13C-caffeine breath test results show high dependence of aryl-hydrocarbon receptor SNPs

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Short Title: Caffeine demethylation depends on s4410790 SNPs and smoking.

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Abbreviations

CBT	^{13}C-caffeine breath test
triCBT	trimethyl-^{13}C-caffeine breath test
n3CBT	N-3-methyl-^{13}C caffeine breath test
CYP1A2	cytochrome P4501A2
SNP	single nucleotide polymorphism

AhR	aryl-hydrocarbon receptor

DECLARATIONS

Ethics

The study was approved by the Research Ethics Board of Nihon University School of Medicine (Approval No. 29-5-0, 1). The purpose of the study was explained to the subjects verbally, and the subjects provided written consent after confirming an absence of allergies to caffeine. (www.umin.ac.jp/identification no. 000036735).

Consent for publication

The corresponding author confirms that the manuscript has been read and approved for submission by all the named authors.

Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The authors' contributions were as follows: Michiko Ishii and Yukimoto Ishii conceived and designed the study, performed the statistical analysis, interpreted the data, critically revised the manuscript, and had the primary responsibility for development of the final content; Tomohiro Nakayama analyzed the SNPs; Yasuo Takahashi designed the statistical analysis and drafted the statistical methods; Satoshi Asai had primary responsibility for development of the final content; all authors read and approved the final manuscript.

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Abstract

Aim: We investigated the relationship between trimethyl-^{13}C-caffeine breath test (triCBT) and single nucleotide polymorphisms (SNPs) that are related to caffeine metabolism and consumption.

Methods: Subjects were 132 young healthy adults (median 21 years: 101 male, 31 female). Subjects completed a questionnaire that enquired about their smoking status, consumption of caffeinated drinks (including coffee, black tea, green tea), height, weight, and body mass index (BMI). DNA was extracted from saliva, and genotyping was performed using TaqMan® SNP Genotyping for cytochrome P4501A2 rs762551, rs2472297, and aryl-hydrocarbon receptor rs4410790. Trimethyl ^{13}C-caffeine (100 mg) was dissolved in distilled water and administered orally. Subsequently, breath samples were collected every 10 mins for 90 mins. Infrared spectroscopy was used to analyze the amount of $^{13}CO_2$ in the expired breath, and the sum ($\Delta^{13}CO_2$) over 90 min (S_{90m}) was calculated.

Results: All subjects had genotype CC for rs2472297. S_{90m} was not significantly different among rs762551 genotypes; however, there was a significant difference in S_{90m} among

rs4410790 genotypes. $\Delta_{13}CO_2$ was significantly affected by rs4410790 SNPs and smoking. The receiver operating characteristic area under the curve was 0.758 when rs4410790 phenotype C was considered positive. When the cutoff value was set to S_{90m} (23.4 ‰), the sensitivity and specificity were 71.4% and 72.1%, respectively. **Conclusions:** Our results suggest that caffeine demethylation is affected by rs4410790 SNPs and smoking, and that triCBT can be used to identify SNPs in rs4410790.

Introduction

The principle of ^{13}C-caffeine breath test (CBT) is based on the administration of either trimethyl-^{13}C-caffeine (triCBT), in which all methyl groups are labelled with ^{13}C, or N-3-methyl-^{13}C-caffeine (n3CBT). The use of CBT has been reported in various studies, including in the evaluation of liver function and measurement of cytochrome P450 1A2 (CYP1A2) activity [1-11].

Recent evidence suggests that caffeine intake is associated with central nervous system diseases [12-16], cardiovascular diseases [17,18], and cancer [19,20]. These studies suggest the involvement of single nucleotide polymorphisms (SNPs) that are related to

caffeine metabolism and sensitivity [12-20].

Caffeine metabolism is primarily dependent on CYP1A2 enzyme, and is affected by age, sex, smoking status, and consumption of caffeinated beverages including coffee [2,21-27]. There are various polymorphisms in CYP1A2, and SNPs rs762551 and rs2472297 are known to affect the efficiency of caffeine metabolism. Specifically, individuals with rs762551 alleles AA, AC, and CC are characterized as extensive, intermediate, and poor metabolizers of caffeine, respectively [26-28].

Caffeine metabolism is dependent on the amount of CYP1A2 enzyme, and aryl-hydrocarbon receptor (AhR) plays an important role in increasing the amount of the enzyme. AhR-coding genome exists on chromosome 7q21, which has various polymorphisms (rs4410790, rs6968865) as with CYP1A2. In particular, rs4410790 was shown to influence caffeine metabolism and the level of caffeinated beverage consumption [29].

When consumed orally, caffeine primarily undergoes N-demethylation and C-8 oxidation in the body. CBT is based on labelling of methyl groups of caffeine by ^{13}C, and the outcome of CBT is dependent on whether SNPs that are related to caffeine metabolism

affect demethylation. However, there are no studies to date that examined how CBT may be affected by SNPs that are related to caffeine metabolism and consumption. Therefore, in the present study, we performed triCBT among healthy adults to determine the effect of three SNPs that are associated with caffeine metabolism and consumption levels (rs762551, rs2472297, and rs4410790).

Materials and Methods

Study subjects

Participants were included in the study if they 1) did not have any history of hospital visits or admission in the past 6 months, 2) did not have any diseases that require medication, 3) did not have any habit of taking specific supplements, 4) did not have any abnormalities in the liver and kidney function as determined by blood tests performed within the past 6 months, and 5) tested negative for hepatitis B and C viruses. Blood pressure was measured prior to triCBT to confirm that it falls within the normal range.

