using the TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA). The final volume of polymerase chain reaction (PCR) was 5ul, containing 10ng of genomic DNA and 2.5ul TaqMan Universal PCR Master Mix, with 0.13ul of 40X Assay Mix (Assay ID C_1845287_10 for CYP3A4, C_1917976_1 for CYP2J2, C_1941752_10 for P2RY12). Thermal cycle conditions were as follows: 50℃ for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95℃ for 10 min to activate the DNA polymerase, followed by 45 cycles of 95℃ for 15 s and 60℃ for 1 min. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA) and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

D. Statistical Analysis

Values were expressed as mean ± SD. χ^2 test for goodness of fit was used to verify agreement with Hardy-Weinberg equilibrium using Fisher's exact test. Comparison of discrete variables was performed using the Chi-square analysis or Fisher's exact test. Comparison of continuous variables between the two

study groups was performed using the Student's t-test. Multivariate logistic regression analysis was performed to determine the independent association of CYP gene polymorphism with clopidogrel resistance. Statistical analysis was performed with SPSS 15.0 (SPSS Inc, Chicago, Il., USA).

III. RESULTS

A. Baseline characteristics

CYP genotypes and clopidogrel resistance of 383 coronary artery disease patients with drug-eluting stent insertion were analyzed in this study. Clopidogrel resistance was found in 111 patients (29.0%). Baseline characteristics of the studied population are shown in table 2.

Table 2. Baseline characteristics of the whole population (n=383)

characteristic	value
Age (years)	61.1±10.2
Body mass index (kg/m^2)	24.8±3.0
Men	278(72.6%)
Clopidogrel resistance	111(29.0%)
Diabetes mellitus	104(27.2%)
Hypertension	153(39.9%)
Old cerebrovascular accident	7(1.8%)
Smoker	135(35.2%)

B. Comparing between clopidogrel resistant and responsive group

The population was divided into two groups according to the presence of clopidogrel resistance assessed by VeryfyNow-P2Y12 assay. No significant differences in age, sex, body mass index (BMI), history of diabetes mellitus, history of hypertension, and smoking status were seen between clopidogrel resistant group and clopidogrel responsive group. In clopidogrel responsive group, there was a significant higher proportion of cilostazol use (Table 3). It is a consistent finding with previous report that addition of cilostazol to conventional dual antiplatelet regimen can attenuate clopidogrel resistance[37].

Table 3. Characteristics of clopidogrel resistant and responsive groups

Characteristics	no resistance	resistance	
Age (years)	61±10.4	61.4±9.7	0.695
Body mass index (kg/m^2)	24.7±3.0	25.0±3.1	0.387
Men	196(72.3%)	82(74.5%)	0.658
Diabetes mellitus	80(30.2%)	24(22.4%)	0.131
Hypertension	113(42.6%)	40(37.4%)	0.351
Old cerebrovascular accident	6(2.3%)	1(0.9%)	0.678
Smoker	101(38.1%)	34(31.8%)	0.25
Cilostazol	160(58.8%)	43(38.7%)	0.0003

C. **Association of clopidogrel resistance and SNP**

Genetic distribution of the 10 SNPs are shown in table 4-1. The distribution of the genetic polymorphisms did not deviate significantly from the Hardy-Weinberg equilibrium, except for CYP2C19*2 (P=0.141). Repetition of genotyping was done and did not find genotyping error. Among the 10 SNPs, CYP2C19*3(rs4986893) polymorphism demonstrated a significantly higher proportion of minor allele in the clopidogrel resistant group compared with responsive group (GG:GA:AA = 235:35:1 vs. 79:31:1, respectively, P=0.001). Because the cut-off value of clopidogrel resistance was not exactly defined by previous studies, t-test was done for comparing of % inhibition according to each genotype (table 4-2). Dominant model of CYP1A1(rs1048943), and CYP2C19*2(rs4244285) polymorphism showed significant difference in % inhibition. CYP19*3(rs4986893) polymorphism demonstrated significantly different % inhibition in codominant and dominant model.

Table 4-1. Gene variant distribution in clopidogrel resistance and responsive groups

Variant	rs no.			no resistance (n=272)	resistance (n=111)	P value
CYP1A1	rs1048943	Codominant	AA	142	68	0.232
			AG	114	37	
			GG	15	4	
		Dominant	AA	142	68	0.770

			AG/GG	129	41	
		Recessive	AA/AG	256	105	0.605
			GG	15	4	
CYP1A2	rs2470890	Codominant	CC	192	72	0.500
			CT	71	35	
			TT	9	4	
		Dominant	CC	192	72	0.272
			CT/TT	80	39	
		Recessive	CC/CT	263	107	1.000
			TT	9	4	
CYP2C19*2	rs4244285	Codominant	GG	153	54	0.536
			GA	92	40	
			AA	26	13	
		Dominant	GG	153	54	0.292
			GA/AA	118	53	
		Recessive	GG/GA	245	94	0.462
			AA	26	13	
CYP2C19*3	rs4986893	Codominant	GG	235	79	0.001
			GA	35	31	
			AA	1	1	
		Dominant	GG	235	79	<0.001
			GA/AA	36	32	
		Recessive	GG/GA	270	110	0.497
			AA	1	1	
CYP3A4	rs2246709	Codominant	TT	62	23	0.987
			TC	88	31	
			CC	22	8	
		Dominant	TT	62	23	0.883
			TC/CC	110	39	
		Recessive	TT/TC	150	54	0.982

