ORIGINAL ARTICLE

Genetic polymorphism of *CYP1A2* increases the risk of myocardial infarction

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Background: There is growing evidence that DNA damage caused by mutagens found in tobacco smoke may contribute to the development of coronary heart disease (CHD). In order to bind to DNA many mutagens require metabolic activation by cytochrome P450 (CYP) 1A1 or CYP1A2. The objective of this study was to determine the effects of *CYP1A1* and *CYP1A2* genotypes on risk of myocardial infarction (MI) and whether smoking interacts with genotype to modify risk.
Methods: Subjects (n = 873) with a first acute non-fatal MI and population based controls (n = 932) living in Costa Rica, matched for age, sex, and area of residence, were genotyped for *CYP1A1*2A* and *CYP1A2*1F* by restriction-fragment length polymorphism (RFLP)-PCR, and smoking status was determined by questionnaire.
Results: After adjusting for matching variables and potential confounders, no association was observed between *CYP1A1* genotype and risk of MI. Compared to individuals with the high inducibility *CYP1A2*1A/*1A* genotype, the adjusted odds ratio and 95% confidence intervals for risk of MI were 1.19 (0.97 to 1.47) for the **1A/*1F* genotype and 1.55 (1.10 to 2.18) for the **1F/*1F* genotype. No significant interactions were observed between smoking and either *CYP1A1* or *CYP1A2* genotype.
Conclusions: The low inducibility genotype for *CYP1A2* was associated with an increased risk of MI. This effect was independent of smoking status and suggests that a substrate of CYP1A2 that is detoxified rather than activated may play a role in CHD.

Atherosclerosis is a major cause of myocardial infarction (MI), one of the leading causes of cardiovascular death in the world.[1] Although smoking is a well established risk factor for atherosclerosis, the mechanism remains unclear. One of the mechanisms that has been proposed is an increase in the formation of DNA adducts by mutagens found in tobacco smoke, which lead to genetic alterations in blood vessels and the heart.[2–4] Indeed, there is evidence that atherosclerotic lesions may develop through monoclonal proliferation of smooth muscle cells in response to a mutational event caused by chemicals.[5 6] Mutagens such as polycyclic aromatic hydrocarbons and aromatic amines found in tobacco smoke increase the development of atherosclerotic lesions in experimental animals.[7–9] Humans and animals exposed to tobacco smoke have elevated levels of DNA adducts in various organs, especially the lungs, heart, and aorta.[9–12] Moreover, the levels of DNA adducts in arterial tissue of patients with atherosclerosis correlate with the number of cigarettes smoked and with disease severity.[11–13]

In order to form DNA adducts, most mutagens present in tobacco smoke must undergo metabolic activation by xenobiotic metabolising enzymes such as cytochrome P450 (CYP). CYP1A1 is the major enzyme that activates polycyclic aromatic hydrocarbons and CYP1A2 primarily activates aromatic amines.[14–16] The genes encoding each of these enzymes are both located on chromosome 15 and their induction is regulated by the aryl hydrocarbon receptor.[17 18] Although the liver is the major site of mutagen activation, cells of the vasculature also express a number of CYP enzymes suggesting that the target tissues involved in atherosclerosis are capable of localised activation.[19 20] Single nucleotide polymorphisms (SNPs) in *CYP1A1* and *CYP1A2* are associated with altered enzyme inducibility, which results in altered rates of mutagen activation. A T to C substitution at nucleotide 3801 (*CYP1A1*2A*) in the 3′ non-coding region of *CYP1A1* increases the inducibility of the enzyme,[21] and an A to C substitution in intron 1 at position −163 (*CYP1A2*1F*) downstream of the first transcribed nucleotide of *CYP1A2* is associated with decreased enzyme inducibility.[22 23] Polymorphisms of mutagen activating enzymes have been reported to modify susceptibility to smoking induced cancer,[24] yet few studies have investigated whether these polymorphisms modify the risk of MI associated with smoking.[25] The objective of this study was to determine the effects of *CYP1A1* and *CYP1A2* genotypes on risk of MI and whether smoking interacts with genotype to modify risk.