A total of 132 adults (median age: 21 years, 101 male, 31 female) were included in the study. Prior to triCBT, a questionnaire was administered to obtain information about

smoking status, consumption of caffeinated beverages (e.g., coffee, black tea, green tea), height, weight, and body mass index (BMI). The amount and frequency of caffeine consumption (including coffee, black tea, green tea, and caffeinated alcohol) were determined, and the subjects were categorized into those with low consumption (L, < 200 mg/day), normal consumption (N, 200-399 mg/day), and heavy consumption (H, ≥ 400 mg/day) of caffeine (Table 1).

Study protocol

Prior to triCBT, 2 mL of saliva was collected from each subject into an Oregene®/DNA saliva collection kit (DNA Genotek Inc., Ottawa, ON, Canada).

Subjects were told not to drink any caffeinated beverages and alcohol 24 hours before triCBT. Smokers were also told not to smoke 24 hours before the test. On the day of the test, subjects were not allowed to eat and had free access to water for up to 1 hour before the test.

Control breath (1,800 mL) was collected prior to the oral administration of 100 mg trimethyl-^{13}C caffeine (ISOTEC Laboratories, Inc., IL, USA, chemical purity

specification ≥ 99.2%, molecular weight 197.17 g/mol, HPLC for chemical purity 100%, isotope enrichment 99.2%), which was dissolved in 100 mL distilled water. Subjects remained in the sitting position after the administration of caffeine, and the breath test was performed every 10 minutes for 90 minutes.

Measurement of $\Delta^{13}CO_2$

Analysis of $^{13}CO_2$ in the expired breath was performed by infrared spectroscopy (POCone, Fukuda Denshi Co., Ltd, Tokyo, Japan). The amount $^{13}CO_2$ present in each breath sample was expressed as the ratio of the change divided by the baseline measurement. The results of the breath test were expressed as Δ‰, and were calculated as the sum of change over 90 min ("S_{90m}") using the following calculations:

The results of the breath test were expressed as $\Delta^{13}CO_2$‰, and were calculated as the sum of change over 90 min (S_{90m}) using the following calculations:

The sum of $\Delta^{13}CO_2$ during a specific period (S_T) was calculated as:

$S^T = \sum_{i=0}^{N-1} \Delta^{13}CO_2 \left(\Delta t \cdot i\right)$, Δt = 10 min, N = T/10 min. With $\Delta^{13}CO_2$‰ calculated as:

$\Delta^{13}CO_2$ (‰) = $\{(^{13}CO_2/^{12}CO_{2\ tmin} - {}^{13}CO_2/^{12}CO_{2\ 0min})/ {}^{13}CO_{2\ std}\} \times 10^3$

Genotyping

Three types of SNPs related to caffeine metabolism and consumption (rs762551, rs2472297, and rs4410790) were selected. The minor allele frequency (MAF) and the nucleotide sequences around each SNP are shown in Table2.

Genomic DNA was obtained from saliva mononuclear cells using an Oragene®•DNA OG-500 Kit (DNA Genotek Inc., Ottawa, ON, Canada). The amount of DNA was adjusted to 1 ng/μL using a spectrophotometer [30]. We identified the genotypes using TaqMan® PCR with TaqMan SNP Genotyping Assays or Custom TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

For each reaction, 2 μg of DNA, 2.5 μL of TaqMan® Genotyping Master Mix (Thermo Fisher Scientific, Inc.), 2.375 μL of distilled water as a primer, and 0.125 μL of TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Inc.) as a probe were mixed. PCR reactions were each performed within individual wells of a 96-well plate that was placed in a 2720 thermal cycler (Thermo Fisher Scientific, Inc.). Thermal cycling was set to 95°C for 10 min, followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. Finally,

completed reactions were held at 4°C [30].

An ABI PRISM 7700 Sequence Detector (Thermo Fisher Scientific, Inc.) was used as a fluorescence detector for reading the endpoint of the TaqMan® PCR, and the obtained data were analyzed using Detector v. 1.7 alias (Thermo Fisher Scientific, Inc.).

Statistical analysis

χ_2 and Mann-Whitney's U tests were used to compare outcomes between two groups, and the Kruskal-Wallis test was used to compare outcomes among three groups. Fisher's exact test was used as the test of significance. When needed, dummy variables were assigned as follows: non-smoker (0) vs. smoker (1), and caffeine consumption L (1) vs. N (2) vs. and H (3). The Steel-Dwass test was performed to make multiple comparisons among three groups. The receiver operating characteristic (ROC) analysis was performed, and the area under the curve of 0.7 was set as the threshold.

Normality of $\Delta_{13}CO_2$ could not be demonstrated even after variable transformation. Thus, a generalized linear mixed model was applied to analyze the data under the following conditions:

1) Response variable: $\Delta_{13}CO_2$‰ (measured at baseline and at 10, 20, 30, 40, 50, 60, 70, 80, and 90 mins)

2) Explanatory variable: sex, age, BMI, smoking status, caffeine consumption grade, *CYP1A2 SNP* (rs762551), *AhR SNP* (rs4410790), time of measurement (baseline, 10, 20, 30, 40, 50, 60, 70, 80, and 90 mins), and study subject. Since the degree of metabolism changes overtime, subjects and time were set as random variables and others were set as fixed variables. All analyses were performed using JMP Pro ver14 (SAS Institute Inc., North Carolina, USA).

