Interaction between maternal caffeine intake during pregnancy and *CYP1A2* C164A polymorphism affects infant birth size in the Hokkaido Study

Caffeine intake and *CYP1A2* on birth size

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Abstract

Background: Caffeine, 1,3,7-trimethylxanthine, is widely consumed by women of reproductive age. Although caffeine has been proposed to inhibit fetal growth, previous studies on the effects of caffeine on infant birth size have yielded inconsistent findings. This variability may result from failure to account for individual differences in caffeine metabolism related to polymorphisms in the gene for *CYP1A2*, the major caffeine-metabolizing enzyme.

Methods: Five hundred fourteen Japanese women participated in a prospective cohort study in Sapporo, Japan from 2002 to 2005 and 476 mother-child pairs were included for final analysis.

Results: Caffeine intake was not significantly associated with mean infant birth size. When caffeine intake and *CYP1A2* C164A genotype were considered together, women with the AA genotype and caffeine intake of ≥300 mg/day had a mean reduction in infant birth head circumference of 0.8 cm relative to the reference group after adjusting for confounding factors. In subgroup analysis, only nonsmokers with the AA genotype and caffeine intake of ≥300 mg/day had infants with decreased birthweight (mean reduction, 277 g) and birth head circumference (mean reduction, 1.0 cm).

Conclusion: Nonsmokers who rapidly metabolize caffeine may be at increased risk for having infants with decreased birth size when consuming ≥300 mg caffeine/day.

Introduction

Caffeine, 1,3,7-trimethylxanthine, is widely consumed in the form of coffee, tea, and cocoa. Cytochrome P450 1A2 (*CYP1A2*) is the main enzyme involved in the metabolism of caffeine, accounting for 95% of caffeine clearance. Its primary metabolites are paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine), which account for 82, 11, and 5% of caffeine metabolites, respectively (1, 2).

Although the UK Standards Agency have recommended that women of reproductive age maintain their caffeine intake below 200mg/day (3), many women consume caffeine during pregnancy. In Japan, mean caffeine intake among women aged 30 to 39 years was 213 mg/day and 7% of women were found to consume more than 400mg/day (4) and 18% of UK women of childbearing consumed in excess of 300mg/day (5). Maternal caffeine intake can directly affect the fetus because caffeine and its metabolites readily cross the placental barrier and the fetoplacental unit to rely on maternal metabolism for caffeine clearance (6, 7).

Maternal caffeine intake may increase the risk of fetal growth restriction. Significant associations have been found between high caffeine intake during pregnancy and decreased birthweight or increased risk of small-for-gestational-age infants (8–10). Several studies, however, failed to replicate these associations (11, 12). One study found that pregnant women who consume >200 mg caffeine/day have an increased risk of spontaneous abortion (SA) independent of pregnancy-related symptoms such as nausea (13). A similar result was found in a population-based case-control study of pregnant women, among whom the risk of SA increased in the exposure group consuming >300 mg caffeine/day, but in this case the association did not exist after adjusting for nausea (14). Klonoff-Cohen *et al*. reported a higher risk for preterm birth with increasing caffeine intake during pregnancy (15); however, most studies did not find an association between caffeine intake and length of gestation (8, 12). Recent dose-response meta-analyses examining the association between prenatal caffeine exposure and risk of adverse pregnancy outcomes indicate that greater caffeine

intake during pregnancy may be associated with an increased risk of SA, low birth weight, and small-for-gestational-age infants, but not preterm birth (16, 17).