			CC	22	8	
CYP3A4	Rs2242480	Codominant	GG	170	73	0.751
			GA	89	32	
			AA	13	6	
		Dominant	GG	170	73	0.547
			GA/AA	102	38	
		Recessive	GG/GA	259	105	0.798
			AA	13	6	
CYP3A5	rs776746	Codominant	GG	152	61	0.907
			GA	101	40	
			AA	12	6	
		Dominant	GG	152	61	0.951
			GA/AA	113	46	
		Recessive	GG/GA	253	101	0.661
			AA	12	6	
CYP2J2	rs2280274	Codominant	TT	136	50	0.455
			TA	33	10	
			AA	2	2	
		Dominant	TT	136	50	0.852
			TA/AA	35	12	
		Recessive	TT/TA	169	60	0.288
			AA	2	2	
P2RY12	rs2046934	Codominant	TT	176	80	0.249
			TC	87	26	
			CC	8	4	
		Dominant	TT	176	80	0.143
			TC/CC	95	30	
		Recessive	TT/TC	263	106	0.729
			CC	8	4	
COX	rs5277	Codominant	GG	250	103	0.771

	GC	22	8
	CC	0	0

Table 4-2. % inhibition of platelet activity according to genotypes.

Variant	rs no.		Genotype(n)	% inhiition	P value
CYP1A1	rs1048943	Codominant	AA(210)	32.5±22.4	0.074
			AG(151)	37.6±24.3	
			GG(19)	40.2±24.0	
		Dominant	AA(210)	32.5±22.4	0.025
			AG/GG(170)	37.9±24.3	
		Recessive	AA/AG(361)	34.6±23.3	0.310
			GG(19)	40.2±24.0	
CYP1A2	rs2470890	Codominant	CC(264)	34.9±23.2	0.961
			CT(106)	34.2±23.5	
			TT(13)	35.5±26.7	
		Dominant	CC(264)	34.9±23.2	0.832
			CT/TT(119)	34.4±23.8	
		Recessive	CC/CT(370)	34.7±23.3	0.904
			TT(13)	35.5±26.7	
CYP2C19*2	rs4244285	Codominant	GG(207)	37.3±24.5	0.095
			GA(132)	32.5±21.7	
			AA(39)	30.9±21.7	
		Dominant	GG(207)	37.3±24.5	0.033
			GA/AA(171)	32.1±21.6	
		Recessive	GG/GA(339)	35.4±23.5	0.251
			AA(39)	30.9±21.7	
CYP2C19*3	rs4986893	Codominant	GG(314)	37.0±23.4	<0.001
			GA(66)	24.5±20.3	
			AA(2)	17.0±24.0	

		Dominant	GG(314)	37.0±23.4	<0.001
			GA/AA(68)	24.3±2.5	
		Recessive	GG/GA(380)	34.8±23.4	0.283
			AA(2)	17±24.0	
CYP3A4	rs2246709	Codominant	TT(85)	35.3±24.6	0.874
			TC(119)	36.2±22.4	
			CC(30)	37.9±23.4	
		Dominant	TT(85)	35.3±24.6	0.699
			TC/CC(149)	36.5±22.6	
		Recessive	TT/TC(204)	35.8±23.3	0.657
			CC(30)	37.9±23.4	
CYP3A4	Rs2242480	Codominant	GG(243)	34.1±23.4	0.560
			GA(121)	36.6±23.7	
			AA(19)	32.3±20.4	
		Dominant	GG(243)	34.1±23.4	0.433
			GA/AA(140)	36.0±23.2	
		Recessive	GG/GA(364)	34.9±23.5	0.640
			AA(19)	32.3±20.4	
CYP3A5	rs776746	Codominant	GG(213)	34.8±23.2	0.528
			GA(141)	35.4±23.6	
			AA(18)	28.8±21.5	
		Dominant	GG(213)	34.8±23.2	0.947
			GA/AA(159)	34.7±23.4	
		Recessive	GG/GA(354)	35.1±23.3	0.268
			AA(18)	28.8±21.5	
CYP2J2	rs2280274	Codominant	TT(186)	35.8±23.6	0.969
			TA(43)	36.7±21.0	
			AA(4)	37.3±36.7	
		Dominant	TT(186)	35.8±23.6	0.804
			TA/AA(47)	36.8±22.1	

		Recessive	TT/TA(229)	36.0±23.1	0.916
			AA(4)	37.3±36.7	
P2RY12	rs2046934	Codominant	TT(256)	35.1±24.3	0.677
			TC(113)	34.6±20.7	
			CC(12)	29.0±26.5	
		Dominant	TT(256)	35.1±24.3	0.684
			TC/CC(125)	34.1±21.3	
		Recessive	TT/TC(369)	35.0±23.2	0.386
			CC(12)	29±26.5	
COX	rs5277	Codominant	GG(353)	34.7±23.3	0.877
			GC(30)	35.4±23.8	
			CC(0)		

Because cilostazol influent clopidogrel resistance significantly, we examined the association of SNPs and clopidogrel resistance in dual antiplatelet therapy group and triple antiplatelet group, respectively.