Results

Characteristics of the study subjects

Table 1 summarizes the characteristics of the study subjects, including sex, age, BMI, smoking status, and caffeine consumption grade. There was a significant difference in BMI between male and female subjects ($p = 0.010$) (Table 1).

Characteristics of the study subjects and types of SNPs

All subjects had CC allele for rs2472297. There were no significant differences in age, BMI, sex, smoking status, and caffeine consumption grade between rs762551 and rs4410790 genotypes (Table 3).

Caffeine (trimethyl-^{13}C) breath test graph (triCBT graph)

Results of all breath tests are shown as mean ± standard error. Both male (■) and female (●) subjects showed a similar trend for $\Delta^{13}CO_2$‰ characterized by an increase in the first 30-40 minutes (Figure 1a). The curves for male and females followed a similar pattern after 40 minutes.

Compared to non-smokers (●), smokers (■) showed a steep increase in $\Delta^{13}CO_2$‰ within the first 30 minutes and continued rise in $\Delta^{13}CO_2$‰ after 40 minutes (Figure 1b).

Among different caffeine consumption grades, $\Delta^{13}CO_2$‰ showed a steep increase in the heavy (H, ▲) and normal (N, ●) consumption groups compared with the low (L, ■) consumption group. However, the pattern became similar to the L group after 40 minutes (Figure 1c).

For different alleles of rs762551, all groups showed a steep increase in $\Delta^{13}CO_2$‰ in the

first 30 minutes followed by a plateau. Allele CC (▲) had a relatively lower level of $\Delta_{13}CO_2$‰ compared with alleles AA (■) and AC (●) (Figure 1d).

Allele CC (■) in rs4410790 showed a steep increase in $\Delta_{13}CO_2$‰ compared with alleles TC (●) and TT (▲). After 40 minutes, $\Delta_{13}CO_2$‰ plateaued in all groups, and allele CC had a relatively higher level of $\Delta_{13}CO_2$‰ compared with alleles TC and TT (Figure 1e).

S_{90m}

There was no difference in S_{90m} between sexes, caffeine consumption grades, and rs762551 alleles. Compared with non-smokers, smokers had a significantly higher level of S_{90m} ($p = 0.031$). There was also a significant difference in S_{90m} between rs4410790 alleles ($p < 0.0001$) (Table 4), such that S_{90m} was significantly higher for CC allele compared with TT ($p < 0.0001$) and TC ($p = 0.031$) alleles, and for TC allele compared with TT allele ($p = 0.004$) (Figure 2a). S_{90m} for rs4410790 was significantly higher for C allele compared with T (TT + TC) alleles ($p = 0.0002$) (Figure 2b).

Analysis based on a generalized linear mixed model

Factors that significantly affected $\Delta_{13}CO_2$ in expired breaths included both random variables (time and change in the metabolism overtime) and fixed variables (smoking status and rs4410790 SNPs) (Table 5). There was no significant association between $\Delta_{13}CO_2$ and age, sex, BMI, caffeine consumption grade, and rs762551 SNPs (Table 5).

The receiver operating characteristic (ROC) analysis

Figure 3 shows the ROC curves for rs4410790 genotypes and phenotypes (Figure 3a, b). AUC for genotypes CC and TT were 0.758 and 0.715, respectively, and were over the threshold level of 0.7 (Table 6). AUC was 0.758 when phenotype C was considered positive. Sensitivity and specificity were 71.4 and 72.1%, respectively, when S_{90m} of 23.4‰ was set as the cut-off value (Table 7).

Discussion

In the present study, we demonstrated that the outcomes of triCBT was affected by smoking status and rs4410790 SNPs (Tables 3, 4).

Caffeine metabolism is affected by various factors including age, sex, smoking status,

and the level of caffeine consumption [2, 21-27]. Since our subjects were within a similar age range, there was a minimal effect of age on caffeine metabolism in our findings. Similarly, there was no difference in S_{90m} between male and female subjects (Table 4). The level of CYP1A2, which is the primary enzyme involved in caffeine clearance, is higher in males than in females. However, a study demonstrated that there is no difference between males and females when caffeine metabolism is measured by the amount of urinary caffeine metabolites [31]. Our findings with triCBT were consistent with this evidence. However, caffeine metabolism can change with the estrus cycle [32], and we did not examine the estrus cycle prior to triCBT in the present study. Thus, it is still unclear whether the estrus cycle should be considered when performing triCBT in young female subjects.

Beverages that contain caffeine, such as coffee, black tea, and green tea, have a significant impact on caffeine metabolism. In the present study, we prohibited intake of caffeinated beverages 24 hours before triCBT. Although we expected that S_{90m} would be different based on the level of daily caffeine consumption, our findings did not demonstrate any significant difference (Table 4). This may be attributed to the fact that

we only had 10 subjects who were categorized as heavy consumers of caffeine (≥ 400 mg/day), and that our subjects were relatively young with a median age of 21 years. Additional studies are needed with subjects with a wider age range and caffeine consumption levels.