The inconsistency among these results may be due to different methods for estimation of caffeine intake, retrospective assessment of caffeine intake, or variations in maternal caffeine metabolism. Previous studies have demonstrated the importance of metabolic differences in maternal caffeine intake for fetal development. Of the several known *CYP1A2* polymorphisms with associated *in vivo* functional changes, the C164A polymorphism is of particular interest as it leads to a slow caffeine metabolizer phenotype (CC/CA) and a fast phenotype (AA) with higher ratios of caffeine metabolites. Maternal caffeine intake of ≥100 mg/day is associated with increased risk of SA as compared with an intake of ≤99 mg/day among women with the AA genotype (18). A significantly increased risk of recurrent pregnancy loss (RPL) has been found in women with the AA genotype who consume ≥300 mg caffeine/day, as compared with those who consume ≤99 mg caffeine/day (19). This genotype is also associated with an increased risk of neural tube defects (20). These results suggest that the risk of maternal caffeine intake during pregnancy impacting fetal development is higher among women with fast caffeine metabolism than among those with slow caffeine metabolism. However, the issue of whether this genotype is a modifying factor in the association between caffeine intake during pregnancy and infant birth size has not yet been investigated. To clarify the effects of caffeine on fetal growth, data from a prospective cohort study were analyzed to ascertain whether increased caffeine intake was associated with decreased infant birth size when considering the *CYP1A2* C164A polymorphism and controlling for confounding factors such as smoking status.

Methods

Study participants

From July 2002 to October 2005, women who were residents of Sapporo and surrounding areas were asked to participate in a prospective cohort study (the Hokkaido Study on Environment and

Children's Health), which aimed to investigate whether environmental factors combined with genetic predisposition contribute to numerous adverse development and health effects in childhood, as described previously (21) and this work was a part of the study. Participants were native Japanese women who enrolled at 23–35 weeks of gestation during a routine obstetric visit and delivered at Sapporo Toho Hospital.

A total of 1796 pregnant women were invited to take part in the study. Of these women, 22% were excluded because they had decided to enroll in the Japanese cord blood bank, and 3% were excluded because they delivered their baby at another hospital. Finally, 514 women agreed to participate (participation rate of 28.6 %). Among these 514 women, 10 dropped out due to stillbirth, relocation, or voluntary withdrawal from the study before follow-up. Another 7 women with multiple pregnancies were excluded from analysis, 13 women were excluded due to pregnancy complications or illness (11 for pregnancy-induced hypertension, 1 for diabetes, 1 for fetal heart failure), and blood samples could not be collected for 8 women; thus 476 mother–child pairs were left for final analysis.

Data collection

A self-administered questionnaire was used to collect relevant information at enrollment (weeks 23–35 of pregnancy). Information relating to smoking status, alcohol consumption, caffeine intake during pregnancy, annual household income, and education was included on the questionnaire. Information drawn from maternal and infant medical records included details of pregnancy complications, maternal age, maternal pre-pregnancy BMI, parity, infant gender, gestational age, and infant birth size. A 40-mL blood sample was drawn from the peripheral vein of the mother. All samples were stored at −80°C until analysis.

Caffeine intake during pregnancy was estimated using a modified version of the self-administered questionnaire used by Nagata *et al* (22). and described in detail by Washino *et al* (23). Average daily caffeine intake during pregnancy was estimated in milligrams of caffeine per day. The

questionnaire included categories on beverages popular in Japan, including instant coffee (60 mg caffeine/cup); regular coffee (50 mg/cup); decaffeinated coffee (0 mg/cup); canned coffee (50 mg/cup); black tea (60 mg/cup); oolong tea (30 mg/cup); powdered green tea (200 mg/cup); gyokuro (high-quality green tea, 100 mg/cup); other tea varieties including natural leaf teas, coarse green tea, brown rice tea, and roasted green tea (30 mg/cup); cocoa (5 mg/cup); cola (60 mg/glass); and caffeinated energy drinks (50 mg/bottle). Frequency of intake was divided into five categories: rarely/never, once or twice per month, once or twice per week, three to four times per week, or every day. The amount consumed per occasion was divided into four categories by cups (or glasses or bottles): one cup or less, two cups, three cups, or four or more cups. Average caffeine intake per day during pregnancy was calculated as the caffeine content per beverage multiplied by the amount consumed per occasion multiplied by the frequency of intake per day. In this study, caffeine exposure was categorized as <100, 100–299, and ≥300 mg/day (19, 24).

