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Cytochrome P450 Epoxygenase *CYP2J2* G-50T Polymorphism is an Independent Genetic Prognostic Risk Factor and Interacts with Smoking Cessation After Index Premature Myocardial Infarction

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1. Introduction

Cytochrome P450 epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs). One human cytochrome P450 enzyme, *CYP2J2*, is abundantly expressed in coronary artery endothelial and smooth muscle cells, and in cardiac myocytes (Wu et al., 1996; Imig, 2000). One of the primary products of the NADPH-dependent epoxidation of arachidonic acid by CYP2J2 is the production of 11, 12-EET. This eicosanoid has potential anti-inflammatory effects by inhibiting endothelial nuclear factor- B, a transcription factor associated with the induction of many pro-inflammatory gene products in the vasculature (Imig, 2000; Node et al., 1999). Other EETs, including 5,6-, 8,9-, 11,12-, and 14,15-EETs have important vasodilatation properties via mechanism of smooth muscle cells relaxation (Fang et al., 2002; Pinto et al., 1987; Spieker & Liao, 2005; Liu et al., 2006). More recently, the additional vascular protective effects of EETs, including anti-thrombotic, antimigratory, antioxidant, and antiapoptotic effects, have also been observed (Gauthier et al., 2004; Sun et al., 2002).

Recently, a novel genetic variant G-50T of this novel gene *CYP2J2* was found to be associated with coronary artery disease (CAD) (Spieker et al., 2004). This mutation could functionally result in the loss of binding of the Sp1 transcription factor to the *CYP2J2* promoter and a decreased activity in *CYP2J2* promoter. The plasma concentrations of stable EETs metabolites were also lower in individuals with the G-50T polymorphism. However, the role of this novel gene variant in myocardial infarction (MI), especially premature MI, is still not well investigated.

Of the common environmental factors known to be associated with risk of acute MI, smoking is widely acknowledged to make a major contribution (Manson et al., 1992; Teng et al., 1994). Smoking can disturb lipoprotein metabolism by increasing insulin resistance and lipid intolerance, and is implicated in the production of small dense low-density lipoprotein (Craig et al., 1989; Eliasson et al., 1997; Barua et al., 2002). The smoking-associated risk of MI

has been reported to be greater in subgrouping subjects with several genetic variants background (Li et al., 2002; Liu et al., 2005; Humphries et al., 2002).

We thus hypothesized that those who with the T allele may have higher inflammatory status, and thus have higher risk for plaque rupture or occurrence of MI, especially at a younger age. We also speculated that there should be an additive interaction between the effect of smoking behavior and the genetic variation for the onset of premature MI.

Smoking cessation could gradually improve the endothelial function and fibrinolytic status (Tsiara et al., 2003). However, successful cessation of smoking after MI and its interaction with this candidate gene for the subsequent events after index MI was still undefined. Thus, we hypothesized that premature MI patients carrying genetic polymorphism, especially genetic variant G-50T of this novel gene *CYP2J2*, might have a higher risk for subsequent coronary events. In addition, smoking cessation might interact with these gene variations for the prognosis after patients' index MI in Taiwan.

2. Methods

2.1 Study subjects

2.1.1 Study population

We enrolled 200 patients (mean age 42.2±2.5 years; 84% men) with documented MI onset prior to age of 45 years. The patients were recruited after their first MI. Diagnosis of MI was based on ischemic chest symptoms, typical electrocardiographic changes and elevation of serum creatine kinase and its MB isoenzyme, when more than twice the upper level of normal. Coronary angiography was performed using the Judkin's method within 2 weeks after the onset of symptoms. Coronary stenosis is defined as ≥ 50% diameter narrowing.

2.1.2 Control population

The control group was recruited by sex-matched 200 patients (mean age 42.5±2.1 years) from consecutive subjects admitted to our hospital for routine health examinations. They did not show any clinical or electrocardiographic evidence of MI or CAD. They also had no history of cerebrovascular disease or peripheral arterial disease. Written informed consents were obtained from all patients and this study was in agreement with guidelines approved by the research committee of National Cheng-Kung University Hospital.

2.1.3 Background of population

All patients and controls included in this study are Han Chinese/Taiwanese from the same geographic area. The demographic data and the presence of traditional coronary risk factors, including hypertension, diabetes mellitus, smoking and serum cholesterol, were collected from all study participants.

2.1.4 Data collection & history recording

For patients with MI, these data were taken from the medical records at the time of admission for acute MI; for control subjects, they were collected at the time of hospital admission for routine health examinations. They were considered to have hypertension if

elevated blood pressure (>140/90 mmHg) were measured on 3 occasions or if they were already being treated with anti-hypertensive agents. They were defined as having diabetes mellitus (DM) if they had a fasting blood glucose level > 110 mg/dl or were already being treated for DM. All study participants were classified as either smokers (including current or ex-smokers) or non-smokers. The total cholesterol level was determined at the beginning of the study.

2.1.5 Blood collection

The blood sampling time in the study group was at least 2 weeks after the onset of acute MI. In the control group, the blood samples were taken during coronary angiography study. All patients with impaired renal function, malignancy, connective tissue disease or chronic inflammatory disease were excluded. The blood samples were drawn into a 5-ml EDTA glass tube and centrifuged at 2200 g for 15 minutes to separate the plasma contents. The buffy coat after centrifugation was obtained and deoxyribonucleic acid (DNA) in each sample was isolated by the method we used before (Liu et al., 2007). The DNA samples were stored at –70 degree C until use.

