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Caffeine intake and *CYP1A2* variants associated with high caffeine intake protect non-smokers from hypertension

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The 15q24.1 locus, including *CYP1A2*, is associated with blood pressure (BP). The *CYP1A2 rs762551 C* allele is associated with lower CYP1A2 enzyme activity. CYP1A2 metabolizes caffeine and is induced by smoking. The association of caffeine consumption with hypertension remains controversial. We explored the effects of *CYP1A2* variants and CYP1A2 enzyme activity on BP, focusing on caffeine as the potential mediator of *CYP1A2* effects. Four observational ($n = 16\,719$) and one quasi-experimental studies ($n = 106$) including European adults were conducted. Outcome measures were BP, caffeine intake, CYP1A2 activity and polymorphisms rs762551, rs1133323 and rs1378942. *CYP1A2* variants were associated with hypertension in non-smokers, but not in smokers (*CYP1A2*-smoking interaction $P = 0.01$). Odds ratios (95% CIs) for hypertension for rs762551 CC, CA and AA genotypes were 1 (reference), 0.78 (0.59–1.02) and 0.66 (0.50–0.86), respectively, $P = 0.004$. Results were similar for the other variants. Higher CYP1A2 activity was linearly associated with lower BP after quitting smoking ($P = 0.049$ and $P = 0.02$ for systolic and diastolic BP, respectively), but not while smoking. In non-smokers, the *CYP1A2* variants were associated with higher reported caffeine intake, which in turn was associated with lower odds of hypertension and lower BP ($P = 0.01$). In Mendelian randomization analyses using rs1133323 as instrument, each cup of caffeinated beverage was negatively

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associated with systolic BP [−9.57 (−16.22, −2.91) mmHg]. The associations of *CYP1A2* variants with BP were modified by reported caffeine intake. These observational and quasi-experimental results strongly support a causal role of CYP1A2 in BP control via caffeine intake.

INTRODUCTION

Recent meta-analyses of genome-wide association studies (GWASs) have identified the *CYP1A2* locus, on chromosome 15q24.1, as being robustly associated with blood pressure (BP) and hypertension (1,2).

In humans, CYP1A2, encoded by the *CYP1A2* gene, is responsible for ∼13% of the cytochrome P450 activity of the liver (3). CYP1A2 is the main enzyme responsible for the metabolism of caffeine (1,3,7-trimethylxanthine, 137X), a purine alkaloid that occurs naturally in coffee beans. Caffeine intake is heritable (4), and this heritability appears to be quite specific to caffeine (5). Recently, a GWAS has identified the *CYP1A2* gene as being associated with caffeine consumption (6). Although several *CYP1A2* genetic variants have been identified (http://www.cypalleles.ki.se/, last accessed date 1 August 2011), their effects on the CYP1A2 enzyme activity are not clear (7).

On the short term (i.e. <3 months), regular coffee or caffeine intake increases BP (8). Also, acute consumption of caffeine at dietary levels appears to raise BP (9). Yet, a tolerance to the acute cardiovascular effects of caffeine has been described (10) and there is no clear evidence that regular caffeine intake over long periods of time increases the incidence of hypertension, as reflected by an absence of significant positive association in large-scale prospective studies (11–13) and inconsistent results in cross-sectional studies (14–16).

Smoking is a well-known inducer of CYP1A2 activity (17) and quitting smoking decreases CYP1A2 activity (18,19). The aims of this study were to analyze the associations of *CYP1A2* variants with BP and hypertension in the general adult population, exploring the potential modification of these associations by smoking, and focusing on caffeine as the potential mediator of *CYP1A2* effects on BP.

RESULTS

Table 1 lists the characteristics of the participants to the observational ($n = 16\,719$ independent people) and the experimental study ($n = 106$) including European adults. There were no evidence of departure from Hardy–Weinberg proportions ($P = 0.94$, $P = 0.58$ and $P = 0.86$, for rs762551, rs1133323 and rs1378942, respectively). Adjusting for population stratification did not alter any of the genetic associations presented in what follows (data not shown).

Association of *CYP1A2* variants with hypertension, by smoking status

Table 2 shows adjusted associations of *CYP1A2* variants with hypertension, by smoking status. Among non-smokers, the three single-nucleotide polymorphisms (SNPs) were linearly associated with hypertension. The protective allele is T for rs1133323 and A for rs1378942 and rs762551. Individuals with genotypes CT and TT at rs1133323 were 24 and 35% less likely to have hypertension compared with individuals with a CC genotype ($P = 0.0006$). Rs1378942 CA and AA individuals were 18 and 33% less likely to have hypertension than rs1378942 CC individuals ($P = 0.002$), and rs762551 AC and AA individuals were 22 and 34% less likely to have hypertension than rs762551 CC individuals ($P = 0.004$). Interactions between smoking and each *CYP1A2* variant on hypertension were significant (*P*-values for interaction: $P = 0.009$, $P = 0.01$ and $P = 0.01$ for rs1133323, rs1378942 and rs762551). *CYP1A2* genotypes were not associated with hypertension among smokers. Results were similar among never and ex-smokers (interaction tests were not significant, data not shown). Associations and trends of *CYP1A2* variants with hypertension were confirmed in HYPERGENES, with statistically significant results for rs1133323 and rs1378942 among non-smokers only (Supplementary Material, Table S1). However, the statistical interactions were not significant.