METHODS

Study design and participants

The catchment area for this study was 7071 km^2 and contained 2 057 000 people, ethnically Mestizo (as a result of four centuries of tripartite mixing of Europeans, Africans, and Amerindians) and culturally Hispanic American.[26] This area included 36 counties in the Central Valley of Costa Rica and covered a full range of socioeconomic levels, as well as urban, peri-urban, and rural lifestyles. Medical services in this area were provided by six large hospitals, which are part of the National Social Security System. Eligible cases were men and women who were survivors of a first acute MI as diagnosed by a cardiologist at any of the six recruiting hospitals in the catchment area between 1994 and 2000. In order to achieve 100% ascertainment, the hospitals were visited daily by the study fieldworkers. All cases were confirmed by two independent cardiologists according to the World Health Organization criteria for MI, which require typical symptoms plus either elevation in cardiac enzyme levels or diagnostic change in ECG.[27] Enrolment was carried out while patients were in the hospital's step down unit.

Abbreviations: CHD, coronary heart disease; MI, myocardial infarction; OR, odds ratios; RFLP, restriction-fragment length polymorphism; 95% CI, 95% confidence intervals

Patients were ineligible if they died during hospitalisation, were ⩾75 years of age on the day of their first MI, were physically or mentally unable to answer the questionnaire, had a previous hospital admission related to cardiovascular disease, or had previously participated in the study as a control.

One control for each case, matched for age (±5 years), sex, and area of residence (county), was randomly selected using information available from the National Census and Statistics Bureau of Costa Rica. Because of the comprehensive social services provided in Costa Rica, all persons living in the catchment areas had access to medical care irrespective of income. Therefore, control subjects come from the source population that gave rise to the cases and are not likely to have had cardiovascular disease that was not diagnosed because of poor access to medical care. Controls were ineligible if they were physically or mentally unable to answer the questionnaires or if they had ever had an acute MI.

The final participation rate for cases and controls was 97% and 89%, respectively. All subjects were visited at their homes for the collection of dietary and health information, anthropometric measurements, and biological specimens. Patients and controls gave informed consent approved by the Ethics Committee of the Harvard School of Public Health, the National Institute of Health Research at the University of Costa Rica, the Office of Protection from Research Risk at the NIH, and the Ethics Review Committee at the University of Toronto.

Trained interviewers administered two questionnaires consisting of closed ended questions regarding smoking, sociodemographic characteristics, socioeconomic status, physical activity, diet, use of medication, and medical history including personal history of diabetes and hypertension. Subjects were grouped into three categories of smoking status: non-smoker (never+past smoker), smoking one to nine cigarettes per day, or smoking ⩾10 cigarettes per day. Blood samples were collected in the morning at the subject's home after an overnight fast and were centrifuged to separate the plasma and leukocytes for DNA isolation by standard procedures. Detailed procedures used in this study have been previously described elsewhere.[28]