Various studies to date demonstrated the association between caffeine metabolism and smoking, suggesting that smoking promotes caffeine metabolism [23,24]. We also demonstrated that S_{90m} was significantly higher in smokers (n = 13) (Table 4). Our analysis based on the generalized linear mixed model also demonstrated that smoking affected the amount of $^{13}CO_2$ in the expired breath (Table 5). Polycyclic aromatic hydrocarbon in cigarette smoke induces the activity of aryl hydrocarbon hydroxylase, which subsequently increases the activity of CYP1A2 and promotes caffeine metabolism [33,34]. Thus, it is critical to confirm the smoking status of subjects prior to triCBT.

Studies demonstrated that caffeine metabolism is affected by A>C substitution at position 163 of *CYP1A2* (rs762551), as well as rs2472297-T SNP which is located between *CYP1A1* and *CYP1A2* on 15q24 and is not found in the Asian population [26,27]. In our study population, all subjects had CC genotype for rs2472297. The ratio of caffeine

and its metabolites in plasma and urine after caffeine consumption is known to affect rs762551 polymorphism. Specifically, individuals with genotypes AA, AC, and CC are known as extensive, intermediate, and poor metabolizers of caffeine, respectively [26-29]. Although we found that genotype CC had a relatively low degree of change in S_{90m} and Δ‰ compared with genotypes AA and AC, we did not observe significant differences among the rs762551 genotypes. This finding suggests that rs762551 SNPs have little impact on triCBT.

However, our findings may be attributed to the fact that 100 mg/body of ^{13}C caffeine was administered and that the expired breath was collected within a relatively short period of time (90 minutes). Previous studies that examined the association between CBT and CYP1A2 used only 2 - 5 mg/kg of ^{13}C-caffeine and collected samples of expired breath for a much longer time frame of over 8 hrs [1-11]. In order to elucidate the impact of rs762551 on triCBT, additional studies are needed to examine the impact of the amount of ^{13}C-caffeine and experimental duration.

AhR in the cytosol is required to activate CYP1A2. An increase in the amount of CYP1A2 enzyme is attributed to the amount of mRNA. The gene is activated when

aromatic hydrocarbons bind to receptor-type transcription factor AhR. The complex then binds to DNA, specifically the transcription regulatory region for CYP1 family, and increases the level of gene transcription [35]. Gene coding for AhR is found on chromosome 7q21, and is polymorphic (rs4410790, rs6968865) as with CYP1A2. *AhR* polymorphism affects the activity of *CYP* gene; in particular, rs4410790 acts as the on/off signal for CYP1A2 and affects caffeine metabolism and consumption. We demonstrated that demethylation of caffeine is primarily affected by rs4410790 SNPs (Tables 4, 5), suggesting that rs4410790 allele has an important role in regulating demethylation during caffeine metabolism. We also demonstrated that S_{90m} is significantly different among rs4410790 genotypes and phenotypes (Figure 2a, b). Collectively, our findings suggest that rs4410790 SNPs should be considered when performing triCBT.

In terms of S_{90m} determined by triCBT, it is of note that the AUC in the ROC curve for rs4410790 genotypes CC and TT were over the threshold level of 0.7 (0.758 and 0.715, respectively), and that the AUC was 0.758 when phenotype C was considered positive (Table 6). Furthermore, we demonstrated that the sensitivity and specificity were 71.4 and 72.1%, respectively, when S_{90m} of 23.4‰ was set as the cut-off value (Table 7). These

findings suggest that triCBT may be used to determine the phenotype of rs4410790.

Our findings indicate that the outcomes of triCBT is highly dependent on rs4410790 SNPs, suggesting that triCBT may be used to determine the phenotype of rs4410790. We previously obtained similar results for n3CBT [36]. However, our study subjects were limited to young Japanese adults. Additional studies are needed to validate our findings in individuals with different ages and race.

Söderberg *et al*. demonstrated that rs762551 SNP did not influence the metabolism of olanzapine, which is an atypical antipsychotic metabolized by CYP1A2. They further demonstrated that rs4410790 polymorphism is an effective genetic marker for olanzapine metabolism [37]. This suggests that triCBT may also be used to identify genetic markers of drug metabolism. Further studies are warranted to examine the association between CBT and SNPs that are associated with caffeine metabolism.

Conclusions

Our results suggest that caffeine demethylation is affected by rs4410790 SNPs and smoking, and that triCBT can be used to identify SNPs in rs4410790. This finding suggests that rs762551 SNPs have little impact on triCBT. However, our study subjects

were limited to young Japanese adults. Additional studies are needed to validate our findings in individuals with different ages and race.

References

1. Arnaud MJ, Thelin-Doerner A, Ravussin E, Acheson KJ. Study of the demethylation of [1,3,7-Me-13C] caffeine in man using respiratory exchange measurements. *Biomed Mass Spectrom*. 1980; 7:521-524.

2. Kotake AN, Schoeller DA, Lambert GH, Baker AL, Schaffer DD, Josephs H. The caffeine CO_2 breath test: dose response and route of N-demethylation in smokers and nonsmokers. *Clin Pharmacol Ther*. 1982 ;32:261-269.

3. Rost KL, Brosicke H, Brockmoller J, Scheffler M, Helge H, Roots I. Increase of cytochrome P450IA2 activity by omeprazole: evidence by the ^{13}C-[N-3-methyl]-caffeine breath test in poor and extensive metabolizers of S-mephenytoin. *Clin Pharmacol Ther.* 1992; 52:170-180.