Association of CYP1A2 activity with BP before and after smoking cessation

Figure 1 shows the adjusted mean systolic (SBP) and diastolic BP (DBP) by tertiles of CYP1A2 activity before and after smoking cessation. No linear relationship was seen before smoking cessation. After smoking cessation, the adjusted mean SBP decreased from 131.5 (standard error, SE, 2.4) to 125.0 (2.4), and to 124.7 (2.4) mmHg, with increasing tertiles of CYP1A2 activity (*P*-value for trend = 0.049). The adjusted mean DBP decreased from 82.7 (1.8) to 79.5 (1.7), and to 76.6 (1.8) mmHg, with increasing tertiles of CYP1A2 activity (*P*-value for trend = 0.02).

CYP1A2 variants and caffeine consumption

Among non-smokers, the three SNPs were associated with high reported caffeine intake. Rs1133323 CT and TT individuals were, respectively, 40 and 60% more likely than rs1133323 CC individuals to report high caffeine intake (*P*-value = 0.0001). *CYP1A2* genotypes were not associated with high reported caffeine intake in smokers (Supplementary Material, Table S2).

Reported caffeine intake and hypertension or BP

Table 3 shows associations of reported caffeine intake with hypertension, by smoking status. Among non-smokers, reported caffeine intake showed a negative dose–effect relationship with hypertension. Compared with subjects who report 0 cup/day of caffeine intake, individuals who reported 1–3 cups/day, 4–6 cups/day and >6 cups/day were 13, 22 and 41% less likely to have hypertension (*P*-value for

Table 1. Demographic and risk factor characteristics for all participants, by study ($n = 16\,719$)

	CoLaus ($N = 6127$)	Bus Santé ($N = 7573$)	HYPERGENES: controls ($N = 1396$)	HYPERGENES: cases ($N = 1517$)	GenSmoke ($N = 106$)
Age (years), mean (SD)	53.1 (10.8)	56.2 (11.4)	63.8 (12.01)	49.0 (9.43)	40.9 (10.7)
Men, *n* (%)	2909 (47.5)	3793 (50.1)	812 (58.17)	1001 (65.99)	55 (51.9)
High reported caffeine intake (4+ cups/day), *n* (%)	1764 (28.8)	3928 (51.9)[a]	NA[b]	NA	NA
Current smokers, *n* (%)	1647 (26.9)	1386 (18.3)	408 (29.23)	451 (29.73)	106 (100.0)[c]
Current alcohol consumption, *n* (%)	1552 (25.3)	2339 (30.9)	NA	NA	NA
Diabetes, *n* (%)	386 (6.3)	390 (5.2)	NA	NA	NA
Contraceptive use (women), *n* (%)	261 (8.1)	NA	NA	NA	23 (45.1)
Body mass index (kg/m^2), mean (SD)	25.8 (4.5)	25.5 (4.2)	25.5 (3.55)	27.2 (3.94)	25.1 (4.2)
Hypertension, *n* (%)	2197 (35.9)	2519 (33.3)	0 (0)	1517 (100)	35 (33.0)
SBP (mmHg), mean (SD)	128.3 (17.9)	128.3 (18.8)	123.3 (9.44)	153.7 (13.94)	128.4 (15.8)
DBP (mmHg), mean (SD)	79.3 (10.8)	75.7 (10.9)	77.2 (6.45)	99.1 (8.51)	81.6 (10.6)
eGFR CKD-epi[d] (ml/min per 1.73 m^2), mean (SD)	85.7 (15.2)	NA	82.0 (14.90)	88.7 (16.95)	NA
Triglycerides (mmol/l), mean (SD)	1.40 (1.2)	1.3 (0.9)	1.4 (0.65)	1.4(0.71)	NA
Total cholesterol (mmol/l), mean (SD)	5.6 (1.0)	5.6 (1.0)	5.6 (1.01)	5.5(1.00)	NA

[a]High reported caffeine intake defined as 2+ cups/day.
[b]NA, not available.
[c]All subjects are smokersat baseline.
[d]eGFR, glomerular filtration rate estimated using the CKD-EPI formula.