2.1.6 Genomic amplification by PCR

Patient's DNA was isolated from the whole-blood samples by the phenol-chloroform extraction method. A 273-bp promoter region proximal to the transcriptional start site was amplified with primers described previously (King et al., 2002). The sequence products were resolved on an ABI 377 automated sequencer. The promoter polymorphism G-50T was verified by direct sequencing. The numbering of the polymorphisms refers to the GenBank sequence AF272142 (accession number).

2.1.7 Functional EET analysis

Eicosanoids were extracted from plasma samples 3 times with ethyl acetate after acidification with acetic acid. After evaporation, saponification with 0.4*N* KOH in methanol, and re-extraction, concentrations of the stable EETs metabolite 14,15-dihydroxyeicosatrienoic acid (DHET) were determined by an ELISA kit (Detroit R&D) (Liu et al., 2007; Spieker et al., 2004).

2.1.8 Smoking habits definition

Individuals were classified according to their smoking status; a current smoker was defined as any person who smoked regularly (at least one cigarette per day and/or one cigar or one pipe per week). Subjects who had smoked at least one cigarette per day and/or one cigar or one pipe per week in the past were classified as former smokers. Never smokers were those who had never smoked any tobacco product regularly. Subjects were considered to have achieved smoking cessation if they were reported non-smoking from the quitting day until the end of the 6-month period.

2.1.9 Follow-up study

Patients received regular follow-up care in our cardiology ward or clinics for at least 6 months with a maximum of 13 years or until occurrence of one of the following coronary events: recurrent angina pectoris, non-fatal MI, or cardiac death. Recurrent angina pectoris

was defined as recurrent chest pain with ischemic ECG changes lasting >10 min despite antiangina therapy. Diagnosis of recurrent MI was the same as for index MI (see Study population). Cause of death was determined from hospital records. In this study, the follow-up data were available for a total of 162 (95.3%) premature MI patients. Eight patients (4.7%) were not available for follow-up. The reasons included: 3 (1.8%) patients moved back to their primary residency region and we were unable to follow-up; 3 (1.8%) patients died of non-cardiovascular events, including two (1.2%) in traffic accidents and one (0.6%) by suicide; two (1.2%) patients were lost or changed their telephone numbers without detailed medical records. Those event-free patients who we were unable to follow-up completely were included in the event-free group. Their follow-up periods were defined between the index MI and their last clinic visit.

2.1.10 Statistical analysis

Data on age and cholesterol levels were presented as mean value ± standard deviation (SD). The values of DHET were presented as median ± SD. The difference between the groups was analyzed by the unpaired Student's t test. The differences in the frequencies of smoking, hypertension, hyperlipidemia, diabetes mellitus, and *CYP2J2* G-50T genotypes were analyzed by Fisher's exact test. χ^2 analyses were used to test deviations of genotype distribution from Hardy-Weinberg equilibrium and to determine allele or genotype frequencies between patients and control groups. The risk factors that appear to be possible significant predictors ($p<0.05$) in the single-variant analyses were included in the multiple logistic regression analyses. Multivariate analyses were conducted with multiple logistic regression methods, and adjusted estimations of conditioned relative risk and 95% confidence intervals (CIs) were done. The Kaplan–Meier method (log-rank test) was applied in subsequent event-free analysis. All statistical analyses were performed using SPSS Advanced Statistics 13.0 for Window. In this study, a value of $p<0.05$ was taken to be statistical significance.

3. Results

3.1 Comparison of traditional CAD risk factors between MI and control groups

We compared the control and premature MI group for traditional CAD risk factors, including hypertension, diabetes mellitus, smoking and serum cholesterol levels (Table 1).

Characteristics	Control (n=200)	Premature MI (n=200)
Age (yrs)	42.5±2.1	42.2±2.1
Men/Women	167/33	168/32
Systemic hypertension (%)	46 (23.0)	60 (30.0) †
Diabetes mellitus (%)	9 (4.5)	26 (13.0)*
Smoker (%)	89 (44.5)	154 (77.0)*
Total cholesterol (mg/dL)	182±37.5	210±34.5
Triglycerides (mg/dL)	135±32.1	136±30.8
HDL cholesterol (mg/dL)	46±10.5	41±10.1
LDL cholesterol (mg/dL)	120±29.3	128±21.5

Data are presented as number (%) of patients or mean ± standard deviation. HDL = high-density lipoprotein; LDL = low-density lipoprotein; MI = myocardial infarction. *: $p<0.001$; †: $p<0.01$.

Table 1. Clinical characteristics of study subjects

There were no significant differences in the age between these 2 groups (p = 0.890). The frequency of smoking (p <0.001), diabetes mellitus (p <0.001) and hypertension (p <0.01) were significantly higher in premature MI patients. However, the traditional CAD risk factor such as total cholesterol level was similar between 2 groups.

3.2 Distribution of CYP2J2 G-50T genotypes

Table 2 shows the distribution of *CYP2J2* G-50T genotype in both premature MI patients and control subjects. The frequency of the T allele was significantly higher in the premature MI than the control group (16.0% vs. 12.0%, p<0.01; odds ratio (OR) 2.15, 95% [CI] 1.30 to 6.80). There was a significantly higher prevalence of the T allele genotype (GT+TT) among patients with premature MI in comparison to the control subjects (28.0% vs. 22.0%; OR 2.0, 95% [CI] 1.3 to 6.8, p = 0.01). The distributions of genotype in both the premature MI group and control group were compatible with the Hardy-Weinberg equilibrium.