In this study, nonsmokers were defined as women who never smoked during pregnancy or those who quit during the first trimester, and smokers were defined as those who smoked throughout pregnancy or quit after the first trimester, based on self-reporting. Smoking status was classified in this manner because the mean birthweight of infants born to women who quit smoking during the first trimester was 3123 g, similar to the mean birthweight of infants born to women who never smoked (3060 g). Because infant mean birthweight decreased to 2922 and 2911 g when women quit smoking during the second and third trimester, respectively, these women were grouped with women who smoked throughout pregnancy. This agrees with previous findings that quitting smoking early in pregnancy does not greatly affect infant birth size (25).

Genotyping analyses

CYP1A2 (C164A, rs762551) polymorphisms were determined by real-time PCR, using minor groove binder (MGB) probes. DNA amplification was carried out in batches in a 96-well MicroAmp reaction plate on a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City,

CA, USA). The validated TaqMan probes were prepared and each of the reporters was quenched by excess MGB. PCR was performed and the fluorescence of the products was measured using a 7300/7500 Real-Time PCR system (Applied Biosystems) (26, 27). This resulted in the clear identification of the CC, CA, and AA genotypes of *CYP1A2*. A *CYP1A2* genotype of CC or CA indicated a slow metabolizer phenotype, and AA indicated the high inducible phenotype. These two phenotypes were used for statistical analyses.

Statistical analyses

Maternal and infant characteristics were analyzed in relation to categories of maternal caffeine intake using the χ^2 test for categorical variables and ANOVA or the Kruskal–Wallis test for continuous variables. The individual effect of maternal caffeine intake on infant birth size and that of the *CYP1A2* genotype (CC/CA vs. AA) on infant birth size were considered in separate regression models, with adjustment for maternal age, maternal pre-pregnancy BMI (kg/m^2), maternal education level (≤12 vs. ≥13 years), maternal smoking status during pregnancy (smoker vs. nonsmoker), maternal alcohol intake during pregnancy (grams per day), parity (0, defined as if woman has had no previous viable pregnancies vs. ≥1), mode of delivery (Vaginal vs. Cesarean section), infant gender, and gestational age (weeks). Because none of the women reported caffeine intake of 0 mg/day, the <100 mg/day intake group was used as the reference group for analysis. Additionally, the individual effect of smoking on birth size was analyzed because of its known impacts on fetal growth (25, 28). The main linear regression model included mean infant birth size as the dependent variable, caffeine intake as a categorical variable, the *CYP1A2* genotype (CC/CA vs. AA), and the *CYP1A2* and caffeine gene–environment interaction variable (the product term of maternal caffeine intake and *CYP1A2* genotype) adjusted by the confounders listed above. To further assess confounding effects of smoking, the regression analysis was stratified by maternal smoking status. Pregnant women at 23-35 weeks of gestation were asked to participate in our prospective cohort study and most women completed a self-administered questionnaire in the third

trimester. They were asked the daily alcohol and caffeine intake throughout pregnancy, and their answers might be an average consumption of all three trimesters of pregnancy. Smoking status was asked in each trimester and smokers were defined as women who smoked throughout pregnancy or quit after the first trimester as described above. So the timing of smoking exposure was after the second trimester. Intermediate variable is a consequence of exposure and it lies on the causal pathway between the exposure and the outcome. The onset of the exposure leads to changes in the intermediate variable which occur prior to the onset of the outcome (29). In our study, the exposure and the outcome were caffeine intake and birth size, respectively. As alcohol and tobacco smoke exposure might occur prior to caffeine exposure, and they were associated with both exposure and outcome, both alcohol intake and smoking status were used as confounders in our study.

Hardy–Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies using the χ^2 test. All p-values were two-sided and statistical significance was defined as $p < 0.05$. All statistical analyses were performed using SPSS for Windows, version 20.0J (SPSS, Inc., Chicago, IL, USA).

Ethical approval

This study was approved by the Institutional Ethics Board for Human Gene and Genome Studies of the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences. Informed consent was obtained from all participants.