In the dual antiplatelet therapy population, CYP2C19*3 polymorphism still demonstrated a significantly higher proportion of minor allele in clopidogrel resistant group compared with responsive group (GG:GA:AA = 97:14:0 vs. 49:19:0, respectively, P=0.01). No significant associations between any other SNPs and clopidogrel resistance were seen. Table 5-1 demonstrates the distribution of genotype in clopidogrel resistant and responsive group received dual antiplatelet therapy. In t-test, CYP2C19*3 showed significantly lower % inhibition in minor allele group than wild type group. CYP2C19*2 showed no difference in % inhibition in dual antiplatelet group.

Table 5-1. Cytochrome gene variant distribution in population received dual antiplatelet therapy

Variant	rs no.			no resistance (n=112)	resistance (n=68)	P value
CYP1A1	rs1048943	Codominant	AA	60	41	0.545
			AG	46	22	
			GG	5	3	
		Dominant	AA	60	41	0.294
			AG/GG	51	25	

		Recessive	AA/AG	106	63	1.000
			GG	5	3	
CYP1A2	rs2470890	Codominant	CC	83	45	0.157
			CT	27	21	
			TT	2	2	
		Dominant	CC	83	45	0.255
			CT/TT	29	23	
		Recessive	CC/CT	110	66	0.634
			TT	2	2	
CYP2C19*2	rs4244285	Codominant	GG	67	34	0.621
			GA	38	26	
			AA	7	5	
		Dominant	GG	67	34	0.330
			GA/AA	45	31	
		Recessive	GG/GA	105	60	0.761
			AA	7	5	
CYP2C19*3	rs4986893	Codominant	GG	97	49	0.01
			GA	14	19	
			AA	0	0	
		Dominant	GG	97	49	0.010
			GA/AA	14	19	
		Recessive	GG/GA	111	68	N/A
			AA	0	0	
CYP3A4	rs2246709	Codominant	TT	22	17	0.552
			TC	35	18	
			CC	7	6	
		Dominant	TT	22	17	0.463
			TC/CC	42	24	
		Recessive	TT/TC	57	35	0.575
			CC	7	6	

CYP3A4	Rs2242480	Codominant	GG	68	44	0.898
			GA	38	21	
			AA	6	3	
		Dominant	GG	68	44	0.592
			GA/AA	44	24	
		Recessive	GG/GA	106	65	1.000
			AA	6	3	
CYP3A5	rs776746	Codominant	GG	62	39	0.936
			GA	41	23	
			AA	7	4	
		Dominant	GG	62	39	0.723
			GA/AA	48	27	
		Recessive	GG/GA	103	62	1.000
			AA	7	4	
CYP2J2	rs2280274	Codominant	TT	53	33	0.651
			TA	11	7	
			AA	0	1	
		Dominant	TT	53	33	0.763
			TA/AA	11	8	
		Recessive	TT/TA	64	40	0.390
			AA	0	1	
P2RY12	rs2046934	Codominant	TT	72	49	0.443
			TC	35	15	
			CC	5	3	
		Dominant	TT	72	49	0.221
			TC/CC	40	18	
		Recessive	TT/TC	107	64	0.997
			CC	5	3	
COX	rs5277	Codominant	GG	106	61	0.243
			GC	6	7	

			CC	0	0

Table 5-2. % inhibition of each genotype in dual antiplatelet group

Variant	rs no.		Genotype(n)	% inhiition	P value
CYP1A1	rs1048943	Codominant	AA(101)	25.6±17.4	0.342
			AG(68)	29.9±19.9	
			GG(8)	21.1±7.5	
		Dominant	AA(101)	25.6±17.4	0.146
			AG/GG(76)	29.8±19.9	
		Recessive	AA/AG(169)	27.4±18.5	0.865
			GG(8)	28.5±21.1	
CYP1A2	rs2470890	Codominant	CC(128)	28.1±18.8	0.438
			CT(48)	25.9±18.6	
			TT(4)	17.3±6.8	
		Dominant	CC(128)	28.1±18.8	0.351
			CT/TT(52)	25.2±18.1	
		Recessive	CC/CT(176)	27.5±18.7	0.280
			TT(4)	17.3±6.8	
CYP2C19*2	rs4244285	Codominant	GG(101)	28.7±18.8	0.616
			GA(64)	26.0±18.2	
			AA(12)	25.4±20.5	
		Dominant	GG(101)	28.7±18.8	0.327
			GA/AA(76)	25.9±18.5	
		Recessive	GG/GA(165)	27.7±18.6	0.688
			AA(12)	25.4±20.5	
CYP2C19*3	rs4986893	Codominant	GG(146)	29.0±18.7	0.003
			GA(33)	24.5±20.3	