	Control (n=200)	Premature MI (n=200)	OR (95% CI)	P value
TT	4 (2.0)	8 (4.0)		
GT	40 (20.0)	48 (24.0)		
TT+GT	44 (22.0)	56 (28.0)	2.0 (1.3-6.8)	0.01
GG	156 (78.0)	144 (72.0)		
T allele frequency	0.12	0.16		0.01

Data are presented as number (%) of patients. CI = confidence interval; GG = homozygous G allele of *CYP2J2* G-50T gene; GT = heterozygous allele of *CYP2J2* G-50T gene; MI = myocardial infarction; TT = homozygous T allele of *CYP2J2* G-50T gene; OR = odds ratio.

Table 2. Frequency of genotypes of *CYP2J2* G-50T gene in control subjects and patients with premature myocardial infarction

3.3 Identification of independent risk factors of MI

Table 3 shows the results of multiple logistic regression analysis for identifying the independent risk factors of premature MI. Hypertension, DM, smoking and *CYP2J2* genotype were all used as independent variables. Multiple logistic regression analysis showed that the T allele was an independent risk factor (OR 1.78, 95% CI 1.12 to 6.40, p = 0.02), as well as smoking (OR 3.05, 95% CI 1.55 to 7.25, p<0.01), diabetes mellitus (OR 3.24, 95% CI 1.22 to 6.55, p<0.01) and hypertension (OR 1.95, 95% CI 1.13 to 5.73, p<0.01) for the premature onset of MI.

	OR for MI	95% CI	P value
Smoking	3.05	1.55-7.25	<0.01
Diabetes mellitus	3.24	1.22-6.55	<0.01
Hypertension	1.95	1.13-5.73	<0.01
CYP2J2 G-50T polymorphism	1.78	1.12-6.40	0.02

CI = confidence interval; GG = homozygous G allele of *CYP2J2* G-50T gene; GT = heterozygous allele of *CYP2J2* G-50T gene; MI = myocardial infarction; TT = homozygous T allele of *CYP2J2* G-50T gene; OR = odds ratio.

Table 3. Risk factors of premature myocardial infarction identified by multiple logistic regression analysis

Moreover, there was a synergistic effect between smoking and T allele of *CYP2J2* genotype on the occurrence of MI. (Table 4.) Among patients who did not smoke, the T allele was associated with a higher risk of young MI (OR 1.43, 95% CI 1.2 to 6.2). Smoking carrier with the G allele was associated with a 3-fold higher risk for premature MI (OR 3.78, 95% CI 3.3 to 10.6). Furthermore, smoking carriers of the T allele of *CYP2J2* allele had a significantly 5.6-fold higher risk of premature MI (OR 5.55, 95% CI 4.3 to 13.7) when compared with non-smoking and G allele genotype carriers.

Smoking	*CYP2J2* G-50T genotype	Control (n=200)	Premature MI (n=200)	OR	95% CI
No	GG	87	36	1	
No	GT+TT	24	10	1.43	1.2-6.2
Yes	GG	69	108	3.78	3.3-10.6
Yes	GT+TT	20	46	5.55	4.3-13.7

Data are presented as number of patients. CI = confidence interval; GG = homozygous G allele of *CYP2J2* G-50T gene; GT = heterozygous allele of *CYP2J2* G-50T gene; MI = myocardial infarction; TT = homozygous T allele of *CYP2J2* G-50T gene; OR = odds ratio

Table 4. Association between smoking and *CYP2J2* G-50T genotype on premature myocardial infarction

3.4 Functional analysis of EET metabolites

To further investigate the functional role of the G-50T polymorphism, we measured the plasma concentrations of the major *CYP2J2*-dependent epoxidation product from arachidonic acid. Given the instability of the primary products, EETs, concentration of the stable metabolite 14,15-DHET was determined after extraction from plasma samples. Median DHET plasma concentrations were significantly lower in samples from premature MI subjects with the G-50T polymorphism when compared with G allele individuals (6.2 ± 1.2 ng/mL vs. 10.8 ± 2.5 ng/mL; p = 0.025). Among premature MI subjects, the median DHET plasma concentrations were significantly lower among smoking carriers with the G-50T polymorphism (3.3 ± 1.0 ng/mL vs. 6.8 ± 1.3 ng/mL; p = 0.001). However, this effect was not significant for subjects without gene variation (G allele carriers) (10.2 ± 1.3 ng/mL vs. 10.8 ± 2.4 ng/mL; p = 0.18). (Fig 1)

Median DHET plasma concentrations were significantly lower in samples from premature MI subjects with the G-50T polymorphism when compared with G allele individuals. Among premature MI subjects, the median DHET plasma concentrations were significantly lower among smoking carriers with the G-50T polymorphism. However, this effect was not significant for subjects without gene variation (G allele carriers). Median DHET levels were significantly lower among *CYP2J2* G-50T polymorphism compared with G allele individuals. The median DEHT levels were significantly lower among smoking carriers with T allele subjects, but not among G allele ones. DHET = dihydroxyeicosatrienoic acid; MI = myocardial infarction.

