CYP1A2 and coffee intake and the modifying effect of sex, age, and smoking[1–3]

Eline M Rodenburg, Mark Eijgelsheim, Johanna M Geleijnse, Najaf Amin, Cornelia M van Duijn, Albert Hofman, Andre G Uitterlinden, Bruno H Stricker, and Loes E Visser

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, age, 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).

Conclusion: rs2472299G>A, linked to rs762551A>C, sex, age, and smoking significantly contribute to coffee intake. *Am J Clin Nutr* 2012;96:182–7.

INTRODUCTION

Cytochrome 1A2 (CYP1A2)[4] 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 paraxathine (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). Coffee 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

[1] From the Department of Epidemiology, Erasmus Medical Center, Rotterdam, Netherlands (EMR, ME, NA, CMvD, AH, AGU, BHS, and LEV); Inspectorate of Health Care, The Hague, Netherlands (EMR and BHS); the Department of Human Nutrition, Wageningen University Agrotechnology and Nutrition Science, Wageningen, Netherlands (JMG); The Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands (AGU, BHS, and LEV); Member of the Netherlands Consortium on Healthy Ageing, Leiden/Rotterdam, Netherlands (AH, AGU, and BHS); and the Department of Hospital Pharmacy, Erasmus Medical Center, Rotterdam, Netherlands (LEV).

[2] No financial support was received for this study.

[3] Address reprint requests to EM Rodenburg and correspondence to BH Stricker, Department of Epidemiology, Erasmus Medical Center, PO Box 2040, 3000 CA, Rotterdam, Netherlands. E-mail: e.rodenburg@erasmusmc.nl or b.stricker@erasmusmc.nl.

[4] Abbreviations used: CYP1A2, cytochrome 1A2; RS, Rotterdam Study; SNP, single nucleotide polymorphism.

Received September 21, 2011. Accepted for publication April 6, 2012.
First published online May 30, 2012; doi: 10.3945/ajcn.111.027102.

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 dietitian 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>C was linked to the G allele of rs2472299G>A (30). In addition, SNPs rs2472297 and rs2470893 were extracted to include in the analysis as covariables. These SNPs are from previous genome-wide association studies and were associated with coffee intake (4–6).

Covariables

Information on potential confounders and effect modifiers, namely genotype, sex, age, and smoking, were gathered at baseline. Smoking status was classified as current and noncurrent smoking, because of its direct induction of the CYP1A2 enzyme (10).

Data analysis

The association of SNP rs2472299G>A, sex, age, and smoking on coffee intake (in cups/d) was assessed cross-sectionally at baseline by using linear regression models, including 95% CIs. The major allele G was used as the reference to study the genotype effect (GG, GA, or AA) of the SNP. In the analyses, we adjusted for cohort (RS-I or RS-II) and the other covariables (sex, age, and smoking). Effect modification was tested for genotype, sex, age, and smoking by using interaction terms, adjusted and unadjusted for the other covariables. To illustrate the differences between the strata, adjusted geometric means were calculated per genotype, sex, and smoking stratum by using multivariable general linear models. To study the contribution of tea in the association with coffee (tea containing half of the amount of caffeine compared with coffee), we performed an additional analysis in which coffee and tea were combined as an outcome. To study the contribution of the factors genotype, sex, and smoking on intake, we created a risk model. Different variables were created of all possible combinations of the contributing factors genotype, sex, and smoking. The combination associated with the lowest coffee intake was considered baseline risk. A P value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS software (version 15.0; SPSS Inc).

RESULTS

Of the population of RS-I and RS-II, data on genotype were available for 8126 participants. Within this group, coffee intake was known for 6698 of the participants. After exclusion of participants who do not drink coffee (n = 410), 6288 participants were included in the study. Mean age was slightly lower in RS-II, and RS-II contained more current smokers than did the first cohort. Compared with the total cohort of the RS, the study population was slightly younger and consisted of relatively more men and more smokers (**Table 1**). 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 rs2472297G>A and rs2470893G>A were significantly associated with coffee intake in our population ($P = 1.7 \times 10^{-8}$ and $P = 1.1 \times 10^{-7}$, 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 ($P = 7.7 \times 10^{-6}$), 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