Genotyping

Genotyping was conducted by restriction-fragment length polymorphism (RFLP)-PCR, without knowledge of case-control status. Approximately 1 ng of DNA was amplified by thermal cycling using the HotStar (Qiagen, Mississauga, Canada) DNA polymerase kit with PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 U Taq, and 8 pmol of each primer set. All primers were synthesised by ACGT (Toronto, Canada). The *CYP1A1*2A* (rs4646903) polymorphism was detected as previously described[29] using the following primers: (forward) 5′-CAGGAAGAGGTGTAGCCG CT-3′ and (reverse) 5′-TAGGAGTCTTGTCTCATGCCT-3′. The primers amplify a 340 bp fragment that is cut into 200 and 140 bp fragments with the *CYP1A1*2A* allele by the *Msp*I restriction enzyme. PCR conditions included an initial denaturation at 95℃ for 15 min followed by 40 cycles of 94℃ for 30 s, 65℃ for 1 min, and 72℃ for 1 min, with a final extension at 72℃ for 7 min. The *CYP1A2*1F* (rs762551) polymorphism was detected using either of two previously described methods.[22, 23] The following primers: (forward) 5′-CACCCTGCCAATCTCAAGCAC-3′ and (reverse) 5′-AGAAGC TCTGTGGCCGAGAAGG-3′, amplify a 920 bp fragment that is cut into 709 and 211 bp fragments with the *CYP1A2*1F* allele by the *Apa*I restriction enzyme.[22] The alternative set of primers (forward: 5′-CCCAGAAGTGGAAACTGAGA-3′ and reverse: 5′-GGGTTGAGATGGAGACATTC-3′) amplify a 243 bp fragment that is cut into 124 and 119 bp fragments with the *CYP1A2*1F* allele by the *Apa*I restriction enzyme.[23] PCR conditions for genotyping *CYP1A2* using either set of primers included an initial denaturation at 95℃ for 15 min followed by 35 cycles of 94℃ for 30 s, 60℃ for 30 s, and 72℃ for 30 s, with a final extension at 72℃ for 7 min. PCR products were digested with 2 U of the corresponding restriction enzyme, resolved by 2% agarose gel electrophoresis, and stained with ethidium bromide. Bands were visualised using a FluorChem UV imaging system (Alpha Innotech, San Leandro, CA, USA).

Statistical analyses

All data were analysed using SAS version 8.1 (SAS Institute, Cacy, NC, USA). DNA was available from 2132 subjects (1034 cases and 1098 controls). A total of 327 subjects were excluded because they had missing data on potential confounders (92 cases, 90 controls) or they could not be genotyped (69 cases, 76 controls), leaving 873 cases and 932 controls for the final analysis. Differences in health characteristics and potential confounders were assessed by *t* test or χ^2 test. Risk of MI associated with genotype was estimated by calculating odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression, with the wild type as the reference.[30] The effect of combined *CYP1A1* and *CYP1A2* genotypes on risk of MI was also examined. Subjects were stratified into two groups based on the presence or absence of the *CYP1A1*2A* allele, and the effect of *CYP1A2* genotype on risk of MI was assessed within each of these groups using the wild type *CYP1A2*1A/*1A* group as the reference. The distribution of *CYP1A2* genotypes within each of the two *CYP1A1* groups was compared using χ^2 test. To evaluate potential gene-smoking interactions the risk of MI associated with genotype was determined for each category of smoking with the wild type as the reference. Interactions were tested for significance by fit models comparing −2 log (likelihood) in models that included main effects (gene and smoking) with and without the interaction term. Univariate analyses (model 1) included genotype and the matching variables age, sex, and area of residence. Multivariate analyses (model 2) included genotype, matching variables, smoking (three categories), waist to hip ratio (quintiles), physical activity (quintiles), income (quintiles), history of diabetes (yes/no), and history of hypertension (yes/no). Smoking was not adjusted for when the data were analysed for gene-smoking interactions.

RESULTS

General subject characteristics are shown in table 1. Cases had a significantly higher waist to hip ratio, lower income,

Table 1 Subject characteristics

Characteristic	Controls (n = 932)	Cases (n = 873)
Age, years (SD)	57.4 (11.3)	57.5 (11.0)
Male, n (%)	704 (76)	667 (76)
Urban residence, n (%)	658 (71)	619 (71)
Waist to hip ratio (SD)*	0.95 (0.07)	0.97 (0.07)
Smoking status, n (%)		
Never+past smokers*	712 (76)	508 (58)
1–9 cigarettes/day	98 (11)	90 (10)
⩾10 cigarettes/day*	122 (13)	275 (32)
Income, US $/month (SD)*	548 (457)	457 (405)
Physical activity, METS (SD)	1.5 (0.8)	1.5 (0.8)
History of hypertension, n (%)*	256 (27)	329 (38)
History of diabetes, n (%)*	110 (12)	201 (23)

Values are mean (standard deviation, SD) for continuous variables and number (%) for categorical variables. METS, metabolic equivalents.
*p<0.05 for cases compared to controls.