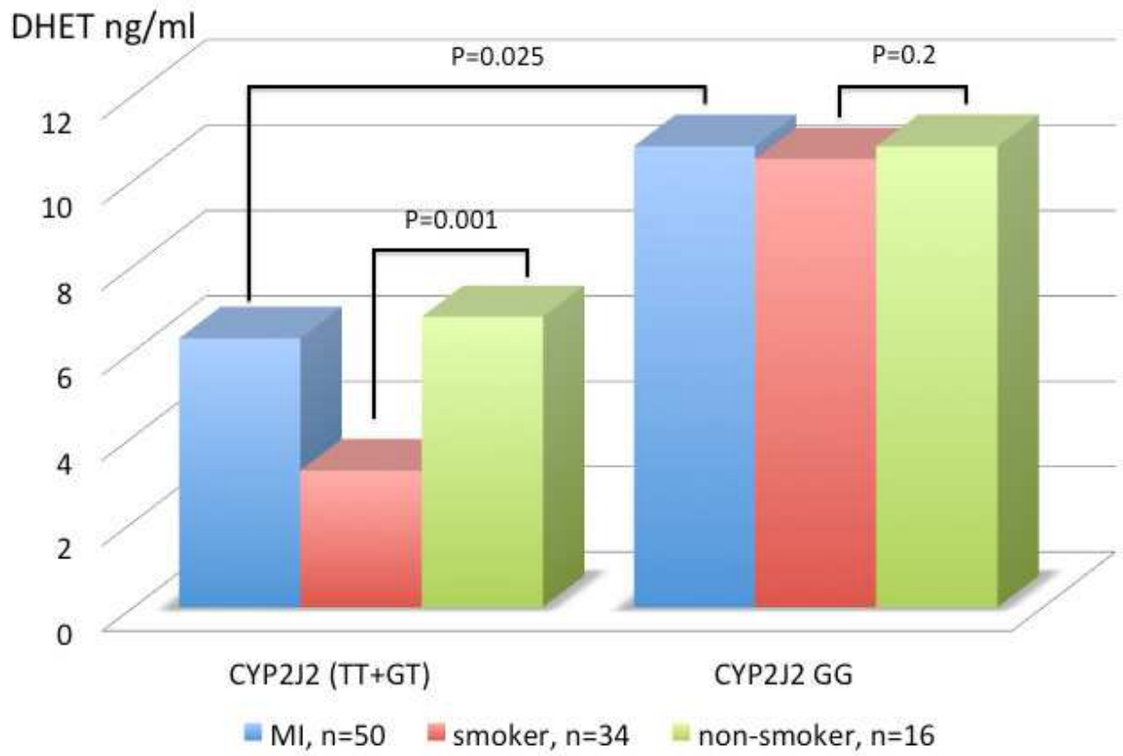

Fig. 1. Plasma concentrations of the 14, 15-dihydroxyeicosatrienoic acid (DHET), a major *CYP2J2*-dependent epoxidation product from arachidonic acid among premature myocardial infarction subjects.

3.5 The follow-up clinical and angiographic characteristics analyses

During a mean period of 4.43 years (from 0.5 to 13 years) follow-up, cardiac events occurred in 48 (28.2%) patients, including four (2.4%) with cardiac death, 24 (14.1%) with recurrent MI, and 20 (11.7%) with recurrent angina pectoris. The baseline and the available follow-up clinical and angiographic characteristics are shown in Table 5.

With similar mean follow-up periods, most of the clinical manifestations and treatment regimens were no different before and after follow-up in both groups that with-event and without-event, except that usage frequencies of both angiotensin-converting enzyme inhibitors (ACEI) and statin were higher after follow-up in both groups ($P < 0.05$, compared with their baseline data). Under these therapeutic profiles, patients' blood pressures and fasting sugar levels were also similar in both groups after follow-up. Almost 92% of our follow-up subjects received catheterization study. The prevalence distribution of the culprit coronary artery lesion changed among patients receiving follow-up coronary angiography (Table 5).

During the first catheterization study, most (48/84, 57.1%) patients had the culprit lesion located in the left anterior descending artery, followed by a right coronary artery lesion. However, during the late angiographic study, 31.3% of the initial culprit lesions regressed, while 33.8% of the new *De novo* lesions became culprit ones.

	With cardiac events (n=48, 30.0%)			Without cardiac events (n=114, 70.0%)		
	Initial treatment	Final treatment	P value	Initial treatment	Final treatment	P value
Hypertension	29 (60.4)	31 (64.5)	0.14	36 (31.5)	38 (33.3)	0.15
Diabetes mellitus	19 (39.5)	21 (43.8)	0.08	20 (17.5)	28 (24.5)	0.06
Smoking	40 (83.3)	32 (66.7)	0.04	100 (87.7)	43 (37.7)	0.01
Total cholesterol, mg/dL	204±33.5	208±32.7	0.24	207±36.1	207±40.7	0.36
HDL-cholesterol, mg/dL	42±10	44±11	0.38	43±9	44±12	0.33
LDL-cholesterol, mg/dL	135±32	137±40	0.28	136±29	137±39	0.54
Triglycerides, mg/dL	132±36	137±56	0.42	135±43	136±56	0.77
LVEF (%)	58.8±9.8	57.9±10.1	0.35	60.1±13.5	59.1±11.5	0.40
Angiography	43 (89.9)	44 (91.6)	0.67	100 (87.7)	94 (82.4)	0.58
PCI	25 (52.0)	29 (60.4)	0.44	60 (52.6)	59 (51.7)	0.38
CABG	6 (12.5)	8 (16.7)	0.40	18 (15.8)	19 (16.7)	0.28
CAD						
Single-vessel	24 (50.0)	26 (54.1)	0.32	64 (56.1)	60 (52.6)	0.85
LAD	16	10		34	26	
LCX	3	7		9	15	
RCA	5	9		21	19	
Double-vessel	13 (27.1)	13 (27.1)	1.00	30 (26.3)	32 (28.0)	0.61
Triple-vessel	10 (20.8)	8 (16.7)	0.44	22 (19.2)	23 (20.1)	0.22
Medications						
β-blocker	19 (39.5)	24 (50.0)	0.06	91 (79.8)	97 (85.0)	0.10
ACEI	10 (20.8)	20 (41.6)	0.04	69 (60.5)	80 (70.1)	0.06
Statin	10 (20.8)	18 (37.5)	0.03	42 (36.8)	60 (52.6)	0.04

