CYP1A2 and coffee intake and the modifying effect of sex, age, and smoking

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ABSTRACT

Background: The enzyme CYP1A2 (cytochrome 1A2) is involved in the metabolism of certain drugs and caffeine, and its activity can be influenced by factors such as sex, age, and smoking. The single nucleotide polymorphism (SNP) rs762551A>C, which has also been studied for its modifying effect on cardiovascular disease, has been reported to alter enzyme activity.

Objective: The objective was to study the effect of CYP1A2, sex, age, and smoking on coffee intake.

Design: Within the Rotterdam Study, a population-based cohort, all coffee drinkers for whom genome-wide association data were available were selected. Because SNP rs762551 was not on the Illumina 550 platform, SNP rs2472299 was used as a proxy, with the A allele of rs762551 linked to the G allele of rs2472299. Linear regression analyses were used to determine the effect and interaction of rs2472299, sex, and smoking on coffee intake. Adjusted geometric means of coffee intake were calculated per genotype for the different smoking and sex strata by using multivariable general linear models. A combined analysis, with the use of a “risk score,” was performed to determine the contribution of each separate factor.

Results: rs2472299G>A, female sex, and nonsmoking were significantly inversely related to coffee intake. Coffee intake was lowest in nonsmoking women homozygous for rs2472299G>A (3.49 cups/d; 436 mL). All factors contributed almost linearly to the intake of coffee, with the highest coffee intake in smoking men without the A allele (5.32 cups/d; 665 mL).


INTRODUCTION

Cytochrome 1A2 (CYP1A2) accounts for 13% of the total hepatic content of cytochrome isoenzymes and plays a role in the metabolism of various drugs, such as clozapine, olanzapine, omeprazole, erythromycin, propranolol, and paracetamol (1, 2). CYP1A2 is encoded by the CYP1A2 gene located on chromosome 15q24.1. The single nucleotide polymorphism (SNP) rs762551A>C has been reported to alter enzyme activity. The C allele, the variant allele in whites, is associated with a decrease in enzyme activity (2, 3). Polymorphisms within the CYP1A2 gene were recently shown to be associated with coffee intake (4–6). The pharmacologic effect of these variants remains to be proven with experimental studies.

The activity of CYP1A2 can be influenced by factors such as sex, age, and smoking. Sex has an influence that mainly seems to be caused by the effect of the sex steroids. Overall, women have a lower enzyme activity than men (7). Fluctuations in estradiol concentrations cause fluctuations in enzyme activity; higher estradiol concentrations result in a lower metabolic rate (8). Increasing age was previously shown to be associated with a decrease in enzyme activity, although not consistently (2, 9). Smoking is responsible for induction of CYP1A2 enzyme activity (10, 11).

Caffeine is commonly used to measure the enzyme activity of CYP1A2 by urinary caffeine metabolic ratios (3, 12, 13). Caffeine is almost completely metabolized via CYP1A2 to paraxanthine (82%), theobromine (11%), and theophylline (5%) (14). Caffeine may have several negatively experienced effects on the body, such as tachycardia, tremor, and flushing. These adverse effects are influenced by CYP1A2 activity, sex, and smoking (15). Cofee is one of the most widely consumed caffeine-containing beverages. Certain diseases or health effects have been related to coffee intake, such as a decrease in bone mineral density, a decreased risk of hormone-related cancer in women, and protection against Parkinson disease and development of diabetes mellitus (16–20). Regarding cardiovascular effects, coffee is associated with an increased risk of hypertension (21), modified by rs762551A>C (22). The effect on coronary artery disease is multifactorial and inconclusive (23).

We conducted a study to investigate the effects of CYP1A2 on coffee intake and the modifying effects of sex, age, and smoking. We hypothesized that all of these factors, possibly by
influencing enzyme activity, are related to coffee intake. The aim of our study was to confirm the results from small experimental studies with population-based data.