			AA(0)		
CYP3A4	rs2246709	Codominant	TT(39)	27.6±23.5	0.578
			TC(53)	27.6±16.6	
			CC(13)	21.7±12.6	
		Dominant	TT(39)	27.6±23.5	0.784
			TC/CC(66)	26.5±16.0	
		Recessive	TT/TC(92)	27.6±19.7	0.294
			CC(13)	21.7±12.6	
CYP3A4	Rs2242480	Codominant	GG(112)	26.9±19.4	0.556
			GA(59)	28.7±18.2	
			AA(9)	21.8±10.6	
		Dominant	GG(112)	26.9±19.4	0.751
			GA/AA(68)	27.8±17.4	
		Recessive	GG/GA(171)	27.5±18.9	0.163
			AA(9)	21.8±10.6	
CYP3A5	rs776746	Codominant	GG(101)	27.3±18.4	0.496
			GA(64)	28.8±19.7	
			AA(11)	21.6±13.0	
		Dominant	GG(101)	27.3±18.4	0.870
			GA/AA(75)	27.7±19.0	
		Recessive	GG/GA(165)	27.9±18.9	0.284
			AA(11)	21.6±13.0	
CYP2J2	rs2280274	Codominant	TT(86)	26.8±18.6	0.504
			TA(18)	28.8±21.3	
			AA(1)	6.0	
		Dominant	TT(86)	26.8±18.6	0.865
			TA/AA(19)	27.8±21.3	
		Recessive	TT/TA(104)	27.1±19.0	0.272
			AA(1)	6.0	
P2RY12	rs2046934	Codominant	TT(121)	26.6±19.1	0.625

			TC(50)	29.4±17.3	
			CC(8)	24.9±20.3	
		Dominant	TT(121)	26.6±19.1	0.465
			TC/CC(58)	28.8±17.6	
		Recessive	TT/TC(171)	27.4±18.6	0.705
			CC(8)	24.9±20.3	
COX	rs5277	Codominant	GG(167)	27.6±18.4	0.316
			GC(13)	22.2±21.0	
			CC(0)		

In the triple antiplatelet therapy group, CYP2C19*3 polymorphism still demonstrated a significantly higher proportion of minor allele in clopidogrel resistant group compared with responsive group (GG:GA:AA = 138:21:1 vs. 30:12:1, respectively, P=0.028). No significant associations between any other SNPs and clopidogrel resistance were seen (Table 6-1). However, the results of the t-test showed significantly less inhibition of platelet in CYP2C19*2 and *3 polymorphism.

Table 6-1. Cytochrome gene variant distribution in population received triple antiplatelet therapy

Variant	rs no.			no resistance (n=160)	resistance (n=43)	P value
CYP1A1	rs1048943	Codominant	AA	82	27	0.358
			AG	68	15	

			GG	10	1	
		Dominant	AA	82	27	0.178
			AG/GG	78	16	
		Recessive	AA/AG	150	42	0.464
			GG	10	1	
CYP1A2	rs2470890	Codominant	CC	109	27	0.780
			CT	44	14	
			TT	7	2	
		Dominant	CC	109	27	0.509
			CT/TT	51	16	
		Recessive	CC/CT	153	41	1.000
			TT	7	2	
CYP2C19*2	rs4244285	Codominant	GG	86	20	0.469
			GA	54	14	
			AA	19	8	
		Dominant	GG	86	20	0.455
			GA/AA	73	22	
		Recessive	GG/GA	140	34	0.230
			AA	19	8	
CYP2C19*3	rs4986893	Codominant	GG	138	30	0.028
			GA	21	12	
			AA	1	1	
		Dominant	GG	138	30	0.011
			GA/AA	22	13	
		Recessive	GG/GA	159	42	0.380
			AA	1	1	
CYP3A4	rs2246709	Codominant	TT	40	6	0.635
			TC	53	13	
			CC	15	2	
		Dominant	TT	40	6	0.459

			TC/CC	68	15	
		Recessive	TT/TC	93	19	0.738
			CC	15	2	
CYP3A4	Rs2242480	Codominant	GG	102	29	0.569
			GA	51	11	
			AA	7	3	
		Dominant	GG	102	29	0.653
			GA/AA	58	14	
		Recessive	GG/GA	153	40	0.444
			AA	7	3	
CYP3A5	rs776746	Codominant	GG	90	22	0.726
			GA	60	17	
			AA	5	2	
		Dominant	GG	90	22	0.612
			GA/AA	65	19	
		Recessive	GG/GA	150	39	0.638
			AA	5	2	
CYP2J2	rs2280274	Codominant	TT	30	14	0.449
			TA	20	6	
			AA	2	2	
		Dominant	TT	30	14	0.634
			TA/AA	22	8	
		Recessive	TT/TA	50	20	0.577
			AA	2	2	
P2RY12	rs2046934	Codominant	TT	104	31	0.566
			TC	52	11	
			CC	3	1	
		Dominant	TT	104	31	0.409
			TC/CC	55	12	
		Recessive	TT/TC	156	42	1.000