With similar mean follow-up periods, most of the clinical manifestations and treatment regimens were no different before and after follow-up in both groups that with-event and without-event, except that usage frequencies of both angiotensin-converting enzyme inhibitors and statin were higher after follow-up in both groups. Under these therapeutic profiles, patients' blood pressures and fasting sugar levels were also similar in both groups after follow-up. Values are expressed as number (%) or mean ± SD. ACEI = angiotensin-converting enzyme inhibitor; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; CI = confidence intervals; ECG = electrocardiograms; HDL = high density lipoproteins; LAD = left anterior descending artery; LCX = left circumflex artery; LDL = low density lipoproteins; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PCI = percutaneous coronary interventions; RCA = right coronary artery.

Table 5. The initial and follow-up clinical and angiographic characteristics of patients with premature myocardial infarction

Compared with event-free group, subjects with event during the follow-up period had significantly higher genetic prevalence rate of T allele (TT+GT) (Event vs. event-free subjects: 58.3% vs. 42.1%, p=0.02) as well as the whole T allele frequency (Event vs. event-free: 42.7% vs. 29.8%, p=0.02).

CYP2J2 genotypes	With event (n=48)	Event-free (n=114)	P value
TT+GT	11+17 (58.3)	16+30 (42.1)	0.02
GG	20 (41.6)	68 (60.0)	
T allele relative frequency	41/96 (42.7)	68/228 (29.8)	0.02

Compared with event-free group, subjects with event during the follow-up period had significantly higher genetic prevalence rate of T allele (TT+GT) as well as the whole T allele frequency. Values are expressed as n (%).

Table 6. Frequencies of *CYP2J2* G-50T genotypes in groups with or without cardiac events after index myocardial infarction

Kaplan–Meier analysis demonstrated a significantly lower probability (23.5% vs. 34.6%, log-rank P=0.04) of developing clinical coronary events among patients with the polymorphism of *CYP2J2* promoter G-50T genotype (Fig. 2).

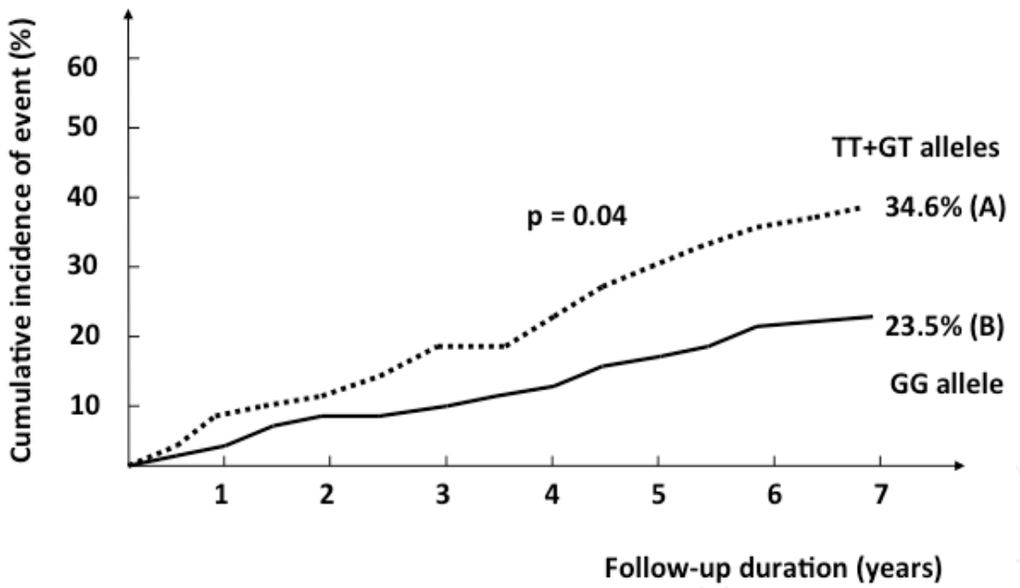

Fig. 2. Kaplan-Meier survival curve for subsequent coronary events after index acute myocardial infarction for the patients carrying TT or GT allele (A) (or GG allele for (B)) at their *CYP2J2* gene (after modified risk with age, diabetes mellitus, smoking, hypertension, and medication usage with ACEI or β-blockers).