4. Fuhr U, Rost KL. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. *Pharmacogenetics*. 1994; 4:109-116.

5. Park GJ, Katelaris PH, Jones DB, Seow F, Le Couteur DG, Ngu MC. Validity of the ^{13}C-caffeine breath test as a noninvasive, quantitative test of liver function. *Hepatology*. 2003; 38:1227-1236.

6. Park GJ, Katelaris PH, Jones DB, Seow F, Lin BP, Le Couteur DG, et al. The C-caffeine breath test distinguishes significant fibrosis in chronic hepatitis B and reflects response to lamivudine therapy. *Aliment Pharmacol Ther*. 2005; 22:395-403.

7. Schmilovitz-Weiss H, Niv Y, Pappo O, Halpern M, Sulkes J, Braun M, et al. The ^{13}C-caffeine breath test detects significant fibrosis in patients with nonalcoholic steatohepatitis. *J Clin Gastroenterol*. 2008; 42:408-412.

8. Park GJ, Wiseman E, George J, Katelaris PH, Seow F, Fung C, et al. Non-invasive estimation of liver fibrosis in non-alcoholic fatty liver disease using the ^{13}C-caffeine breath test. *J Gastroenterol Hepatol*. 2011; 26:1411-1416.

9. Rost KL, Roots I. Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios: coincidence with plasma clearance and breath test. *Clin Pharmacol Ther*. 1994; 55:402-411.

10. Rost KL, Brosicke H, Heinemeyer G, Roots I. Specific and dose-dependent enzyme induction by omeprazole in human beings. *Hepatology*. 1994; 20:1204-1212.

11. Oshikoya KA, Smith K, Sammons H, Choonara I. Decreased metabolism of ^{13}C-caffeine via hepatic CYP1A2 in marasmus and kwashiorkor based on breath test. *J Basic Clin Physiol Pharmacol*. 2015; 26:105-113.

12. Popat RA, Van Den Eeden SK, Tanner CM, Kamel F, Umbach DM, Marder K, et al. Coffee, ADORA2A, and CYP1A2: the caffeine connection in Parkinson's disease. *Eur J Neurol*. 2011; 18:756-765.

13. Chuang YH, Lill CM, Lee PC, Hansen J, Lassen CF, Bertram L, et al. Gene-Environment Interaction in Parkinson's Disease: Coffee, ADORA2A, and CYP1A2. *Neuroepidemiology*. 2016;47:192-200.

14. Facheris MF, Schneider NK, Lesnick TG, de Andrade M, Cunningham JM, Rocca WA, et al. Coffee, caffeine-related genes, and Parkinson's disease: a case-control study. *Mov Disord*. 2008; 23:2033-2040.

15. Yamada-Fowler N, Fredrikson M, Soderkvist P. Caffeine interaction with glutamate receptor gene GRIN2A: Parkinson's disease in Swedish population. *PLoS One*. 2014;9:e99294.

16. Yamada-Fowler N, Soderkvist P. Coffee, Genetic Variants, and Parkinson's Disease: Gene-Environment Interactions. *J Caffeine Res*. 2015; 5:3-10.

17. Nordestgaard AT, Nordestgaard BG. Coffee intake, cardiovascular disease and all-cause mortality: observational and Mendelian randomization analyses in 95 000-223 000 individuals. *Int J Epidemiol.* 2016; 45:1938-1952.

18. Zhou A, Hypponen E. Long-term coffee consumption, caffeine metabolism genetics, and risk of cardiovascular disease: a prospective analysis of up to 347,077 individuals and 8368 cases. *Am J Clin Nutr*. 2019; 109:509-516.

19. Inoue M, Tsugane S. Coffee Drinking and Reduced Risk of Liver Cancer: Update on Epidemiological Findings and Potential Mechanisms. *Curr Nutr Rep*. 2019; 8:182-186.

20. Sartini M, Bragazzi NL, Spagnolo AM, Schinca E, Ottria G, Dupont C, et al. Coffee Consumption and Risk of Colorectal Cancer: A Systematic Review and Meta-Analysis of Prospective Studies. *Nutrients*. 2019; 11:694-704.

21. Lambert GH, Schoeller DA, Kotake AN, Flores C, Hay D. The effect of age, gender, and sexual maturation on the caffeine breath test. *Dev Pharmacol Ther*. 1986; 9:375-388.

22. Pons G, Blais JC, Rey E, Plissonnier M, Richard MO, Carrier O, et al. Maturation of caffeine N-demethylation in infancy: a study using the $^{13}CO_2$ breath test. *Pediatr Res*. 1988; 23:632-636.

23. Rodenburg EM, Eijgelsheim M, Geleijnse JM, Amin N, van Duijn CM, Hofman A, et al. CYP1A2 and coffee intake and the modifying effect of sex, age, and smoking. *Am J Clin Nutr*. 2012; 96:182-187.

24. Bjorngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafo MR, et al. Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. *Int J Epidemiol*. 2017; 46:1958-1967.

25. Tantcheva-Poor I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. *Pharmacogenetics*. 1999; 9:131-144.

26. Djordjevic N, Ghotbi R, Bertilsson L, Jankovic S, Aklillu E. Induction of CYP1A2 by heavy coffee consumption in Serbs and Swedes. *Eur J Clin Pharmacol*. 2008; 64:381-385.

27. 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; 47:445-449.