Table 2. Association of CYP1A2 variants with hypertension, by smoking status, odds ratio (95%CI), in the CoLaus study

CYP1A2 variants	Non-smokers				Smokers			
rs1133323 genotype	CC	CT	TT	*P*-value	CC	CT	TT	*P*-value
N	1046	1789	767		387	668	253	
Unadjusted	Ref	0.81 (0.74–0.95)	0.71 (0.59–0.87)	0.002	Ref	1.18 (0.89–1.56)	1.16 (0.82–1.65)	0.49
Model 1[a]	Ref	0.76 (00.63–0.91)	0.64 (0.51–0.80)	<0.001	Ref	1.27 (0.92–1.74)	1.21 (0.81–1.81)	0.33
Model 2[b]	Ref	0.76 (0.63–0.92)	0.65 (0.52–0.82)	<0.001	Ref	1.27 (0.93–1.75)	1.22 (0.81–1.82)	0.32
Model 3[c]	Ref	0.76 (0.63–0.92)	0.65 (0.52–0.82)	<0.001	Ref	1.26 (0.92–1.74)	1.21 (0.81–1.81)	0.35
rs1378942 genotype	CC	CA	AA	*P*-value	CC	CA	AA	*P*-value
N	497	1826	1712		197	696	553	
Unadjusted	Ref	0.86 (0.71–1.05)	0.79 (0.64–0.97)	0.06	Ref	0.85 (0.60–1.20)	1.06 (0.74–1.50)	0.19
Model 1[a]	Ref	0.80 (0.63–1.02)	0.66 (0.52–0.84)	0.002	Ref	0.82 (0.55–1.21)	1.08 (0.72–1.62)	0.12
Model 2[b]	Ref	0.81 (0.64–1.03)	0.67 (0.53–0.86)	0.003	Ref	0.82 (0.55–1.22)	1.09 (0.73–1.63)	0.13
Model 3[c]	Ref	0.82 (0.64–1.04)	0.67 (0.53–0.85)	0.002	Ref	0.81 (0.55–1.20)	1.09 (0.72–1.62)	0.12
rs762551 genotype	CC	CA	AA	*P*-value	CC	CA	AA	*P*-value
N	366	1693	1958		148	623	664	
Unadjusted	Ref	0.86 (0.69–1.09)	0.80 (0.64–1.01)	0.14	Ref	0.90 (0.61–1.34)	1.06 (0.72–1.57)	0.42
Model 1[a]	Ref	0.77 (0.59–1.01)	0.66 (0.50–0.86)	0.004	Ref	0.80 (0.51–1.25)	1.06 (0.68–1.65)	0.13
Model 2[b]	Ref	0.78 (0.60–1.03)	0.67 (0.51–0.88)	0.006	Ref	0.80 (0.51–1.26)	1.07 (0.68–1.66)	0.13
Model 3[c]	Ref	0.78 (0.59–1.02)	0.66 (0.50–0.86)	0.004	Ref	0.79 (0.50–1.24)	1.06 (0.68–1.65)	0.11

[a]Model 1 was adjusted for age, sex, BMI, contraceptive use, cholesterol, triglycerides, diabetes, alcohol and CKD-EPI.
[b]Model 2 was adjusted as full model 1 + reported caffeine intake.
[c]Model 3 was adjusted as full model 2 + menopause.
Model 3-adjusted *P*-values for interaction tests: $P = 0.009$, $P = 0.01$ and $P = 0.01$ for rs1133323, rs1378942 and rs762551, respectively.

trends = 0.03). Reported caffeine intake was not associated with hypertension among smokers. Associations and trends were similar in never smokers and ex-smokers (Supplementary Material, Table S3). These results were confirmed in the independent population-based Bus Santé study (Supplementary Material, Table S4). Supplementary Material, Figure S1 illustrates the adjusted mean SBP and DBP in smokers and non-smokers, by number of reported caffeinated cups/day. The mean adjusted SBP and DBP decreased with the number of reported caffeinated cups/day in non-smokers (both *P*-value for trends<0.05), but not in smokers.

Association of reported caffeine intake and BP using a Mendelian randomization approach with instrumental variables

All three SNPs were appropriate instrumental variables ($F >$ 10 in the first-stage regression) (Table 4). The rs1133323 variant was the best instrument ($F = 15$). The negative