			CC	3	1	
COX	rs5277	Codominant	GG	144	42	0.130
			GC	16	1	
			CC	0	0	

Table 6-2. % inhibition of each genotype in triple antiplatelet group

Variant	rs no.		Genotype(n)	% inhiition	P value
CYP1A1	rs1048943	Codominant	AA(109)	38.9±24.5	0.238
			AG(83)	43.9±25.9	
			GG(11)	48.7±23.2	
		Dominant	AA(109)	38.9±24.5	0.113
			AG/GG(94)	44.5±25.5	
		Recessive	AA/AG(192)	41.0±25.2	0.324
			GG(11)	48.7±23.2	
CYP1A2	rs2470890	Codominant	CC(136)	41.4±25.0	0.963
			CT(58)	41.2±25.0	
			TT(9)	43.7±28.4	
		Dominant	CC(136)	41.4±25.1	0.977
			CT/TT(67)	41.5±25.2	
		Recessive	CC/CT(194)	41.3±25.0	0.787
			TT(9)	43.7±28.4	
CYP2C19*2	rs4244285	Codominant	GG(106)	45.4±26.5	0.040
			GA(68)	38.6±23.0	
			AA(27)	33.3±25.1	
		Dominant	GG(106)	45.4±26.5	0.018
			GA/AA(95)	37.0±22.8	
		Recessive	GG/GA(174)	42.8±25.3	0.069
			AA(27)	33.3±22.2	
CYP2C19*3	rs4986893	Codominant	GG(168)	43.9±24.9	0.006

			GA(33)	30.4±22.9	
			AA(2)	17.0±24.0	
		Dominant	GG(168)	43.9±24.9	0.002
			GA/AA(35)	29.6±22.8	
		Recessive	GG/GA(201)	41.7±25.0	0.167
			AA(2)	17±24.0	
CYP3A4	rs2246709	Codominant	TT(46)	41.8±23.8	0.451
			TC(66)	43.1±24.2	
			CC(17)	50.2±23.8	
		Dominant	TT(46)	41.8±23.8	0.535
			TC/CC(83)	44.6±23.9	
		Recessive	TT/TC(112)	42.6±32.9	0.217
			CC(17)	50.2±22.3	
CYP3A4	Rs2242480	Codominant	GG(131)	40.2±24.9	0.606
			GA(62)	44.1±25.9	
			AA(10)	41.8±25.1	
		Dominant	GG(131)	40.2±24.9	0.334
			GA/AA(72)	43.8±25.4	
		Recessive	GG/GA(193)	41.4±25.2	0.964
			AA(19)	41.8±22.9	
CYP3A5	rs776746	Codominant	GG(112)	41.6±24.9	0.974
			GA(77)	40.9±25.2	
			AA(7)	40.1±28.0	
		Dominant	GG(112)	41.6±24.9	0.828
			GA/AA(84)	40.8±25.3	
		Recessive	GG/GA(189)	41.3±25.0	0.902
			AA(7)	40.1±28.0	
CYP2J2	rs2280274	Codominant	TT(100)	43.7±24.8	0.933
			TA(25)	42.3±19.2	
			AA(3)	47.7±37.0	

		Dominant	TT(100)	43.7±24.8	0.905
			TA/AA(28)	43.0±20.8	
		Recessive	TT/TA(125)	43.4±23.7	0.762
			AA(3)	47.7±37.0	
P2RY12	rs2046934	Codominant	TT(135)	42.7±26.0	0.565
			TC(63)	38.8±22.3	
			CC(4)	37.2±38.6	
		Dominant	TT(135)	42.7±26.0	0.287
			TC/CC(67)	38.7±23.2	
		Recessive	TT/TC(198)	41.4±24.9	0.742
			CC(4)	37.3±38.6	
COX	rs5277	Codominant	GG(186)	41.1±25.4	0.491
			GC(17)	45.5±5.1	
			CC(0)		

Finally, multiple logistic regression analysis was performed for the 10 SNPs after controlling for age, sex, history of diabetes, smoking status, cilostazol use, and BMI. The results demonstrated that CYP2C19*3 rs4986893 SNP is an independent predictor of clopidogrel resistance. Cilostazol attenuates clopidogrel resistance significantly as previously reported (Table 7).

Table 7. Multiple logistic regression analysis of the association of various gene polymorphisms with clopidogrel resistance

	Dominant		Codominant		Recessive	
	OR	P value	OR	P value	OR	P value
CYP1A1 rs1048943	0.666 (0.411-1.079)	0.099	0.707 (0.467-1.068)	0.1	0.669 (0.210-2.126)	0.669
CYP1A2 rs2470890	1.588 (0.860-2.933)	0.139	1.554 (0.906-2.665)	0.110	2.188 (0.424-11.282)	0.349
CYP2C19*2 rs4244285	1.441 (0.893-2.325)	0.135	1.315 (0.927-1.866)	0.124	1.505 (0.702-3.222)	0.293
CYP2C19*3 rs4986893	2.510 (1.415-4.452)	0.002	2.462 (1.426-4.250)	0.001	5.839 (0.333-102.264)	0.227
CYP3A4 rs2246709	0.967 (0.508-1.840)	0.919	0.991 (0.621-1.582)	0.970	1.026 (0.414-2.540)	0.957
CYP3A4 rs2242480	1.059 (0.581-1.932)	0.851	1.183 (0.715-1.958)	0.513	2.584 (0.661-10.104)	0.172
CYP3A5 rs776746	0.947 (0.586-1.530)	0.823	0.982 (0.660-1.462)	0.982	1.126 (0.397-3.194)	0.823
CYP2J2 rs2280274	1.146 (0.530-2.479)	0.730	1.275 (0.651-2.494)	0.478	4.152 (0.507-34.025)	0.185
P2RY12 rs2046934	0.655 (0.390-1.100)	0.110	0.698 (0.443-1.098)	0.12	0.730 (0.182-2.931)	0.657
COX rs5277	0.864 (0.360-2.076)	0.864	0.864 (0.360-2.076)	0.864	N/A	N/A