SUBJECTS AND METHODS

Cohort
This study was embedded in the Rotterdam Study (RS)—a prospective population-based cohort study of neurologic, cardiovascular, locomotor, and ophthalmologic diseases. All inhabitants of Ommoord, a suburb of the city of Rotterdam in the Netherlands, aged ≥55 y were invited in 1990 to participate in the study. The medical ethics committee of the Erasmus Medical Center approved the study, and informed consent was obtained from all participants. The rationale and design of the study were described elsewhere (24). The first cohort included 7983 individuals who were all interviewed and investigated at baseline in the period 1990–1993. This cohort is designated RS-I. In addition, in 2000 a second cohort (RS-II) was enrolled. All inhabitants of Ommoord aged ≥55 y at that time and not yet participating in RS-I were invited. This cohort encompasses 3011 individuals, who entered the study after consent. We included all participants of RS-I and RS-II with successful genotyping and positive coffee intake at baseline.

Outcome
Dietary intake data were collected at baseline between 1990 and 1993 for RS-I and between 2000 and 2001 for RS-II. Participants were interviewed by a trained diettian at the study center, who used a validated semiquantitative food-frequency questionnaire (25). In the interview, information on the amount and consumption frequency of food items was collected. The participants reported their habitual coffee and tea intake as number of cups per day or week, which was recalculated into cups per day (1 cup of coffee is assumed to contain 125 mL). Intake of non-caffeine-containing coffee was defined as noncoffee intake. One cup of tea was considered to contribute the caffeine content of 0.5 cups of coffee (26, 27).

Genotyping
Microarray genotyping was performed in the whole original RS cohort with proper quality DNA samples by using the Infinium II HumanHap550K Genotyping BeadChip version 3 (Illumina). These methods were described in detail previously (28). Because SNP rs762551 was not on the Illumina 550 platform, we extracted SNP rs2472299, which was in complete linkage disequilibrium with rs762551, according to HapMap data (release 22) (29). The A allele of rs762551A had a minor allele (A) frequency of 0.0005. Including the noncoffee drinkers in the analysis showed no significant difference in genotype pattern was found between the coffee drinkers and the participants who did not drink coffee (data not shown).

Genetic effect
SNP rs2472299G>A had a minor allele (A) frequency of 27.0%. The distribution of the SNP did not deviate from Hardy-Weinberg equilibrium. Intake of coffee was ~0.19 cups/d (95% CI: 0.29, 0.09; P < 0.0002) lower in carriers of one minor allele (GA) than in carriers of 2 major alleles (GG). Carriers of 2 minor alleles (AA) drank ~0.34 cup/d less (95% CI: 0.53, 0.15; P < 0.0005). In a univariate analysis, SNPs rs2472297A and rs2470893G were significantly associated with coffee intake in our population (P = 1.7 × 10−8 and P = 1.1 × 10−7, respectively). In a multivariate analysis, after adjustment for the individual effects of the SNPs, the SNP of interest, rs2472299G>A, remained significantly associated with coffee intake (P = 7.7 × 10−8), but the effect of SNP rs2472297G>A and rs2470893G>A was not significant (P = 0.14 and P = 0.10, respectively). The results for the total population and stratified for sex, age, and smoking are shown in Table 2. Including the noncoffee drinkers in the analysis showed
similar results (GA: \(-0.20\) cup/d, 95% CI: \(-0.30, -0.09\); AA: \(-0.40\) cup/d, 95% CI: \(-0.60, -0.40\)).

**Sex, age, and smoking effect**

Women drank less coffee than men, with a difference of 0.38 cup/d (95% CI: \(-0.48, -0.28\)). Age was associated with a significantly decreased coffee intake in both men and women. Per year of age, coffee intake declined by 0.07 cup/d (95% CI: \(-0.07, -0.06\)). Coffee intake declined by 0.08 cup/d (95% CI: \(-0.09, 0.07\)) in men and by 0.06 cup/d (95% CI: \(-0.06, 0.05\)) in women per year of age. Smoking was associated with a higher coffee intake than was nonsmoking. Smokers drank almost 1 cup of coffee (0.90 cup) per day more than did nonsmokers (95% CI: 0.79, 1.01).

**Effect modification**

Men showed a greater decline in coffee intake per variant allele; statistical interaction between sex and genotype was significant (P = 0.049; Table 2). This difference was more pronounced within the nonsmoking stratum, but this higher-level interaction could not be statistically confirmed (P = 0.05). No significant interaction was found between genotype and age (P = 0.16) or between genotype and smoking (P = 0.79).