TABLE 1
Baseline characteristics of the participants[1]

	Total cohort		Study population	
	RS-I (n = 7983)	RS-II (n = 3011)	RS-I (n = 4473)	RS-II (n = 1815)
Female sex [n (%)]	4878 (61)	1693 (56)	2608 (58)	966 (53)
Age (y)	70.6 ± 9.8[2]	65.2 ± 8.4	67.5 ± 7.6	64.6 ± 7.9
Smoking [n (%)]	1725 (22)	942 (31)	1079 (24)	607 (33)
Genotype rs2472299G>A [n (%)]				
GG	3202 (54)	1123 (52)	2383 (53)	950 (52)
GA	2359 (40)	862 (40)	1790 (40)	721 (40)
AA	409 (7)	171 (8)	300 (7)	144 (8)
Coffee intake (cups/d)	3.8 ± 1.9	3.8 ± 2.9	4.0 ± 1.8	4.5 ± 2.5
Tea intake (cups/d)	2.9 ± 2.0	3.0 ± 2.4	2.9 ± 2.0	2.8 ± 2.3

[1] Differences in the characteristics sex, age, and smoking between the study population and the total cohort and between RS-I and RS-II were significant ($P < 0.05$). Differences in coffee and tea intake were significant between all groups, except for RS-I and RS-II in the total cohort for coffee and for RS-I (total cohort and study population) for tea. The analyses were performed by using the chi-square test for binary variables and Student's t test for continuous variables. G = 73%, A = 27%; Hardy-Weinberg equilibrium: $\chi^2 = 0.96$. RS, Rotterdam Study.
[2] Mean ± SD (all such values).

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 ± 0.11 cup/d compared with 3.49 ± 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[1]

	Change in coffee intake[2]						
	GG		GA		AA		
	n	cups/d	n	cups/d	n	cups/d	P-interaction
Total	3315	1.00	2505	−0.19 (−0.29, −0.09)[3]	441	−0.34 (−0.52, −0.16)[3]	
Men	1449	1.00	1079	−0.30 (−0.46, −0.13)[3]	176	−0.50 (−0.82, −0.17)[3]	0.049
Women	1866	1.00	1426	−0.10 (−0.23, −0.02)[3]	265	−0.24 (−0.47, −0.02)[3]	
Nonsmoking	2426	1.00	1836	−0.18 (−0.28, −0.08)[3]	312	−0.33 (−0.53, −0.13)[3]	0.79
Smoking	889	1.00	669	−0.21 (−0.45, 0.03)	129	−0.37 (−0.81, 0.08)	
Age ≤65 y[4]	1640	1.00	1232	−0.20 (−0.37, −0.04)[3]	233	−0.44 (−0.74, −0.14)[3]	0.16
Age >65 y[4]	1675	1.00	1273	−0.15 (−0.26, −0.03)[3]	208	−0.25 (−0.47, −0.02)[3]	

[1] rs2472299G>A was used as a proxy for the rs762551A>C genotype; smoking data are missing for 27 participants. The analyses were performed by using the linear regression analysis.
[2] 95% CI in parentheses. Adjusted for sex, age, smoking, and cohort.
[3] Statistically significant.
[4] Age categories based on median; lower half and upper half.