IV. DISCUSSION

The primary finding from this study was that CYP2C19*3 polymorphism significantly affected clopidogrel resistance in patients with coronary disease treated with coronary angioplasty and DES implantation. We found that CYP2C19*3 minor allele carriers had a higher proportion of clopidogrel resistance. The association remained significant after adjustment for other clinical factors such as age, sex, BMI, smoking status, and history of diabetes. Multiple logistic regression demonstrated that it is the independent predictor of clopidogrel resistance. CYP2C19*2 did not showed significance in the chi-square analysis based on the assumption of the cut off value of clopidogrel resistance as less than 20% inhibition. However, in the t-test, CYP2C19*2 showed significant association in clopidogrel resistance in dominant model of overall group, and codominant and dominant model of triple antiplatelet group. It did not showed significance in dual antiplatelet therapy group. CYP2C19*2 is mainly studied in Caucasian, and various studies reported that its polymorphism reduces the inhibition of platelet activity by clopidogrel and may associated clinical outcome after coronary artery stenting[24, 25, 30, 38]. In this study, although not statistically significant in chi-square analysis, the trend of the association of clopidogrel 2C19*2 polymorphism and clopidogrel

resistance was seen. However, impact of CYP2C19*2 polymorphism on clopidogrel responsiveness was not as strong as the impact of CYP2C19*3 polymorphism. CYP2C19*3 polymorphism may do more important role in activity of CYP than CYP2C19*2 in Korean.

The exact mechanism of clopidogrel resistance is still unclear, but variances in activity of CYP have been suggested. The active metabolite of clopidogrel arises from biochemical reactions involving several CYP isoforms, so one CYP polymorphism may not explain all the variability of clopidogrel response. Savi et al demonstrated that CYP1A activity plays key role in clopidogrel metabolism[2]. Lau et al found that CYP3A4 activity was associated with variability in clopidogrel responsiveness. Hulot et al reported that cytochrome 2C19*2 polymorphism is associated with clopidogrel resistance which has since been validated in several other studies as well[23-25].

The interesting finding from this study was that CYP2C19*3 was significantly associated with clopidogrel resistance. CYP2C19*3 was first described by De Morais et al in Japanese population[39]. CYP2C19*3 is a G636→A point mutation in exon 4 that produces a premature stop codon[40]. According to the original report, there were marked interracial differences in the frequency of the CYP2C19*3, with 9 of 34 alleles being detected in Japanese poor metabolizers. However, it was not detected in Caucasian poor

metabolizers. Because of the paucity of CYP2C19*3 allele in western population, its role was incompletely studied. In the Hulot's study, no case of CYP2C19*3 minor allele was demonstrated. It is reported that the occurrence of CYP2C19*3 is less than 1% in whites[40]. Our study demonstrated, for the first time, that CYP2C19*3 polymorphism is a significant risk factor for clopidogrel resistance, and may have significant importance in clopidogrel metabolism in the Asian population. Further investigation is needed to confirm the importance of CYP2C19*3. It is interesting to note that although the addition of cilostazol significantly reduces the rate of clopidogrel resistance, the expression of CYP2C19*3 minor allele was an independent predictor of clopidogrel resistance in subjects administered with a triple regimen of antiplatelet. This demonstrates that adding another antiplatelet agent is not enough to overcome the adverse effect of CYP2C19*3 polymorphism on clopidogrel metabolism. Although speculative at this time, an addition of drugs to increase the activity of the CYP2C19 activity may be one way to enhance the potency of clopidogrel and overcome clopidogrel resistance. Also, further investigation is needed to clarify whether the increased clopidogrel resistance in subjects with CYP2C19*3 minor allele polymorphism translates into increased cardiovascular outcomes.

V. CONCLUSION

In conclusion, CYP2C19*3 SNP is an independent risk factor of clopidogrel resistance in Korean subjects with coronary artery disease. The results from this study suggested that CYP2C19*3 may have a more important role than CYP2C19*2 in the metabolism of clopidogrel in Korean. Further investigation is needed to clarify whether the increased clopidogrel resistance in subjects with CYP2C19*3 minor allele polymorphism translates into increased cardiovascular outcomes.

REFERENCES

1) Ding Z, Kim S, Dorsam RT, Jin J, Kunapuli SP. Inactivation of the human P2Y12 receptor by thiol reagents requires interaction with both extracellular cysteine residues, Cys17 and Cys270. Blood 101:3908-3914, 2003

2) Savi P, Combalbert J, Gaich C, Rouchon MC, Maffrand JP, Berger Y, Herbert JM. The antiaggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450-1A. Thrombosis and haemostasis 72:313-317, 1994

3) Savi P, Pereillo JM, Uzabiaga MF, Combalbert J, Picard C, Maffrand JP, Pascal M, Herbert JM. Identification and biological activity of the active metabolite of clopidogrel. Thrombosis and haemostasis 84:891-896, 2000

4) Gurbel PA, Tantry US. Clopidogrel resistance? Thrombosis Research 120:311-321, 2007

5) Steinhubl SR, Berger PB, Mann JT, Fry ET, DeLago A, Wilmer C, Topol EJ. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 288:2411-2420, 2002