The intake of coffee calculated per stratum is shown in Figure 1. Smoking men without a variant allele drank \(50\%\) more coffee than did nonsmoking women with 2 variant alleles (5.32 \(\pm\) 0.11 cup/d compared with 3.49 \(\pm\) 0.11 cup/d). To illustrate the effects after inclusion of tea to total caffeine intake, the analysis was also performed for coffee and tea combined. The effects of the separate determinants were less pronounced in this analysis, indicating that the differences in effect between the strata were less, although the effects show similar patterns (data not shown). The genotype effect was significant for coffee and tea combined (GA: \(-0.11\) cup/d, 95% CI: \(-0.21, -0.01\); AA: \(-0.41\) cup/d, 95% CI: \(-0.60, -0.21\)), but not in all strata in the stratified analysis. Effect modification by sex was not significant (P = 0.70).

### TABLE 2

Effect of rs2472299G>A on coffee intake by genotype

<table>
<thead>
<tr>
<th>Change in coffee intake</th>
<th>n</th>
<th>cups/d</th>
<th>n</th>
<th>cups/d</th>
<th>n</th>
<th>cups/d</th>
<th>P-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3315</td>
<td>1.00</td>
<td>2505</td>
<td>(-0.19 \ (= -0.29, -0.09))</td>
<td>441</td>
<td>(-0.34 \ (= -0.52, -0.16))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Men</td>
<td>1449</td>
<td>1.00</td>
<td>1079</td>
<td>(-0.30 \ (= -0.46, -0.13))</td>
<td>176</td>
<td>(-0.50 \ (= -0.82, -0.17))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Women</td>
<td>1866</td>
<td>1.00</td>
<td>1426</td>
<td>(-0.10 \ (= -0.23, -0.02))</td>
<td>265</td>
<td>(-0.24 \ (= -0.47, -0.02))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Nonsmoking</td>
<td>2426</td>
<td>1.00</td>
<td>1836</td>
<td>(-0.18 \ (= -0.28, -0.08))</td>
<td>312</td>
<td>(-0.33 \ (= -0.53, -0.13))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Smoking</td>
<td>889</td>
<td>1.00</td>
<td>669</td>
<td>(-0.21 \ (= -0.45, 0.03))</td>
<td>129</td>
<td>(-0.37 \ (= -0.81, 0.08))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Age ≤65 y(^a)</td>
<td>1640</td>
<td>1.00</td>
<td>1232</td>
<td>(-0.20 \ (= -0.37, -0.04))</td>
<td>233</td>
<td>(-0.44 \ (= -0.74, -0.14))</td>
<td>(0.049)</td>
</tr>
<tr>
<td>Age &gt;65 y(^a)</td>
<td>1675</td>
<td>1.00</td>
<td>1273</td>
<td>(-0.15 \ (= -0.26, -0.03))</td>
<td>208</td>
<td>(-0.25 \ (= -0.47, -0.02))</td>
<td>(0.049)</td>
</tr>
</tbody>
</table>

\(\text{rs}2472299G>A\) was used as a proxy for the \(\text{rs}762551A>C\) genotype; smoking data are missing for 27 participants.

The analyses were performed by using the linear regression analysis.

\(^{a}\) 95% CI in parentheses. Adjusted for sex, age, smoking, and cohort.

\(^{b}\) Statistically significant.
Of the studied factors, age was responsible for 54% of variation, followed by smoking (28%), sex (11%), and genotype (8%). The effects of every additional determinant on coffee intake, adjusted for age because of its continuous value, are shown in Figure 2. Carriage of 2 variant alleles—female sex and nonsmoking—were considered “baseline risk.” Carriage of one or no variant alleles, male sex, and smoking were considered as contributing to coffee intake. A linear effect could be distinguished in contribution of determinants.

**DISCUSSION**

Our study showed the contributing effects of genetic variance in the CYP1A2 gene, sex, age, and smoking on coffee intake. Intake was lowest in nonsmoking women with 2 variant alleles of rs2472299G>A (AA genotype) and increased with the addition of each of these factors. Smoking and sex were responsible for the largest difference in coffee intake, followed by genetic effect. Activity of CYP1A2, which is involved in the metabolism of caffeine, explains only part of these results, because the effect of sex, age, and smoking was independent of genotype and had a larger effect on the intake than this genotype.