For traditional risk factors, patients in event groups had significantly higher prevalence rates of DM, hypertension and initial severe Killip's status (>II) (all $P < 0.05$, see Table 7). The mean cholesterol level was also higher in the event group. Compared with the event group, patients without events received more medications such as ACEI, β-blocker and statin. The success rate of smoking cessation was higher in the event-free group (52.0% vs. 19.5%).

However, the event-free group patients received more frequent procedures of coronary bypass surgery in our MI group.

	Without cardiac events (n=114, 70.0%)	With cardiac events (n=48, 30%)	p value
Age (years)	40.3 ± 4.8	39.7 ± 4.4	0.43
Sex (male)	91 (79.8)	42 (87.5)	0.50
Family history	23 (20.1)	10 (20.8)	0.73
Hypertension	36 (28.9)	29 (60.4)	<0.01
Diabetes mellitus	20 (17.5)	19 (39.5)	<0.01
Smoking	100 (87.7)	42 (87.5)	0.77
Total cholesterol, mg/dL	191.4 ± 33.2	209.0 ± 35.3	<0.01
HDL-cholesterol, mg/dL	42 ± 11	44 ± 9	0.22
LDL-cholesterol, mg/dL	133 ± 32	136 ± 30	0.34
Triglycerides, mg/dL	135 ± 37	138 ± 44	0.25
Status of MI			
Q wave in EKG	98 (85.9)	36 (75.0)	0.47
Peak CK level (U/L)	2845 ± 1988	2975 ± 2354	0.38
LVEF (%)	55.4 ± 11.5	54.8 ± 12.3	0.64
LVEF <45%	26 (22.8)	14 (29.1)	0.06
Thrombolytic therapy	89 (78.0)	36 (75.0)	1.00
Primary PTCA	19 (16.6)	8 (16.7)	1.00
Coronary angiography	107 (93.8)	44 (91.6)	1.00
PCI	61 (53.5)	25 (52.0)	0.89
CABG	18 (15.8)	6 (12.5)	0.81
VT/Vf at MI	9 (7.8)	4 (8.3)	0.91
Killip's classification ≥ II	26 (22.8)	23 (47.9)	<0.01
Medication usage after MI			
β-blocker	91 (79.8)	19 (39.5)	<0.01
ACEI	69 (60.5)	10 (20.8)	<0.01
Statin	42 (36.8)	10 (20.8)	0.03

Patients in event groups had significantly higher prevalence rates of DM, hypertension and initial severe Killip's status (>II). The mean cholesterol level was also higher in the event group. Compared with the event group, patients without events received more medications such as ACEI, β-blocker and statin. The success rate of smoking cessation was higher in the event-free group. The event-free group patients received more frequent procedures of coronary bypass surgery in our MI group. Values are expressed as number (%) or mean ± SD. CK = creatine kinase; Vf = ventricular fibrillation; VT = ventricular tachycardia.

Table 7. Comparison between patient groups with- or without- subsequent cardiac events during follow-up period after index myocardial infarction

Univariate Cox regression analyses of the clinical characteristics and genetic backgrounds of premature MI patients are shown in Table 8. Finally, we included the variables as DM, hypertension, smoking cessation after MI, multiple (>2-vessel) coronary disease, medical therapies with β-blockers, ACEI, or statins in traditional risk factors; and the polymorphism of *CYP2J2* promoter G-50T genotype in genetic factors in the multiple logistic regression analysis. For clinical consideration, we also included factors such as treatment by thrombolysis or primary angioplasty or none into this survival analyses. That analysis showed that the polymorphism of *CYP2J2* promoter G-50T genotype, DM, smoking cessation and use of ACEI were independent survival predictors (Table 9).

	Hazard ratios (95% CI)	p value
Genetic variables		
CYP2J2 G-50T	2.78 (1.50-5.00)	<0.01
Traditional variables		
Age (>40 years-old)	1.59 (0.94-3.08)	0.07
Sex (male)	0.66 (0.18-2.18)	0.51
Family history vs. non-history	0.72 (0.35-1.55)	0.38
Systemic hypertension	2.09 (1.39-5.05)	<0.01
Diabetes mellitus	2.71 (1.46-4.89)	<0.01
Smoking behavior before MI	1.89 (0.82-3.01)	0.55
Smoking cessation after MI	0.21 (0.11-0.40)	<0.01
Total cholesterol > 200mg/dL	1.41 (0.50-1.98)	0.22
Anterior MI vs. other wall	1.47 (0.81-1.95)	0.25
LVEF (<45%)	0.68 (0.36-1.30)	0.25
Thrombolytic therapy	1.42 (0.62-6.39)	0.57
Primary PTCA	1.17 (0.93-8.78)	0.10
Coronary angiography	0.46 (0.78-4.76)	0.88
PCI procedure	0.53 (0.39-2.54)	0.70
CABG	0.87 (0.72-1.32)	0.89
VT/Vf at MI	0.77 (0.79-2.69)	0.72
Killip's classification ≥ II	1.88 (1.33-6.62)	<0.01
Multiple (>2-vessel) disease	2.96 (0.84-7.25)	0.42
Medication usage after MI		
Not-using β-blocker	2.34 (1.51-3.17)	0.01
Not-using ACEI	7.19 (2.84-10.2)	<0.01
Not-using statin	1.65 (1.02-2.93)	0.01

Table 8. Univariate analyses of traditional and genetic risk factors with Cox proportional hazards models for subsequent cardiac events. Values are expressed as number (%) or mean ± SD.