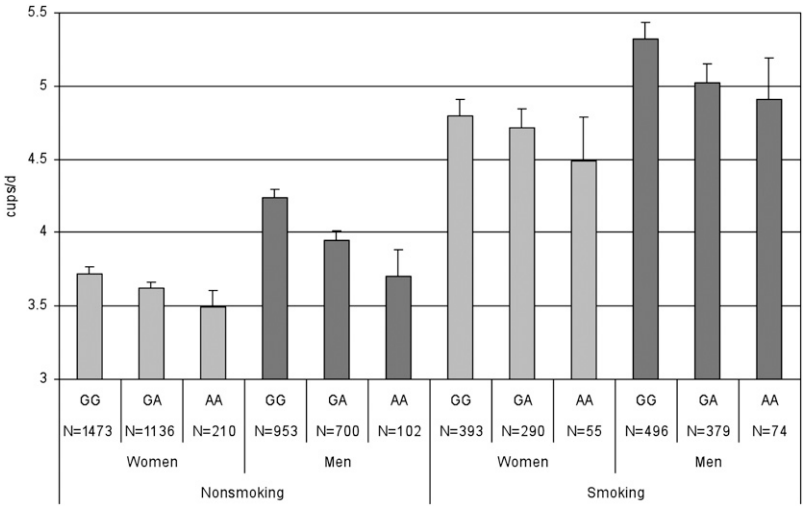

FIGURE 1. rs2472299G>A and coffee intake, per sex and smoking status. Values represent mean (±SE) coffee intake, adjusted for age and cohort; rs2472299G>A was used as a proxy for rs762551A>C, and smoking data are missing for 27 participants. Adjusted geometric means were calculated by using multivariable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

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

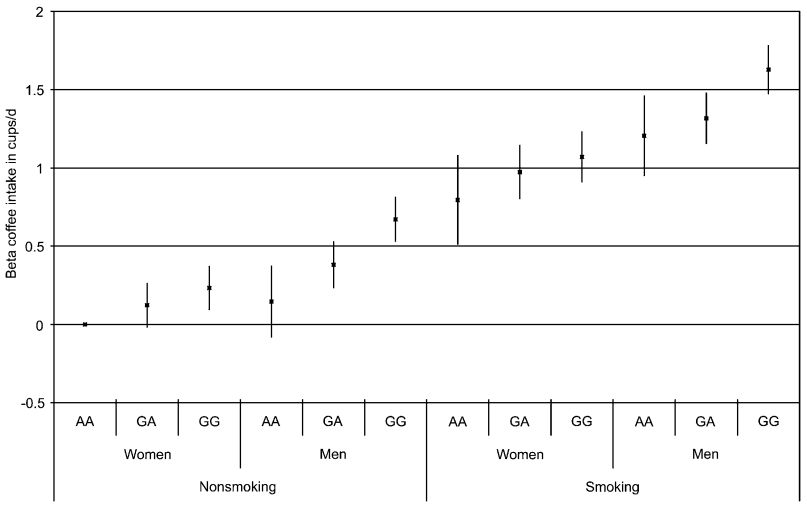

FIGURE 2. Risk scores for rs2472299G>A, sex, and smoking in relation to coffee intake. Values are presented as β (±SE), adjusted for age and cohort; rs2472299 is used as proxy for rs762551A>C. β Values were calculated by using linear regression analyses.

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.