28. Denden S, Bouden B, Haj Khelil A, Ben Chibani J, Hamdaoui MH. Gender and ethnicity modify the association between the CYP1A2 rs762551 polymorphism and habitual coffee intake: evidence from a meta-analysis. *Genet Mol Res*. 2016; 15:74-87.

29. Sulem P, Gudbjartsson DF, Geller F, Prokopenko I, Feenstra B, Aben KK, et al. Sequence variants at CYP1A1-CYP1A2 and AHR associate with coffee consumption. *Hum Mol Genet*. 2011; 20:2071-2077.

30. Shinya K, Nakayama T, Nakayama T, Yamamoto T. A case-control study between the STIM1 gene and hypertensive disorders of pregnancy. *Hypertens Res*. 2018; 41(1):39-44.

31. Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. *Handb Exp Pharmacol*. 2011; (200):33-91.

32. Lane JD, Steege JF, Rupp SL, Kuhn CM. Menstrual cycle effects on caffeine elimination in the human female. *Eur J Clin Pharmacol*. 1992; 43:543-546.

33. Parsons WD, Neims AH. Effect of smoking on caffeine clearance. *Clin Pharmacol Ther.* 1978; 24:40-45.

34. Kalow W, Tang BK. Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. *Clin Pharmacol Ther*. 1991; 49:44-48.

35. Johansson G, Gillner M, Hogberg B, Gustafsson JA. The TCDD receptor in rat intestinal mucosa and its possible dietary ligands. *Nutr Cancer*. 1982; 3:134-144.

36. Ishii M, Ishii Y, Nakayama T, Takahashi Y, Asai S. ^{13}C-caffeine breath test identifies single nucleotide polymorphisms associated with caffeine metabolism. *Drug Metabolism and Pharmacokinetics*. 2020; 35:321-328.

37. Söderberg MM, Haslemo T, Molden E, Dahl ML. Influence of CYP1A1/CYP1A2 and AHR polymorphisms on systemic olanzapine exposure. *Pharmacogenet Genomics.* 2013; 23:279-285.

Figure legends

Figure 1: Results of the caffeine (trimethyl-^{13}C) breath test graph.

The results of the breath test are expressed as Δ‰, Δ‰ calculated as: Δ‰ = $\{(^{13}CO_2/^{12}CO_{2\ tmin} - {}^{13}CO_2/^{12}CO_{2\ 0min})/\ ^{13}CO_{2\ std}\}\times 10^3$.

Caffeine (trimethyl-^{13}C) breath test (mean ± standard error): (a) ■ male vs ● female, (b) ■ smoker vs ● non-smoker, (c) caffeine consumption, ■ Low (caffeine < 200 mg/day) vs ● Normal (caffeine 200-399 mg/day) vs ▲ Heavy (caffeine ≥ 400 mg/day), (d) rs762551 polymorphisms ■ AA ● AC ▲ CC, (e) rs4410790 polymorphisms ■ CC ● TC ▲ TT.

Figure 2: S_{90m}: the sum of $\Delta^{13}CO_2$ during a specific period was calculated as follows. $S^T = \sum_{i=0}^{N-1} \Delta^{13}CO_2\left(\Delta t \cdot i\right)$, Δt = 10 min, N = T/10 min. (a) S_{90m}- rs4410790 genotypes. Values are the median quartile range. (b) S_{90m}- rs4410790 phenotypes. Values are the median quartile range. rs4410790 showed a significant difference between gene

polymorphisms. CC was significantly higher than TT and TC, and TC was higher than TT. In S_{90m} of the phenotype of rs4410790, a significant difference was observed between C and T (TT + TC), and C was significantly high.

Figure 3: The ROC curve for the S_{90m} of alleles C and T of rs4410790. (a) ROC curve for the S_{90m} of genotypes rs4410790, CC, TC, and TT. The area under the ROC curves are shown in table 5. (b) The ROC curve for S_{90m} of phenotype C and T of rs4410790, where the genotype CC is defined as the positive outcome. The area under the ROC curve is 0.757.