6) Gurbel PA, Bliden KP, Hayes KM, Yoho JA, Herzog WR, Tantry US. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. Journal of the American College of Cardiology 45:1392-1396, 2005

7) Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Barrera-Ramirez C, Sabate M, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C. Identification of low

responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. Thromb Res 115:101-108, 2005

8) Dziewierz A, Dudek D, Heba G, Rakowski T, Mielecki W, Dubiel JS. Inter-individual variability in response to clopidogrel in patients with coronary artery disease. Kardiol Pol 62:108-117; discussion 118, 2005

9) Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. J Intern Med 252:233-238, 2002

10) Lepantalo A, Virtanen KS, Heikkila J, Wartiovaara U, Lassila R. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. Eur Heart J 25:476-483, 2004

11) Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 109:3171-3175, 2004

12) Mobley JE, Bresee SJ, Wortham DC, Craft RM, Snider CC, Carroll RC. Frequency of nonresponse antiplatelet activity of clopidogrel during pretreatment for cardiac catheterization. Am J Cardiol 93:456-458, 2004

13) Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost 89:783-787, 2003

14) Levine RL, Dixit SN, Dulli DA, Khasru MA. Aspirin "failures," clopidogrel added to aspirin, and secondary stroke prevention in veterans presenting with TIA or mild-to-moderate ischemic stroke. J Stroke Cerebrovasc Dis 12:37-43, 2003

15) Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M,

Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Banuelos C, Costa MA, Bass TA, Macaya C. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes 54:2430-2435, 2005

16) Müller I, Besta F, Schulz C, Massberg S, Schönig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thrombosis and haemostasis 89:783-787, 2003

17) Järemo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. Journal of internal medicine 252:233-238, 2002

18) Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Barrera-Ramirez C, Sabaté M, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Macaya C. Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. Thrombosis Research 115:101-108, 2005

19) Cuisset T, Frere C, Quilici J, Barbou F, Morange PE, Hovasse T, Bonnet JL, Alessi MC. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. Journal of thrombosis and haemostasis 4:542-549, 2006

20) Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, Bassi AK, Tantry US. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol 46:1820-1826, 2005

21) Gurbel PA, Bliden KP, Tantry US. Effect of clopidogrel with and without eptifibatide on tumor necrosis factor-alpha and C-reactive protein release after elective stenting: results from the CLEAR

PLATELETS 1b study. J Am Coll Cardiol 48:2186-2191, 2006

22) Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study. Circulation 111:1153-1159, 2005

23) Frere C, Cuisset T, Morange PE, Quilici J, Camoin-Jau L, Saut N, Faille D, Lambert M, Juhan-Vague I, Bonnet JL, Alessi MC. Effect of Cytochrome P450 Polymorphisms on Platelet Reactivity After Treatment With Clopidogrel in Acute Coronary Syndrome. The American journal of cardiology 101:1088-1093, 2008

24) Fontana P, Senouf D, Mach F. Biological effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19*2 allele on clopidogrel responsiveness. Thrombosis Research 121:463-468, 2008

25) Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, Valente S, Antoniucci D, Abbate R, Gensini GF. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10흡 and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenetics and genomics 17:1057-1064, 2007

26) Cuisset T, Frere C, Quilici J, Morange PE, Saut N, Lambert M, Camoin L, Vague IJ, Bonnet JL, Alessi MC. Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. Thromb Res 120:893-899, 2007

27) Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang IJ, Lee DS, Sohn DW, Lee MM, Kim HS. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. CMAJ 174:1715-1722, 2006

28) Smith SM, Judge HM, Peters G, Armstrong M, Fontana P, Gaussem P, Daly ME, Storey RF. Common sequence variations in the P2Y12 and CYP3A5 genes do not explain the variability in the inhibitory effects of clopidogrel therapy. Platelets 17:250-258, 2006

29) Lau WC, Gurbel PA. Antiplatelet drug resistance and drug-drug interactions: Role of cytochrome P450 3A4. Pharmaceutical Research 23:2691-2708, 2006

30) Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P, Gaussem P. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 108:2244-2247, 2006

31) Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, Sabaté M, Hernández R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Pignatti PF, Macaya C. Contribution of gene sequence variations of the hepatic cytochrome P450 3A4 enzyme to variability in individual responsiveness to clopidogrel. Arteriosclerosis, thrombosis, and vascular biology 26:1895-1900, 2006

32) Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE, Bates ER. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation 107:32-37, 2003

33) Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, Mahmud E, Atar D, Serebruany V. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. Thrombosis Research 119:277-284, 2007

34) Malinin A, Pokov A, Swaim L, Kotob M, Serebruany V. Validation of a VerifyNow-P2Y12 cartridge for monitoring platelet inhibition with clopidogrel. Methods and findings in experimental and clinical

pharmacology 28:315-322, 2006

35) Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, Ernst A, Sawhney NS, Schatz RA, Teirstein PS. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J 29:992-1000, 2008

36) Jakubowski JA, Payne CD, Li YG, Brandt JT, Small DS, Farid NA, Salazar DE, Winters KJ. The use of the VerifyNow P2Y12 point-of-care device to monitor platelet function across a range of P2Y12 inhibition levels following prasugrel and clopidogrel administration. Thromb Haemost 99:409-415, 2008