Table 2 *CYP1A1* and *CYP1A2* genotypes and risk of MI

Genotype	Controls, n (%)	Cases, n (%)	Model 1, OR (95% CI)†	Model 2, OR (95% CI)‡
CYP1A1				
*1A/*1A	444 (48)	426 (49)	1.00	1.00
*1A/*2A	382 (41)	354 (40)	0.97 (0.80 to 1.18)	0.96 (0.78 to 1.19)
*2A/*2A	106 (11)	93 (11)	0.91 (0.67 to 1.25)	0.92 (0.66 to 1.28)
CYP1A2				
*1A/*1A	461 (49)	385 (44)	1.00	1.00
*1A/*1F	387 (42)	384 (44)	1.19 (0.98 to 1.44)	1.19 (0.97 to 1.47)
*1F/*1F	84 (9)	104 (12)	1.48 (1.07 to 2.03)	1.55 (1.10 to 2.18)*

†Adjusted for age, sex, and area of residence.
‡Adjusted for age, sex, area of residence, smoking status, waist to hip ratio, income, physical activity, and history of diabetes and hypertension.
*$p<0.05$.

and were more likely to have a history of diabetes or hypertension. Controls were more likely to be non-smokers and patients were more likely to smoke ⩾10 cigarettes per day.

Frequencies of the *CYP1A1*2A* allele were 31% and 32% among cases and controls, respectively. The frequency of the *CYP1A2*1F* allele was 34% for cases and 30% for controls. Among controls, both *CYP1A1* and *CYP1A2* genotype distributions were in Hardy-Weinberg equilibrium. *CYP1A2*, but not *CYP1A1* genotype, was significantly associated with risk of MI (table 2). Compared to individuals with the high inducibility *CYP1A2*1A/*1A* genotype, the OR (95% CI) for risk of MI was 1.19 (0.98 to 1.44) for the *1A/*1F genotype and 1.48 (1.07 to 2.03) for the *1F/*1F genotype. In the multivariate models, the OR (95% CI) for risk of MI relative to those with the *1A/*1A genotype was 1.19 (0.97 to 1.47) for the *1A/*1F genotype and 1.55 (1.10 to 2.18) for the *1F/*1F genotype.

We next determined whether *CYP1A1* interacted with *CYP1A2* to modify the risk of MI. As shown in table 3, subjects with the wild type *CYP1A1* (*1A/*1A) were more likely to have the less common *CYP1A2*1F/*1F* genotype compared to carriers of the *CYP1A1*2A* allele ($p<0.001$). Thus, a formal test of interaction was not performed. Nonetheless, stratifying subjects by *CYP1A1* genotype does not appear to significantly alter the association between *CYP1A2* genotype and risk of MI.

After adjusting for the matching variables and potential confounders, smoking 1–9 cigarettes per day was associated with an increased risk of MI [1.45 (1.05 to 2.01)] and smoking ⩾10 cigarettes per day was associated with an even greater risk of MI [3.52 (2.71 to 4.58)] when compared to never+past smokers. As shown in table 4, smoking did not interact with either *CYP1A1* or *CYP1A2* genotype ($p = 0.24$ and 0.17 for interaction, respectively). Because of the reduced sample size within each category of smoking the increased risk observed with the *CYP1A2*1F/*1F* genotype did not remain statistically significant. Analyses were also performed separately for never and past smokers, but results were not materially different (data not shown).