REFERENCES

1. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994;270:414–23.
2. Gunes A, Ozbey G, Vural EH, Uluoglu C, Scordo MG, Zengil H, Dahl ML. Influence of genetic polymorphisms, smoking, gender and age on CYP1A2 activity in a Turkish population. Pharmacogenomics 2009;10:769–78.
3. Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C→A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 1999;47:445–9.
4. Amin N, Byrne E, Johnson J, Chenevix-Trench G, Walter S, Nolte IM, kConFab Investigators, Vink JM, Rawal R, Mangino M, et al. Genome-wide association analysis of coffee drinking suggests association with CYP1A1/CYP1A2 and NRCAM. Mol Psychiatry 2011.
5. Cornelis MC, Monda KL, Yu K, Paynter N, Azzato EM, Bennett SN, Berndt SI, Boerwinkle E, Chanock S, Chatterjee N, et al. Genome-wide meta-analysis identifies regions on 7p21 (AHR) and 15q24 (CYP1A2) as determinants of habitual caffeine consumption. PLoS Genet 2011;7:e1002033.
6. Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KK, Franke B, den Heijer M, Kovacs P, Stumvoll M, et al. Sequence variants at CYP1A1-CYP1A2 and AHR associate with coffee consumption. Hum Mol Genet 2011;20:2071–7.
7. Gunes A, Dahl ML. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics 2008;9:625–37.
8. Lane JD, Steege JF, Rupp SL, Kuhn CM. Menstrual cycle effects on caffeine elimination in the human female. Eur J Clin Pharmacol 1992;43:543–6.
9. Simon T, Becquemont L, Hamon B, Nouyrigat E, Chodjania Y, Poirier JM, Funck-Brentano C, Jaillon P. Variability of cytochrome P450 1A2 activity over time in young and elderly healthy volunteers. Br J Clin Pharmacol 2001;52:601–4.
10. Kalow W, Tang BK. Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. Clin Pharmacol Ther 1991;49:44–8.
11. Backman JT, Schroder MT, Neuvonen PJ. Effects of gender and moderate smoking on the pharmacokinetics and effects of the CYP1A2 substrate tizanidine. Eur J Clin Pharmacol 2008;64:17–24.
12. Kalow W, Tang BK. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. Clin Pharmacol Ther 1991;50:508–19.
13. Kot M, Daniel WA. Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat. Pharmacol Rep 2008;60:789–97.
14. Gu L, Gonzalez FJ, Kalow W, Tang BK. Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. Pharmacogenetics 1992;2:73–7.
15. Carrillo JA, Benitez J. CYP1A2 activity, gender and smoking, as variables influencing the toxicity of caffeine. Br J Clin Pharmacol 1996;41:605–8.
16. Tworoger SS, Gertig DM, Gates MA, Hecht JL, Hankinson SE. Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. Cancer 2008;112:1169–77.
17. van Dam RM, Hu FB. Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 2005;294:97–104.
18. Ganmaa D, Willett WC, Li TY, Feskanich D, van Dam RM, Lopez-Garcia E, Hunter DJ, Holmes MD. Coffee, tea, caffeine and risk of breast cancer: a 22-year follow-up. Int J Cancer 2008;122:2071–6.
19. Hallström H, Melhus H, Glynn A, Lind L, Syvanen AC, Michaelsson K. Coffee consumption and CYP1A2 genotype in relation to bone mineral density of the proximal femur in elderly men and women: a cohort study. Nutr Metab (Lond) 2010;7:12.
20. Hernán MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. Ann Neurol 2002;52:276–84.
21. Farag NH, Whitsett TL, McKey BS, Wilson MF, Vincent AS, Everson-Rose SA, Lovallo WR. Caffeine and blood pressure response: sex, age, and hormonal status. J Womens Health (Larchmt) 2010;19:1171–6.
22. Palatini P, Ceolotto G, Ragazzo F, Dorigatti F, Saladini F, Papparella I, Mos L, Zanata G, Santonastaso M. CYP1A2 genotype modifies the

association between coffee intake and the risk of hypertension. J Hypertens 2009;27:1594–601.
23. Cornelis MC, El-Sohemy A. Coffee, caffeine, and coronary heart disease. Curr Opin Clin Nutr Metab Care 2007;10:745–51.
24. Hofman A, van Duijn CM, Franco OH, Ikram MA, Janssen HL, Klaver CC, Kuipers EJ, Nijsten TE, Stricker BH, Tiemeier H, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol 2011;26:657–86.
25. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, Geleijnse JM, Hofman A, Grobbee DE, Witteman JC. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr 1998;52:588–96.
26. Schreiber GB, Maffeo CE, Robins M, Masters MN, Bond AP. Measurement of coffee and caffeine intake: implications for epidemiologic research. Prev Med 1988;17:280–94.
27. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol 1996;34:119–29.
28. Rodenburg EM, Visser LE, Danser AH, Hofman A, van Noord C, Witteman JC, Uitterlinden AG, Stricker BH. Genetic variance in CYP2C8 and increased risk of myocardial infarction. Pharmacogenet Genomics 2010;20:426–34.
29. SNP Annotation and Proxy Search. Version 2.2. 2008–2012. Available from: http://www.broadinstitute.org/mpg/snap/ldsearch.php (cited September 2011).
30. The NCBI database of genetic variation. Available from: http://www.ncbi.nlm.nih.gov/projects/SNP/ (cited September 2011).
31. Cornelis MC, El-Sohemy A, Campos H. Genetic polymorphism of the adenosine A2A receptor is associated with habitual caffeine consumption. Am J Clin Nutr 2007;86:240–4.
32. Karjalainen MJ, Neuvonen PJ, Backman JT. In vitro inhibition of CYP1A2 by model inhibitors, anti-inflammatory analgesics and female sex steroids: predictability of in vivo interactions. Basic Clin Pharmacol Toxicol 2008;103:157–65.