37) Lee SW, Park SW, Kim YH, Yun SC, Park DW, Lee CW, Hong MK, Kim HS, Ko JK, Park JH, Lee JH, Choi SW, Seong IW, Cho YH, Lee NH, Kim JH, Chun KJ, Park SJ. Drug-eluting stenting followed by cilostazol treatment reduces late restenosis in patients with diabetes mellitus the DECLARE-DIABETES Trial (A Randomized Comparison of Triple Antiplatelet Therapy with Dual Antiplatelet Therapy After Drug-Eluting Stent Implantation in Diabetic Patients). J Am Coll Cardiol 51:1181-1187, 2008

38) Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, Stratz C, Schmiebusch P, Bestehorn HP, Buttner HJ, Neumann FJ. Cytochrome P450 2C19 681G>A Polymorphism and High On-Clopidogrel Platelet Reactivity Associated With Adverse 1-Year Clinical Outcome of Elective Percutaneous Coronary Intervention With Drug-Eluting or Bare-Metal Stents. J Am Coll Cardiol 51:1925-1934, 2008

39) De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. Molecular pharmacology 46:594-598, 1994

40) Xie HG, Kim RB, Wood AJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. Annu Rev Pharmacol Toxicol 41:815-850, 2001

ABSTRACT (IN KOREAN)

약물용출 스텐트 삽입술을 받은 한국인 환자에서 cytochrome P450의 유전자 염기서열변이와 clopidogrel 저항성의 관계

<지도교수 장양수>

연세대학교 대학원 의학과

이정명

클로피도그렐은 전구약물로써 간의 싸이토크롬 P450에 의해 활성화되어 혈소판의 P2Y12 수용체를 차단함으로써 혈소판 응집을 막는다. 클로피도그렐 저항성의 기전은 정확히 알려져 있지 않으나, 싸이토크롬 효소에 의해 활성화 대사물로 전환되는 과정의 차이가 중요한 인자로 생각되고 있다. 최근의 연구에서 싸이토크롬 2C19*2 유전자 염기서열 변이가 클로피도그렐 저항성과 유의한 관계가 있다고 보고되었다. 또한 싸이토크롬 3A4 및 3A5 유전자의 염기서열 변이도 유의한 관계가 있다는 보고가 있다. 기존 연구들은 대부분

서양인을 대상으로 시행되었다. 본 연구에서는 한국인을 대상으로 클로피도그렐 저항성과 관련이 있는 유전자 염기서열 변이가 있는지 찾아보겠다.

2006년 10월부터 2007년 5월까지 450명의 환자들이 연세의료원 심혈관 센터에서 관상동맥성형술 및 약물용출 스텐트 삽입술을 받았다. 환자들은 아스피린, 클로피도그렐의 이중 항혈소판 치료군과, 여기에 실로스타졸을 추가한 삼중 항혈소판 치료군으로 배정되었고, 이중 383명의 환자에서 클로피도그렐 저항성 및 유전체 검사가 시행되었다. 클로피도그렐 저항성은 VeryfyNow P2Y12 를 사용하여 측정하였고, % inhibition 이 20% 미만인 경우 저항성이 있는 것으로 간주되었다. COX2 rs5277, CYP3A5*3 rs776747, CYP1A1 rs1048943, CYP2C19*2 rs4244285, CYP2C19*3 rs4986893, CYP1A2 rs2470890, CYP3A4 rs2242480 는 SNaPShot Multiplex kit 을 사용하여 분석하였고, CYP3A4 rs2246709, CYP2J2 rs2280274, P2RY12 는 TaqMan fluorogenic 5’ nuclease assay 를 사용하여 분석하였다.

클로피도그렐 저항성은 111명 (29%) 의 환자에서 발견되었다. 클로피도그렐 저항성 군과 반응성 군 간에 나이, 성별, 당뇨병, 흡연력, 체질량지수 등의 유의한 차이는

없었으나, 클로피도그렐 반응성 군에서 실로스타졸의 사용률이 유의하게 높았다. 실로스타졸이 클로피도그렐 저항성과 유의한 관련이 있었기 때문에, 연구 대상을 이중 항혈소판 치료군과 삼중 항혈소판 치료군으로 나누어서 분석을 시행하였다. 두 가지 치료군 모두에서 싸이토크롬 2C19*3 유전자 염기서열 변이가 클로피도그렐 저항성 군에서 유의하게 많은 분포를 보였다. 다중 로지스틱 회귀분석에서 싸이토크롬 2C19*3 유전자 염기서열 변이는, 클로피도그렐 저항성의 독립적 예측인자로 나타났다.

결론적으로, 싸이토크롬 2C19*3 유전자 염기서열 변이는 관상동맥 질환으로 약물용출 스텐트를 삽입 받은 한국인에서, 클로피도그렐 저항성의 독립적 예측인자이다. 본 연구의 결과는 한국인에서 CYP2C19*2 보다 CYP2C19*3 의 역할이 더 중요함을 시사한다. CYP2C19*3 유전자 염기서열 변이를 갖고 있는 환자의 클로피도그렐 저항성이 예후와 어떤 관련이 있는지는 추가적인 연구가 필요하다.

핵심 되는 말: 싸이토크롬, 싸이토크롬 2C19*3, 유전자 염기 서열 변이, 클로피도그렐 저항성, 약물 용출 스텐트