	Hazard ratios (95% CI)	p value
Not-using ACEI	10.5 (2.08-14.18)	<0.01
Diabetes mellitus	2.41 (1.23-6.95)	0.01
Smoking cessation after MI	0.33 (0.15-0.81)	0.01
Not-using statin	1.45 (1.02-2.95)	0.04
CYP2J2 G-50T	2.51 (1.09-5.78)	0.03
Not-using β-blocker	1.46 (0.99-3.29)	0.06
Multiple vessel disease	1.76 (0.88-7.56)	0.26
Systemic hypertension	1.57 (0.84-3.57)	0.32
Thrombolytic therapy	1.52 (0.25-8.40)	0.50
LVEF <45%	1.34 (0.87-10.56)	0.55
Primary PTCA	1.08 (0.80-10.12)	0.43
Killip's classification ≥ II	1.48 (0.59-8.76)	0.34
Age (>40 years-old)	1.32 (0.50-2.22)	0.65

The variables as DM, hypertension, smoking cessation after MI, multiple (>2-vessel) coronary disease, medical therapies with β-blockers, ACEI, or statins in traditional risk factors; and the polymorphism of *CYP2J2* promoter G-50T genotype in genetic factors were put in the multiple logistic regression analysis. That analysis showed that the polymorphism of *CYP2J2* promoter G-50T genotype, DM, smoking cessation and use of ACEI were independent survival predictors. Values are expressed as number (%) or mean ± SD.

Table 9. Multivariate analysis with Cox regression method assessing both traditional and genetic risk factors for subsequent cardiac events

3.6 The modification effect and gene–environment interaction of smoking cessation

We also analyzed the effect of smoking cessation after the smoker's index MI. We divided the smoking patients into two groups, based on their successful smoking cessation or not after the index MI and found that successful smoking cessation could improve the outcome (successful smoking cessation: event vs. event-free, 25% vs. 46.3%, HR 0.26, 95% CI 0.11 to 0.42; current smoking after index MI: event vs. event-free, 60% vs. 28.4%, HR 3.91, 95% CI 2.37 to 8.86; P=0.003 for HR difference). Gene–environment interactions were analyzed for the polymorphism of *CYP2J2* promoter G-50T genotype. Among the successful smoking cessation subjects, the risk of subsequent cardiovascular events was 1.6-fold higher among the G allele subgroup when compared with the T allele carrying subjects. With the same genetic background as T genotype, their risk was also 2.1-fold higher among current smokers. However, among patients who carried the G allele, the current smoking behavior increased the risk to 7.2-fold higher (Fig. 3). It seems that the risk could be lower after smoking cessation, even among high-risk gene carrying patients.

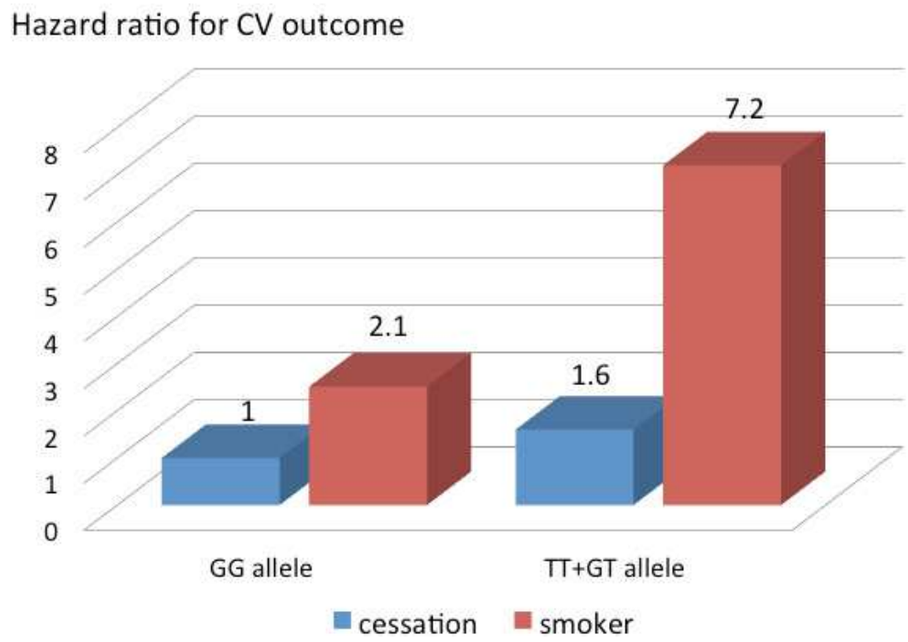

Fig. 3. Modification and interaction effects of the *CYP2J2* G-50T gene polymorphism and the cessation of smoking for the risk of subsequent cardiac events

4. Discussion

The present study investigates the association between the polymorphism of *CYP2J2* promoter G-50T genotype and the onset of premature MI in Taiwanese population. We found a higher frequency of T allele in patients with premature MI than in control subjects. There was a significant synergistic interaction between this polymorphism and the smoking behavior for the onset of MI at younger age in Taiwan.