Figures

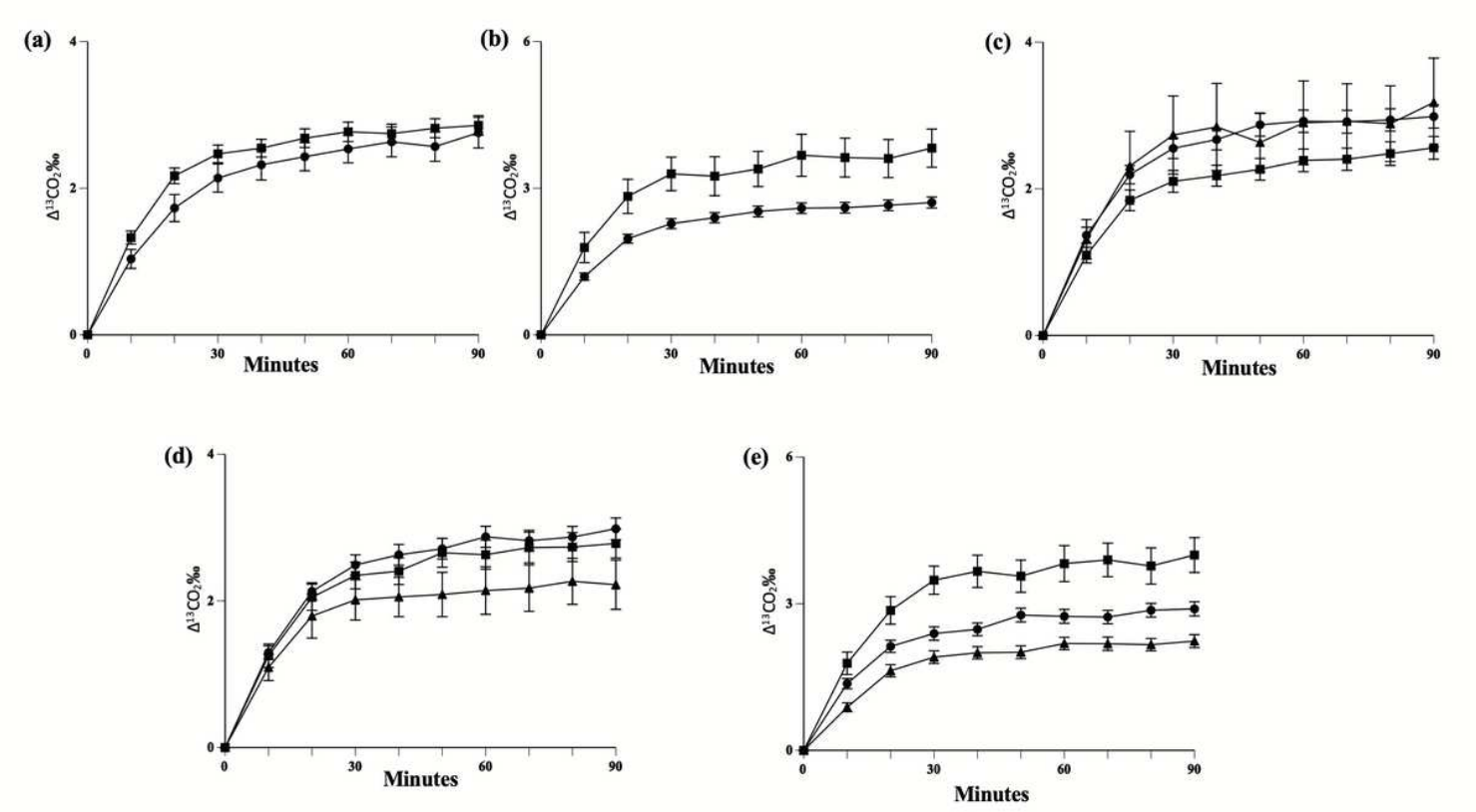

Figure 1

Results of the caffeine (trimethyl-13C) breath test graph.