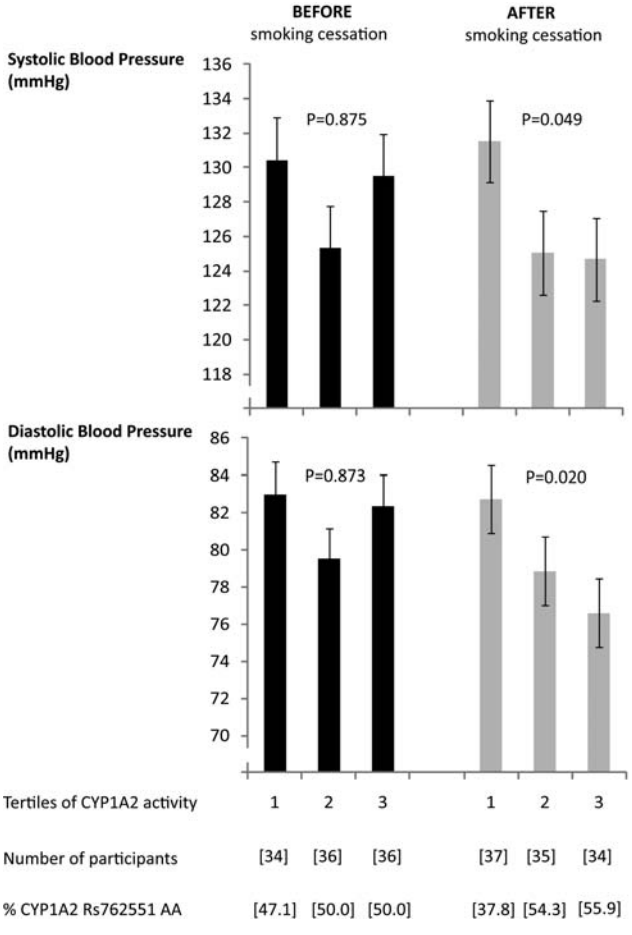

Figure 1. Before and after smoking cessation mean (SE) SBP and DBP, by tertiles of CYP1A2 activity in GenSmoke study ($n = 106$). Adjusted for age, sex, BMI, number of cigarettes smoked at baseline, smoking cessation treatment and contraceptive use.

association of reported cup of caffeine intake with BP observed in ordinary least squares (OLS) analyses was confirmed using two-stage least squares (2SLS). For both SBP and DBP, the 2SLS-based associations were stronger than the OLS-based associations—SBP OLS versus 2SLS: −0.48 (−0.76, −0.21) versus −9.57 (−16.22, −2.91), −0.48 (−0.74, −0.22) versus −9.23 (−16.12, −2.30) and −0.44 (−0.79, −0.18) versus −6.55 (−12.77, −0.33) for rs1133323, rs1378942 and rs762551, respectively (Table 4); DBP OLS versus 2SLS: −0.96 (−0.54, −0.18) versus −5.47 (−9.57,−1.38), −0.37 (−0.54, −0.20) versus −7.83 (−13.02, −2.64) and −0.33 (−0.50, −0.16) versus −6.00 (−10.61, −1.38) for rs1133323, rs1378942, and rs762551, respectively. The three SNPs were not appropriate instrumental variables in smokers (Supplementary Material, Table S5). These results support a negative causal relation between caffeine intake and BP.

Association of *CYP1A2* variants with BP among non-smokers, by caffeine intake

Significant negative associations of *CYP1A2* variants with both SBP and DBP were seen only in the presence of caffeine

Table 3. Association of reported caffeine intake with hypertension, by smoking status, odds ratio (95% CI), in the CoLaus study

	Non-smokers					Smokers				
Reported caffeine intake	0 cups/day	1–3 cups/day	4–6 cups/day	>6 cups/day	*P*-value for trend	0 cups/day	1–3 cups/day	4–6 cups/day	>6 cups/day	*P*-value for trend
N	326	2999	1006	149		69	969	465	144	
Model 1	Ref	0.85 (0.65–1.13)	0.76 (0.56–1.03)	0.64 (0.40–1.04)	0.025	Ref	1.53 (0.80–2.93)	1.56 (0.80–3.05)	0.98 (0.45–2.12)	0.16
Model 2	Ref	0.86 (0.62–1.18)	0.77 (0.54–1.09)	0.61 (0.35–1.05)	0.040	Ref	1.84 (0.84–4.03)	1.82 (0.81–4.08)	0.97 (0.38–2.44)	0.31
Model 3	Ref	0.87 (0.63–1.20)	0.78 (0.54–1.11)	0.59 (0.34–1.02)	0.033	Ref	1.83 (0.84–4.01)	1.82 (0.81–4.07)	0.96 (0.38–2.42)	0.31

Model 1 was adjusted for age, sex, BMI, contraceptive use, total cholesterol, triglycerides, diabetes, alcohol and CKD-EPI.
Model 2 was adjusted as model 1 + *CYP1A2* variants.
Model 3 was adjusted as full model 2 + menopause.
Model 3-adjusted *P*-value for interaction test: $P = 0.19$.

Table 4. Change in SBP and DBP (mmHg) by reported daily caffeinated beverage cups using an instrumental variable approach (rs1133323, rs1378942, rs762551) (the CoLaus study)