4.1 Mechanism of the association between the polymorphism of the *CYP2J2* promoter G-50T genotype and premature MI

The polymorphism of *CYP2J2* promoter G-50T gene has been described among different disease status groups, including hypertension and CAD (Spieker et al., 2004; King et al., 2005). Spieker et al had demonstrated a functional relevance of this genetic variant by the method of electrophoretic mobility shift assays in human umbilical vein endothelial cells (Spieker et al., 2004). They found a functional consequence of reduced Sp1-DNA binding on transcriptional activation of the *CYP2J2* gene by using transfection studies *in vitro*. The construction containing the wild type promoter induced a 2-fold higher promoter activity compared with the mutant G-50T construct cells. In subjects of documented CAD, the frequency of the G-50T polymorphism was much higher. In our study, we also demonstrate that patients with *CYP2J2* G-50T allele have higher possibility of premature MI. In addition, the T allele in the promoter region of *CYP2J2* gene may functionally reduce EETs activities in the atherosclerotic vasculature, which was supported by the evidence of relationship between the genotype G-50T and the reduced EETs activities in these MI patients. This gene-phenotype association of the G-50T mutation in this promoter region could be considered as one of the possible causes to enhance the vulnerability of the atherosclerotic plaque under stimulation.

4.2 The synergistic effects of the *CYP2J2* G-50T genotype and smoking behavior

In our subgroup analysis, we also demonstrate this gene-environment interaction between smoking behavior and the *CYP2J2* G-50T polymorphism. Among non-smoker groups, the risk of MI in patients with T allele (*CYP2J2* GT+TT) is significantly higher when compared with the *CYP2J2* GG genotype patients (OR 1.43 and 1.0, respectively). The smoking behavior alone can increase 3-fold risk of MI in patients with lower activity of *CYP2J2* GG genotype at the promoter region. However, those smokers carrying the T allele polymorphism had a 5.6-fold higher risk of young MI when compared with non-smoking non-carriers.

Arachidonic acid metabolites contribute to the regulation of vascular tone and therefore tissue blood flow (Imig 2000; Gauthier et al., 2004). The vascular endothelium metabolizes arachidonic acid by cytochrome P450 epoxygenases to epoxyeicosatrienoic acids or EETs. In the vasculature, EETs are key components of cellular signaling cascades that cumulate in the activation of smooth muscle potassium channels to induce membrane hyperpolarization and vascular relaxation. Smoking habit might induce the hypercoagulable state by increasing platelet aggregability and had been recognized as a potent risk factor for premature MI (Teng et al., 1994).

In current gene-phenotype functional study, we successfully demonstrated that this genetic variant could influence the active EETs metabolites concentrations among premature MI subjects. Patients carrying T allele at promoter region of *CYP2J2* gene thus had lower median value of 14,15 EETs concentrations, which might protect their coronary vasculatures. In addition, smoking could alter the metabolites of arachidonic acid (Ye et al., 2004). This effect was observed, in our study, more significantly among *CYP2J2* G-50T polymorphism carriers whom probably were more prone to the oxidative stress damage due to their impaired EETs functions. These combination effects might explain the possible mechanism for the synergistic effect of smoking behavior and the functional change of EETs activities by polymorphism with different genotypes in its promoter gene.

4.3 Modification effects of smoking cessation and its association with gene variation on prognosis

Smokers have twice the risk of dying of coronary heart disease or stroke, and the risk diminishes by half in the first year after cessation. After 5–15 years of smoking cessation, the risk of both stroke and heart disease drops to the level of never-smokers. Previous studies usually used the history of smoking rather than current status of smoking for analysis (Li et al., 2002; Sacks et al., 1996). Our study analyzed the influence of smoking cessation on the prognosis following MI and found that those who kept on smoking could have a higher risk for subsequent coronary events when compared with those who stopped smoking. Moreover, among patients carrying higher risk genetic background, which indicated the T allele gene, the benefit was even greater from smoking cessation. It seems that a gene–environment modification relationship exists between smoking behavior and the *CYP2J2* gene variation.

In fact, the smoking behavior alone is a potent risk factor for MI at a young age (Teng et al., 1994; Liu et al., 2003). Smoking, in supporting of our current *in vitro* and *in vivo* findings, can also reduce the activities of DHET and may explain partially the possible mechanism for the smoking behavior alone or its interaction with gene variation to change the *CYP2J2* gene activity. Our findings also suggest that successful smoking cessation is very important and can improve the cardiovascular outcome, especially among those patients carrying high-risk genes.

5. Conclusion

There was a significant association between the polymorphism of G-50T genotype in the promoter region of *CYP2J2* gene and premature MI in Taiwan. Both the *CYP2J2* G-50T genotype and smoking were independent risk factors for young MI population. A synergistic effect between these two risk factors for the premature onset of MI had been shown in subgroup analyses. In addition, there was a significant association between the *CYP2J2* G-50T genotype and the prognosis after index premature MI. Successful smoking cessation after MI also could reduce the incidence of recurrent coronary events, especially among high-risk genetic background populations. Such findings lend credence to the concept that genetics and environment should not be viewed as independent risk factors for a particular disease; rather, environment and genetics interact with each to determine overall health.

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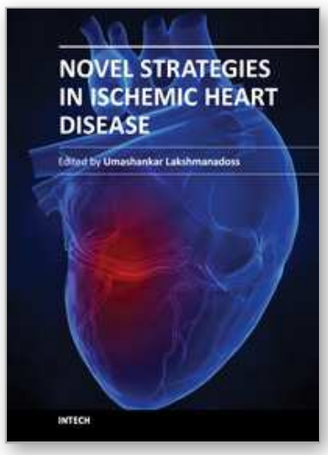

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