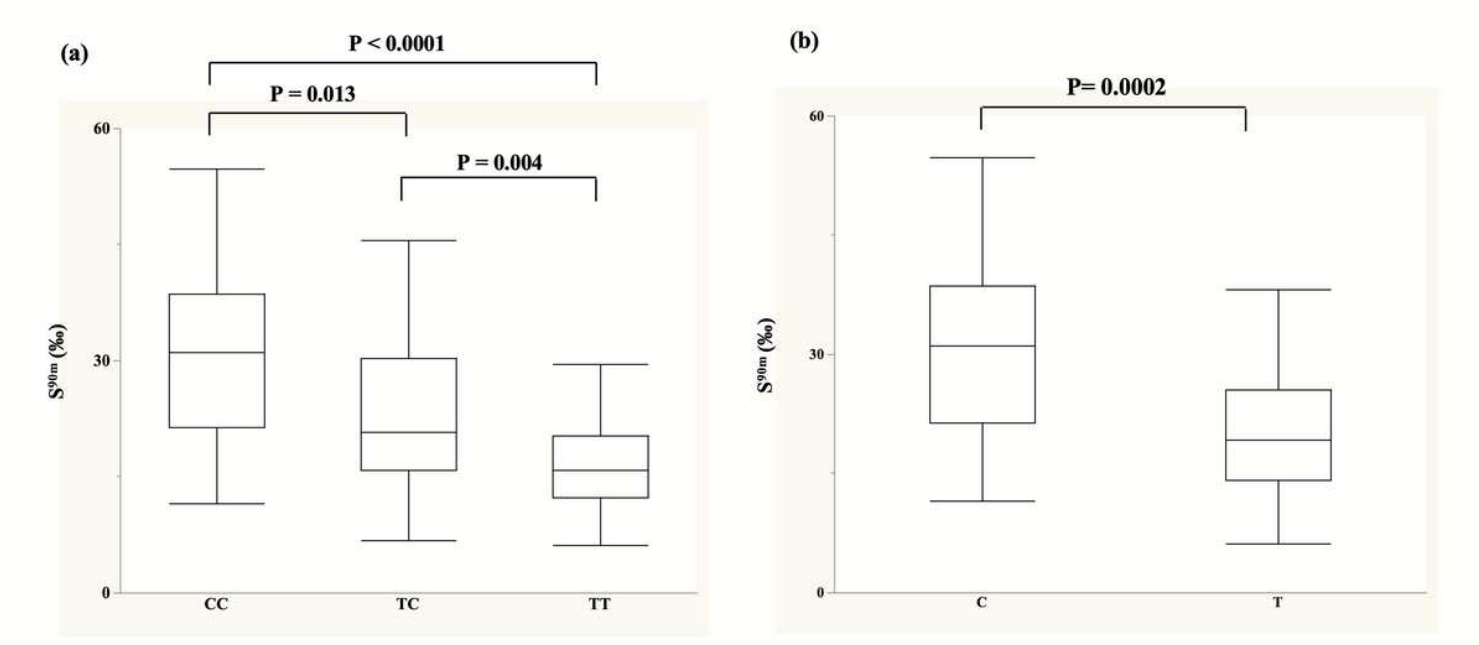

Figure 2

S90m: the sum of Δ13CO2 during a specific period was calculated as follows. S^T=∑_(i=0)^(N-1)⁢⁢Δ^13 ⁢CO⁢_2 (Δt ⁢i) ⁢ , Δt = 10 min, N = T/10 min. (a) S90m- rs4410790 genotypes. Values are the median quartile range. (b) S90m- rs4410790 phenotypes. Values are the median quartile range. rs4410790 showed a significant difference between gene polymorphisms. CC was significantly higher than TT and TC, and TC was higher than TT. In S90m of the phenotype of rs4410790, a significant difference was observed between C and T (TT + TC), and C was significantly high.

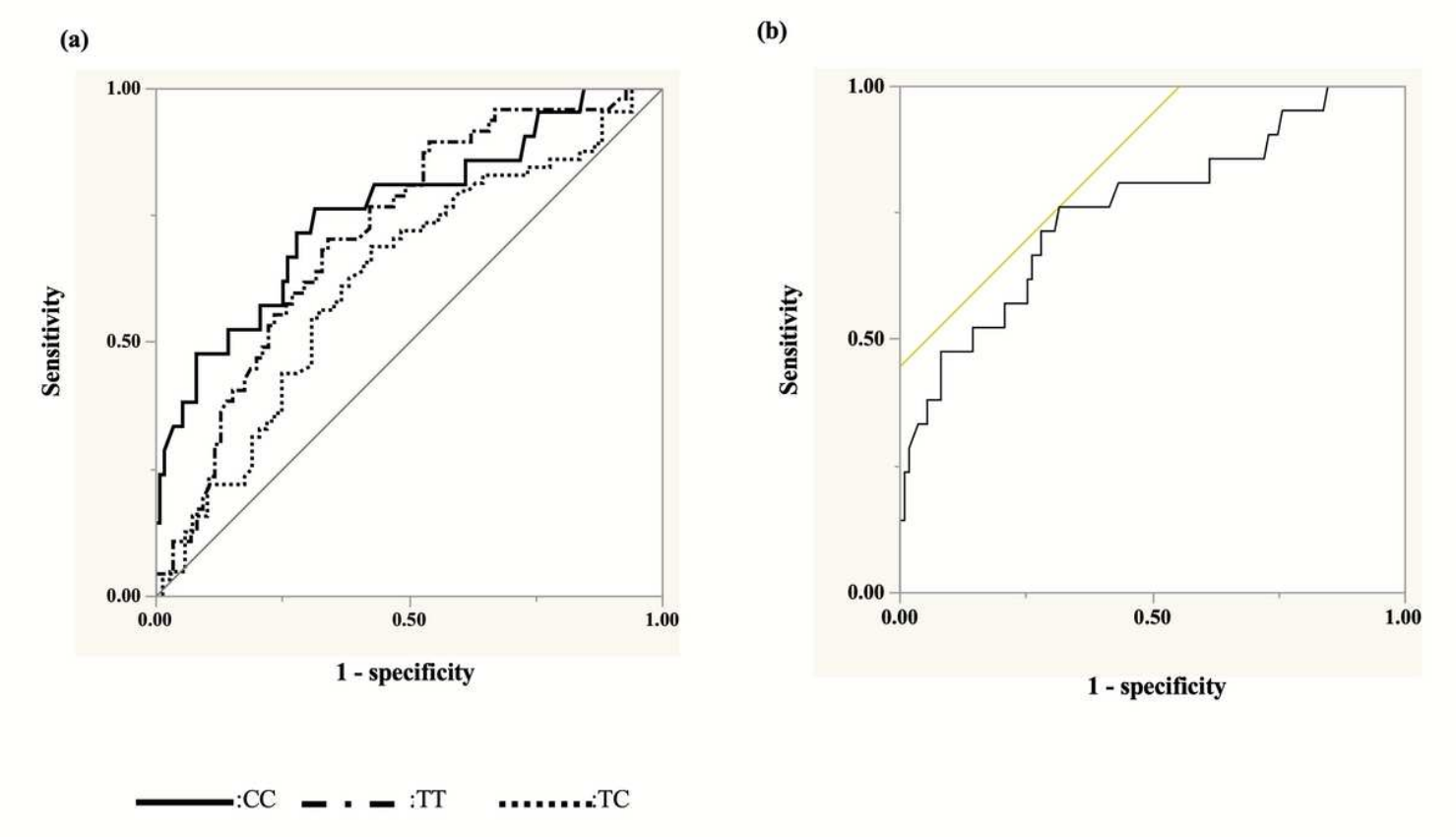

Figure 3

The ROC curve for the S90m of alleles C and T of rs4410790. (a) ROC curve for the S90m of genotypes rs4410790, CC, TC, and TT. The area under the ROC curves are shown in table 5. (b) The ROC curve for S90m of phenotype C and T of rs4410790, where the genotype CC is defined as the positive outcome. The area under the ROC curve is 0.757.

Supplementary Files

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- Tables.docx