SNP	Model	SBP OLS Beta (95% CI)	*P*-value	2SLS Beta (95% CI)	*P*-value	P_{diff}	DBP OLS Beta (95% CI)	*P*-value	2SLS Beta (95% CI)	*P*-value	P_{diff}	*F*-value (first stage)
rs1133323 T allele	Unadjusted ($N = 4910$)	−0.71 (−1.04, −0.38)	<0.001	−9.58 (−17.02, −2.17)	0.019	0.018	−0.38 (−0.57, −0.018)	<0.001	−5.87 (−10.37, −1.36)	0.011	0.017	15.26
	Adjusted[a] ($N = 4887$)	−0.48 (−0.76, −0.21)	0.001	−9.57 (−16.22, −2.91)	0.005	0.008	−0.96 (−0.54, −0.18)	<0.001	−5.47 (−9.57, −1.38)	0.009	0.015	
rs1378942 A allele	Unadjusted ($N = 5481$)	−0.64 (−0.96, −0.33)	<0.001	−6.88 (−14.31, 0.55)	0.69	0.099	−0.36 (−0.54, −0.17)	<0.001	−7.79 (−13.45, −2.13)	0.007	0.010	11.00
	Adjusted[a] ($N = 5454$)	−0.48 (−0.74, −0.22)	<0.001	−9.23 (−16.12, −2.30)	0.009	0.013	−0.37 (−0.54, −0.20)	<0.001	−7.83 (−13.02, −2.64)	0.003	0.005	
rs762551 A allele	Unadjusted ($N = 5454$)	−0.62 (−0.92, −0.30)	<0.001	−3.45 (−10.00, 3.09)	0.301	0.396	−0.32 (−0.51, −0.13)	0.001	−5.21 (−9.80, −0.61)	0.026	0.036	13.44
	Adjusted[a] ($N = 5426$)	−0.44 (−0.79, −0.18)	0.001	−6.55 (−12.77, −0.33)	0.039	0.054	−0.33 (−0.50, −0.16)	<0.001	−6.00 (−10.61, −1.38)	0.011	0.016	

[a]Adjusted for age, sex, BMI, contraceptive use, cholesterol, triglyceride, diabetes, alcohol, eGFR (CKD-EPI) and menopause.
OLS, ordinary least squares; 2-SLS, two-stage least squares.

intake (Supplementary Material, Tables S6 and S7). In the presence of reported caffeinated beverage intake, the coefficients for SBP were −1.83 (−2.63, −1.03), −1.52 (−2.31, −0.73) and −1.44 (−2.27, −0.62) for rs1133323 T, rs1378942 A and rs762551 A alleles, respectively (Supplementary Material, Table S6). The coefficients for DBP were −1.03 (−1.56, −0.50), −1.23 (−1.75, −0.71) and −1.09 (−1.64, −0.55) for rs1133323 T, rs1378942 A and rs762551 A alleles, respectively (Supplementary Material, Table S7). In the absence of caffeine intake, regression coefficients were positive (yet close to zero) and tended to differ significantly from coefficients obtained in the presence of reported caffeine intake (adjusted *P*-value for caffeine intake−CYP1A2 interaction were <0.10 for rs1378942 and rs762551). Given the small number of participants reporting no intake of caffeinated beverage, we have low power for these associations and cannot exclude a small negative effect of rs1133323 T, rs1378942 A and rs762551 A variants on BP. With respect to hypertension, the *P*-values for caffeine–*CYP1A2* variants interactions for rs1133323, rs1378942 and rs762551 were 0.25, 0.01 and 0.08, respectively, in models that included covariates, which suggest that the effects of *CYP1A2* variants on hypertension in the presence differ from those in the absence of reported caffeine intake. We therefore provide evidence that reported caffeine intake modifies the effects of CYP1A2 variants on BP and hypertension.

Our results point toward caffeine intake as a likely mechanism by which the *CYP1A2* gene and enzyme activity may influence BP and risk of hypertension (Supplementary Material, Fig. S2).

DISCUSSION

Our results suggest that alleles in three *CYP1A2* variants (rs762551, rs1133323 and rs1378942) may drive, at least in part, the robust association of the 15q24.1 locus with BP and hypertension (1,2). We found that smoking, a well-known CYP1A2 inducer, modified the association of *CYP1A2* variants with hypertension. In the CoLaus study, non-smokers carrying the AA *CYP1A2* genotype, which is associated with increased CYP1A2 activity, were 35% less likely to have hypertension than non-smokers carrying the reference *CYP1A2* genotype. We did not observe such associations in current smokers. The strength of the association of *CYP1A2* variants with BP reported previously may therefore have been underestimated because results were not stratified by smoking status (1,2). Importantly, using a quasi-experimental design, we found CYP1A2 activity to be negatively associated with SBP and DBP in 106 ex-smokers, but the linear association was absent before these same 106 subjects quit smoking. This supports a causal role of *CYP1A2* variants on BP and highlights the potential functional role of CYP1A2 activity in BP control.

We found that the three selected *CYP1A2* variants, which are not highly correlated with each other, are strongly associated with reported caffeine intake. The latter confirms the results of recent GWASs on caffeine intake (20,21). The identification of *CYP1A2* genetic markers associated with caffeine intake is in line with both the activity of the CYP1A2 enzyme

and the significant heritability for caffeine use, toxicity, tolerance and withdrawal symptoms (4). *CYP1A2* variants may, therefore, represent interesting proxies for reported caffeine intake.