DISCUSSION

There is growing evidence that DNA damage caused by mutagens found in tobacco smoke may accelerate the development of atherosclerosis.[2–4] Many of these mutagens require metabolic activation by xenobiotic metabolising enzymes such as CYP1A1 or CYP1A2.[14–16] Genetic polymorphisms of these enzymes are associated with altered enzyme inducibility and may, in part, explain the differences in susceptibility of individuals to smoking related heart disease. The objective of the present study was to determine whether *CYP1A1* or *CYP1A2* genotypes modify the risk of MI. Given the role of these enzymes in activating mutagens found in tobacco smoke, we also examined whether smoking interacts with genotype to modify risk of MI. We found no association between *CYP1A1* genotype and risk of MI, regardless of the level of smoking. These results are consistent with a recent study that also found no association between the *CYP1A1*2A* polymorphism and risk of MI.[25] In that study, however, the high inducibility *2A* allele was associated with an increased risk of disease progression as assessed by coronary angiography, but only among individuals smoking <20 cigarettes per day.[25] Although we were unable to assess disease progression in our study, classifying subjects into different categories of smoking (including <20 cigarettes per day) did not materially alter any of the results (data not shown). It is possible, however, that other polymorphisms of *CYP1A1* may influence the risk of MI.

To our knowledge, the current study is the first to examine the association between *CYP1A2* genotype and risk of MI. Because this enzyme activates a number of chemicals found in tobacco smoke, we expected smokers with the high inducibility *CYP1A2*1A* allele to have the greatest risk of MI. Unexpectedly, we found that individuals homozygous for the low inducibility *CYP1A2*1F* allele were at increased risk, which was not affected by the level of smoking. Thus, it appears that activation of mutagens by CYP1A2 may not play a significant role in CHD. Our observation that the low inducibility *CYP1A2* genotype increases risk of MI is consistent with a role for this enzyme in detoxification. In

Table 3 Combined *CYP1A1* and *CYP1A2* genotypes and risk of MI

CYP1A1 genotype	CYP1A2 genotype	Controls, n (%)	Cases, n (%)	Model 1, OR (95% CI)†	Model 2, OR (95% CI)‡
*1A/*1A	*1A/*1A	166 (37)	141 (33)	1.00	1.00
*1A/*1A	*1A/*1F	215 (49)	203 (48)	1.12 (0.83 to 1.50)	1.13 (0.82 to 1.56)
*1A/*1A	*1F/*1F	63 (14)	82 (19)	1.54 (1.03 to 2.29)	1.56 (1.01 to 2.42)*
*1A/*2A+*2A/*2A	*1A/*1A	295 (60)	244 (55)	1.00	1.00
*1A/*2A+*2A/*2A	*1A/*1F	172 (35)	181 (40)	1.27 (0.97 to 1.67)	1.28 (0.96 to 1.71)
*1A/*2A+*2A/*2A	*1F/*1F	21 (4)	22 (5)	1.25 (0.67 to 2.34)	1.38 (0.71 to 2.69)

†Adjusted for age, sex, and area of residence.
‡Adjusted for age, sex, area of residence, smoking, waist to hip ratio, income, physical activity, and history of diabetes and hypertension.
*$p<0.05$.

Table 4 *CYP1A1* and *CYP1A2* genotypes and risk of MI by smoking category

Genotype by smoking status	Controls, n (%)	Cases, n (%)	Model 1, OR (95% CI)*	Model 2, OR (95% CI)†
CYP1A1‡				
Never+past smokers				
*1A/*1A	342 (48)	251 (49)	1.00	1.00
*1A/*2A	287 (40)	206 (41)	0.98 (0.77 to 1.25)	1.00 (0.77 to 1.29)
*2A/*2A	83 (12)	51 (10)	0.85 (0.58 to 1.26)	0.89 (0.59 to 1.33)
1–9 cigarettes/day				
*1A/*1A	46 (47)	44 (49)	1.00	1.00
*1A/*2A	41 (42)	40 (44)	0.98 (0.53 to 1.81)	1.02 (0.52 to 2.01)
*2A/*2A	11 (11)	6 (7)	0.61 (0.20 to 1.82)	0.69 (0.20 to 2.38)
⩾10 cigarettes/day				
*1A/*1A	56 (46)	131 (48)	1.00	1.00
*1A/*2A	54 (44)	108 (39)	0.84 (0.53 to 1.33)	0.81 (0.50 to 1.31)
*2A/*2A	12 (10)	36 (13)	1.38 (0.66 to 2.89)	1.32 (0.61 to 2.85)
CYP1A2§				
Never+past smokers				
*1A/*1A	352 (49)	225 (44)	1.00	1.00
*1A/*1F	289 (41)	218 (43)	1.17 (0.91 to 1.49)	1.23 (0.95 to 1.59)
*1F/*1F	71 (10)	65 (13)	1.43 (0.98 to 2.09)	1.44 (0.97 to 2.13)
1–9 cigarettes/day				
*1A/*1A	50 (51)	38 (42)	1.00	1.00
*1A/*1F	41 (42)	39 (43)	1.27 (0.68 to 2.37)	1.15 (0.58 to 2.27)
*1F/*1F	7 (7)	13 (15)	2.41 (0.86 to 6.74)	2.08 (0.66 to 6.52)
⩾10 cigarettes/day				
*1A/*1A	59 (48)	122 (44)	1.00	1.00
*1A/*1F	57 (47)	127 (46)	1.08 (0.69 to 1.70)	1.07 (0.67 to 1.71)
*1F/*1F	6 (5)	26 (10)	2.05 (0.79 to 5.31)	1.93 (0.72 to 5.16)