Influence of rs762551C>A on caffeine metabolism was shown previously in experimental studies (2). In a small study (n = 146) in a Turkish population, the metabolite ratio of caffeine after coffee intake was significantly influenced by carriage of the A allele in smokers (explaining 19% of the variation in the paraxanthine/caffeine metabolite ratio). The effect of age was seen only in nonsmoking women. Smoking and sex explained most of the variation (24% and 10%, respectively), whereas rs762551C>A genotype and age explained <1%. Our study showed similar results regarding the contribution of sex, smoking, and genotype. This is also in agreement with a previous experimental study on the toxicity of caffeine. Women and nonsmokers had the highest risk of toxic symptoms within a group of 120 healthy volunteers (15).

The relation between rs762551A>C genotype and caffeine intake was previously studied in a case-control study (31). No significant association was found between coffee intake and

**FIGURE 1.** rs2472299G>A and coffee intake, per sex and smoking status. Values represent mean (±SE) coffee intake, adjusted for age and cohort; rs2472299G>A was used as a proxy for rs762551A>C, and smoking data are missing for 27 participants. Adjusted geometric means were calculated by using multivariable general linear models.

**FIGURE 2.** Risk scores for rs2472299G>A, sex, and smoking in relation to coffee intake. Values are presented as β (±SE), adjusted for age and cohort; rs2472299 is used as proxy for rs762551A>C. β Values were calculated by using linear regression analyses.
carriage of variant alleles. Three recent genome-wide association studies on coffee consumption identified a relation between SNPs within the gene-coding area of CYP1A1 and CYP1A2 and coffee intake. Two SNPs were significantly associated with coffee intake (rs2472297 and rs2470893) (4–6). These SNPs were not linked to rs762551C>A; nonetheless, this suggests a role of this gene-coding area in coffee intake. The relation between rs762551C>A and coffee intake was present but was not genome-wide significant (6). Our additional analysis showed that the effect of rs2472297 and rs2470893 was not independent of rs762551C>A. We showed the additional role of sex, age, and smoking within our population. A significant effect of SNP rs2472299G>A was present in all strata in both men and women and in both smokers and nonsmokers. Significant effect modification was present between rs2472299G>A and sex (P < 0.05).

Differences in CYP1A2 activity have been attributed to the effects of steroids (8, 32). Our study included only postmenopausal women, which resulted in less of a difference in steroid pattern. Therefore, it is hard to explain the role of these influences, which might be overruled by other factors. In nonsmokers, genetic effects seemed larger in men than in women, although the interaction between sex and genotype within the nonsmoking stratum was not significant (P = 0.05). Previous findings suggest of the influence of genetic variation on caffeine metabolism is larger in smokers (3). Smoking induces CYP1A2 by polycyclic aromatic hydrocarbons in cigarette smoke (11).

One of the strengths of our study was the size of the population with data on genotype and coffee intake. A potential limitation was that the population was selected from the RS. Participants for whom no blood could be drawn were excluded, as were those for whom genotyping was unsuccessful. However, it is highly probable that nonparticipation was independent of genotype, as we showed in comparing our study population with the total population. Selection bias was less likely, because genotype distribution did not differ between coffee drinkers and participants without coffee intake. Random misclassification of the outcome might have occurred because of the subjectivity of the intake questions in the questionnaire. The additional value of caffeine from decaffeinated coffee and tea could have led, most probably nondifferentially, to misclassification because of the definition we used. Furthermore, the participants’ habits may have been temporary. These issues are most probably nondifferential. Caffeinated sodas were not included in the analyses; however, given the age of the population, we did not expect regular consumption. Data on genotype and coffee and tea intake were prospectively recorded without knowledge of the research question. Therefore, information bias is excluded. Drug use of CYP1A2 inducers, such as omeprazole and carbamazepine, was not taken into account. The effect of these drugs is most probably nondifferential, because the use of these drugs might have resulted in either an increase or a decrease in coffee intake because of the indication and effect of these drugs. In conclusion, CYP1A2 genotype, sex, age, and smoking affect coffee intake.

The authors’ responsibilities were as follows—EMR, BHS, and LEV: designed the research; EMR: conducted the research, analyzed the data, and wrote the manuscript; AH and AGU: provided the data; ME, JMG, NA, CMvD, AH, AGU, BHS, and LEV: provided scientific input; and EMR, BHS, and LEV: had primary responsibility for the final content. All authors approved the final manuscript. No conflicts of interest were declared.

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