High reported caffeine intake was associated with a lower prevalence of hypertension only in non-smokers in two independent population-based studies: CoLaus and Bus Santé. Smoking appears to blunt the association of caffeine intake with hypertension, possibly via CYP1A2 induction. Trends were similar in CoLaus participants not aware of having hypertension (4493, 73%), implying that our results do not merely reflect a reduction in caffeine intake following hypertension diagnosis (i.e. reverse causality). Overall, our findings suggest that high caffeine intake might protect non-smokers against hypertension. Failure to account for the modifying effect of smoking may explain why no clear association of regular caffeine intake with the incidence of hypertension has been found in large-scale prospective studies (11,12) and in cross-sectional studies (14–16).

We found that the association of *CYP1A2* variants with BP was significant and negative only in the presence of reported caffeine intake, although we have low power to explore this association in the absence of reported caffeine intake. These results suggest that caffeine intake may mediate the effect of *CYP1A2* on BP. To further explore the causal role of caffeine intake on BP, we conducted a Mendelian randomization analysis, using *CYP1A2* variants as genetic instruments. We found convincing evidence that caffeine intake is causally negatively associated with SBP and DBP and may therefore protect against the risk of developing hypertension. The results of the Mendelian randomization approach are compatible with a clinically relevant effect of caffeine intake on BP (up to 9 and 7 mmHg of SBP and DBP by cup of caffeinated beverages per day, respectively).

Our findings are in line with the results of two recent large-scale prospective studies, showing that high reported coffee consumption may reduce the risk of cerebral infarction (22) or stroke (23,24). In 3837 type 2 diabetic patients, high coffee intake at baseline tended to be associated, although not significantly, with lower risk of stroke after 20 years of follow-up (25). Previous studies showing either no association (26,27) or a positive association (28) between coffee intake and stroke included a much smaller number of strokes ($<$100 events) than more recent studies ($>$1000 events) (22,23). Because hypertension is the single most important modifiable risk factor for ischemic stroke (29), a protective effect of caffeine intake on hypertension could explain part of the observed reduction in stroke in people with elevated coffee intake (22,23). In both studies (22,23), BP and/or hypertension-unadjusted prevalences decreased with higher reported coffee intake. In the Nurse's Health Study (23), the protective effect of long-term coffee consumption on stroke was present in never and past smokers but not in smokers, compatible with the hypothesis that the protective effect of caffeine intake against hypertension-related CV outcomes is obscured in the presence of smoking.

The wide inter-individual variability in CYP1A2 activity can be due to both environmental (e.g. smoking, caffeine intake) and genetic (e.g. *CYP1A2* variants) factors (30,31). In the GenSmoke study, individual change in CYP1A2 activity after smoking cessation ranged from 1.0-fold (no change) to 7.3-fold decrease (19). Yet, none of the currently identified *CYP1A2* polymorphisms seems to explain the large inter-individual variability in CYP1A2 activity (32). Therefore, unidentified genetic variations in the *CYP1A2* gene and/or in other genes controlling CYP1A2 activity, such as the aryl hydrocarbon receptor (33) or the cytochrome P450 oxidoreductase (34), could be responsible for the observed differences in CYP1A2 enzymatic activity.

One of the mechanisms by which *CYP1A2* variants, and thus CYP1A2 activity, could influence BP is via the effect of caffeine on renal segmental tubular sodium handling. Caffeine and its metabolite, paraxanthine, have known diuretic and natriuretic effects (35–37) and belong to the group of methylxanthines that are nonselective adenosine receptor antagonists (38). Caffeine exerts its natriuretic action via the adenosine A1 receptors blockade (39–43), leading to decreased proximal tubular sodium re-absorption (40,41,43).

Clinical and policy implications

Our results suggest that there is no evidence that patients with high BP need to refrain from caffeinated beverages. In contrary, in non-smokers, caffeinated beverages are associated with lower risk of high BP. Given the observed direct link between the CYP1A2 enzyme and BP, factors that modify CYP1A2 activity should be considered in the management of hypertension. These include drugs (e.g. omeprazole, clozapine), habits (smoking, caffeine) and dietary factors (cruciferous vegetables, charcoal-broiled meat) (3).

Considering the widespread use of caffeine and the high prevalence of hypertension, our results may also have large public health implications. There is currently no specific recommendation regarding caffeine intake in hypertension guidelines (44,45). Our analyses suggest that the reported protective effect of caffeine intake on stroke could be mediated via the inverse association between caffeine intake and hypertension—the major modifiable risk factor for stroke. This could guide recommendation on the appropriateness of caffeinated beverage consumption in the context of stroke prevention, which does not currently mention caffeine intake (46).