*Adjusted for age, sex, and area of residence.
†Adjusted for age, sex, area of residence, waist to hip ratio, income, physical activity, and history of diabetes and hypertension.
‡*CYP1A1* and smoking interaction: p = 0.17.
§*CYP1A2* and smoking interaction: p = 0.24.

addition to activating mutagens, CYP1A2 also detoxifies compounds such as caffeine[31], which may contribute to the development of CHD.[32] Recent studies using *CYP1A2* knock-out mice have demonstrated a novel role for this enzyme in oxidative stress[33] and lipid metabolism.[34] Furthermore, cardiac patients with low CYP1A2 activity have elevated levels of inflammatory cytokines that may be involved in the pathogenesis of CHD.[35] These observations suggest that a low level of CYP1A2 has adverse biological effects that may explain the elevated risk of MI among individuals with the low inducibility **1F/*1F* genotype.

The *CYP1A2*1F* polymorphism is located in a non-coding region of the gene, but may result in differential binding of regulatory proteins to the surrounding sequence and alter *CYP1A2* expression levels.[22 23] Although this polymorphism may be in linkage disequilibrium with other SNPs that influence *CYP1A2* inducibility, a recent analysis of six SNPs in *CYP1A2* shows that the **1F* allele is not linked to any other SNP that alters enzyme function.[36] It is also unlikely that linkage disequilibrium confounded the null association we observed between the *CYP1A1*2A* allele and risk of MI. *CYP1A1*2C* is the only common allele known to be linked to *CYP1A1*2A*, but the former does not appear to be functionally significant.[37]

Given that individuals with a low inducibility *CYP1A1* genotype were reported to have lower CYP1A2 activity,[38] we considered whether the increased risk observed among those with the low inducibility *CYP1A2* genotype was even greater among those who also had the low inducibility *CYP1A1* genotype. Although a formal test of interaction was not performed because of the significant linkage between the two SNPs, the association between *CYP1A2* genotype and risk of MI was not modified by *CYP1A1* genotype. Indeed, this linkage likely explains the previously reported association between the low inducibility *CYP1A1* genotype and low CYP1A2 activity.[38]

In addition to being activated by CYP1A1 or CYP1A2, mutagens found in tobacco smoke can also be detoxified by enzymes such as the glutathione *S*-transferases. A number of studies have examined the role of genetic polymorphisms of glutathione *S*-transferases on risk of MI.[39–42] However, the results have been inconclusive. This may be due to genetic differences in other xenobiotic metabolising enzymes or differences in other exposures between the populations studied. Thus, examining genetic variability in both activating and detoxifying enzymes may be important in determining individual susceptibility to CHD caused by mutagens.

In summary, the low inducibility genotype for *CYP1A2* was associated with an increased risk of MI. This observation suggests that a substrate of CYP1A2 that is detoxified rather than activated may play a role in CHD. The role of CYP1A2 in oxidative stress, lipid metabolism, and inflammation suggests that other mechanisms may also be involved.

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