Limitations and strengths

We did not measure serum caffeine levels, but used reported caffeine intake. Although $>$70% of caffeine is provided by coffee consumption, our results are not generalizable to coffee consumption. Yet, results were similar in two independent population-based studies with different questionnaire data. Although the cross-sectional nature of our study limits causal inference for non-genetic associations, genetic associations provide information on cumulative risk even in cross-sectional designs. Oral contraceptive and cigarette smoking have been reported to, respectively, inhibit and increase CYP1A2 activity (47), and we account for that by adjusting and stratifying our analyses accordingly. Given that heavy coffee consumption can also increase CYP1A2 activity (48), we adjusted for reported caffeine intake when appropriate. There are, however, numerous other drugs that are metabolized by CYP1A2 to an extent suggesting clinical relevance (49). To

account for this, we also restricted our analyses to CoLaus individuals who were not taking any drugs ($n = 2539$, 41%). This did not alter the results materially (data not shown). Finally, the effect of coffee compounds other than caffeine on CYP1A2 is unlikely given that caffeine is the only known coffee compound to be detoxified by CYP1A2 (50). As few SNPs are associated with altered CYP1A2 activity (7), we also measured the CYP1A2 activity, using a gold standard method.

There are commonly acknowledged necessary conditions for Mendelian randomization to provide causal inference in observational epidemiology (51). Results for the Mendelian randomization approach should therefore be interpreted cautiously. For example, although the instruments (i.e. *CYP1A2* variants) were clearly correlated with caffeine intake, one condition is that the genetic instruments affect BP in no other way than through caffeine intake. In addition, our Mendelian randomization analyses resulted in wide confidence intervals and low precision as is usually the case for genetic instruments in common complex human disease. Also, we cannot exclude that the true causal variant may be in linkage disequilibrium with these alleles. Finally, the absence of association among smokers could be real or due to a lack of power. If the effect were the same among smokers and non-smokers, the power to detect it among smokers in this study would be $<50\%$. However, (i) the different direction of the associations in smokers and non-smokers, (ii) the absence of clear trends in smokers, (iii) the presence of statistical interactions between smoking status and *CYP1A2* variants and (iv) the presence of significant association of CYP1A2 activity with BP after, but not before, smoking cessation in the same subjects strongly suggest that the effects of *CYP1A2*, CYP1A2 activity and caffeine intake on BP and hypertension differ in non-smokers and in smokers.

In summary, our results based on gene–environment interaction, quasi-experimental data and the Mendelian randomization approach provide strong evidence that caffeine mediates the effect of *CYP1A2* on BP and hypertension, and that smoking modifies these associations. The associations we found are strong, biologically credible, with dose–response relationships and, for the genetic ones, with unambiguous temporal sequence. Overall, our findings may lead to a new area of research for the prevention and treatment of hypertension.

MATERIALS AND METHODS

Details are available in the Supplementary Material.

The CoLaus study: *CYP1A2* variants, reported caffeine intake, BP and hypertension

The CoLaus study complied with the Declaration of Helsinki and was approved by the local Institutional Ethics Committee. All participants gave written informed consent. The sampling procedure of the CoLaus study was population based, with participants aged 35–75 years, and details have been described previously (52).

Assessment process, clinical and biological data

Recruitment began in June 2003 and ended in May 2006. BP was measured three times on the left arm after at least 10 min rest in the seated position, using a clinically validated automated oscillometric device (Omron® HEM-907, Matsusaka, Japan) with a standard cuff, or a large cuff if arm circumference was ≥ 33 cm (53). The average of the last two BP readings was used for analyses. Hypertension was defined as mean SBP ≥ 140 mmHg or mean DBP ≥ 90 mmHg or presence of anti-hypertensive medication. Participants self-reported their consumption of caffeinated beverages as follows: 0 cup/day, 1–3 cups/day, 4–6 cups/day and >6 cups/day. Smoking was defined as present if a participant reported to be a current smoker at the time of examination, regular alcohol consumption was defined as present for participants reporting to drink alcohol at least once a day, and contraceptive pill use was self-reported. Diabetes was defined as a fasting glucose ≥ 7 mmol/l and/or the presence of antidiabetic drug treatment (insulin or oral drugs). Additional information can be found in Supplementary Material.

Genotyping and CYP1A2 variants

Nuclear DNA was extracted from whole blood for whole-genome scan analysis, and genotyping was performed using the Affimetrix 500 K SNP chip, as recommended by the manufacturer. Overall, 91 single-nucleotide polymorphisms (SNPs) were genotyped or imputed within or near the *CYP1A2* gene (Methods in Supplementary Material). Among these, 55 had minor allele frequency $>10\%$. rs762551, a polymorphism shown to have a main effect on CYP1A2 activity (http://www.snpedia.com/index.php/Rs762551), was not among the genotyped SNPs but was imputed with good quality (r^2-hat = 0.92). We selected the *CYP1A2* SNP that is most strongly associated with (i) DBP in the GWAS (rs1378942) (2), (ii) reported caffeine intake in the CoLaus study (rs1133323) and (iii) CYP1A2 enzyme activity (rs762551) (54). Linkage disequilibrium (r^2) of these SNPs were as follows in the CoLaus study: rs762551–rs1133323, $r^2 = 0.23$; rs762551–rs1378942, $r^2 = 0.57$; rs1133323–rs1378942, $r^2 = 0.43$. Allele frequencies were estimated by the gene counting method, and departures from Hardy–Weinberg equilibrium were tested using a χ^2 test.

The GenSmoke study: CYP1A2 phenotyping (experimental study)

GenSmoke is a longitudinal study conducted at the Centre for Psychiatric Neurosciences of the Department of Psychiatry and at the University Outpatient Clinic of Lausanne, Switzerland. The study was approved by the local Institutional Ethics Committee. Written informed consent was obtained from all the participants. The study primarily aimed at assessing the inter-individual variability of the induction of CYP1A2 by smoking, as described elsewhere (19). CYP1A2 activity was determined before and 4 weeks after smoking cessation in volunteers. The 4-week duration was chosen because the inductive effect of smoking on CYP1A2 is expected to disappear within 4 weeks (55), which was confirmed in GenSmoke (19). Smoking abstinence was assessed by self-declaration and by measuring expired carbon monoxide levels (Micro

Smokerlyzer, Bedfont Scientific Ltd, Rochester, UK), and all comedications were recorded. Additional information can be found in Supplementary Material.

Individuals were asked to refrain from caffeine-containing beverages and foods on the night before the day of the scheduled test. Blood was collected 6 h after the intake of a 200 mg caffeine capsule. Before caffeine intake (hour 0) and again before blood sampling (at 6 h), compliance regarding caffeine restriction was assessed by self-declaration and a new test was programmed and performed if compliance was doubtful ($n = 2$). The paraxanthine (17X) and caffeine (137X) plasma levels were measured by gas chromatography/mass spectrometry, using a previously described method (56). The 17X/137X ratio, which is a valid marker of CYP1A2 activity (49,57), was calculated for all individuals. BP was determined by a single measure using the Omron HEM-907 Digital Blood Pressure Monitor machine, in the seated position, after at least 5 min of rest.

Statistical analyses

The CoLaus study

Continuous variables were described with means and standard deviations and categorical variables with percentages. We used multiple logistic regression to test the association between (i) CYP1A2 genotypes and hypertension, (ii) CYP1A2 genotypes and high reported caffeine intake (i.e. 4 cups of more per day) and (iii) reported caffeine intake (i.e. 0 cup/day, 1–3 cups/day, 4–6 cups/day and >6 cups/day) and hypertension, while adjusting for potential confounding factors. We used multiple linear regression to test the association between SBP/DBP and reported caffeine intake while adjusting for potential confounding factors. For genetic association analyses, we adjusted SBP/DBP for antihypertensive treatment by adding a 15/10 mmHg constant, as suggested (58). We used multiple logistic or linear regressions to test for trends. Interactions between smoking and *CYP1A2* variants, and smoking and high reported caffeine intake, were tested using likelihood ratio tests. To ensure the robustness of our findings, we conducted additional analyses in participants without any medication and in participants not aware of having hypertension, adjusting for population stratification principle components. Only those individuals for whom all covariates of interest for the purpose of this study were available were included in the analysis (99.1% of the overall cohort).

The GenSmoke study

To test the association between CYP1A2 activity and BP, we used multiple linear regression. Analyses were adjusted for age (age-squared for DBP), sex, BMI, contraceptive use, smoking cessation treatment and number of cigarettes smoked at baseline as covariates in the models. To illustrate the results graphically, we used dummy variables coding for tertiles of CYP1A2 activity. We tested the association of CYP1A2 activity with BP before and after smoking cessation.

Mendelian randomization

To explore the potential causal effect of caffeine intake on BP, we applied a Mendelian randomization approach using genetic instrumental variables (59,60). We used the number of caffeinated beverage cups as our exposure variable. The number of caffeinated beverage cups were coded as 0, 2, 5 and 7 for the 0 cup/day, 1–3 cups/day, 4–6 cups/day and >6 cups/day categories, respectively. In a first stage, we regressed the number of cups on our instrument (genotypes at rs1133323, rs1378942, rs762551). In a second stage, we regressed the SBP (similarly the DBP) on the fitted values from the first-stage regression. The regression coefficient in this second stage can be interpreted as a causal effect of caffeine intake on BP. We ensured that the instrument was sufficiently strong by checking that the *F*-value obtained in the first-stage regression was >10 (59,60). For each association of interest, we conducted both OLS regression and 2SLS regression, using the ivregress function in Stata (Stata Corporation, College Station, TX, USA). We compared OLS and 2SLS estimates using the Durbin–Hausman test (61).

Statistic methods for HYPERGENES and Bus Santé are in Supplementary Material. All analyses were conducted using Stata, version 11.0 (StataCorp LP, College Station,

TX, USA). Statistical significances for association/trend tests and interaction tests were set at *P*-value <0.05 and <0.10, respectively.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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