Results

As shown in Table 1, the mean maternal age was 30.7 years, with 266 (55.9%) women having more than a high school education. One hundred and two (21.4%) women were classified as smokers during pregnancy and 148 (31.1%) women drank alcohol during pregnancy.

Maternal and infant characteristics of the study population were then reviewed in relation to the three categories of caffeine intake. Maternal pre-pregnancy BMI, annual household income,

education level, parity, infant gender, infant birth size, and gestational age did not differ significantly between the <100, 100–299, and ≥300 mg/day caffeine intake groups. The frequency of *CYP1A2* genotypes was also similar for the <100, 100–299, and ≥300 mg/day caffeine intake groups, and distributions were in Hardy–Weinberg equilibrium (p = 0.863, 0.647, and 0.572, respectively). Genotype frequencies were similar to those published previously for a Japanese population (30).

There was a tendency for smokers to have a high caffeine intake, with 35.5% of smokers consuming ≥300 mg of caffeine per day, compared with 11.1% of them consuming <100mg/day of caffeine. Women who were classified as smokers during pregnancy had significantly lighter babies than nonsmokers (2997 g vs. 3086 g; p=0.032, data not shown) and had infants with birthweights similar to those of mothers consuming ≥300 mg of caffeine per day (2962 g). When considering alcohol intake, a larger proportion of alcohol drinkers (36.2%) consumed 100–299 mg of caffeine per day than those with <100mg /day caffeine intake (24.4%). However, when alcohol intake was considered as a continuous variable among women who drank during pregnancy, the median intake was similar for women in all three caffeine intake groups (Table 1).

The individual effects of caffeine intake and of *CYP1A2* genotype on mean infant birth size were then investigated using linear regression models with adjustment for confounding factors. The individual effect of maternal smoking status during pregnancy on infant birth size was also analyzed. When the effect of caffeine intake alone was analyzed using <100 mg/day consumption as the reference group, there was a nonsignificant decrease in birthweight for caffeine intake of ≥300 mg/day. The maternal *CYP1A2* genotype alone was not significantly associated with mean infant birthweight. When considering maternal smoking status during pregnancy, infants of smokers had a mean reduction in birthweight of 85 g (95% CI −157, −12) as compared with infants born to nonsmokers after adjustment for confounding factors (Table 2A). Mean birth length and head circumference, however, were not affected by maternal smoking status during pregnancy (Table 2B, C).

The mothers were then categorized into six subgroups by *CYP1A2* genotype and caffeine intake. Linear regression analysis was performed, using women with the CC/CA genotype and <100 mg/day caffeine intake as the reference category. Table 3 shows the results from the main model on infant birthweight. When compared to the reference group, mothers with the AA genotype and caffeine intake of ≥300 mg/day had a mean reduction in infant birthweight of 316 g ($p = 0.004$) in the crude model; however, the mean reduction in infant birthweight was not significant in the adjusted model ($p = 0.116$). After stratification by smoking status during pregnancy, only nonsmokers with the AA genotype and caffeine intake of ≥300 mg/day had infants with decreased infant birthweight (mean decrease, 277 g; $p = 0.024$). The interaction between the AA genotype and caffeine intake of ≥300 mg/day was also significant ($p = 0.019$). For infant birth length, we found the largest decrease in infant birth length (0.7 cm) for women with the AA genotype and caffeine intake of ≥300 mg/day among the nonsmoker group, although it was not significant (Table 4).

The results from the main model on infant birth head circumference are presented in Table 5. Women with genotype AA and caffeine intake ≥300 mg/day had a mean reduction in infant birth head circumference of 1.2 cm ($p = 0.002$) compared to the reference group in the crude model, and a mean reduction in infant birth head circumference of 0.8 cm ($p = 0.024$) after adjusting for confounding factors. In the nonsmoker group, infants born to women with the AA genotype and caffeine intakes of ≥300 mg/day showed a decrease in infant birth head circumferences of 1.0 cm in the adjusted model stratified by smoking status during pregnancy ($p = 0.027$), whereas no *CYP1A2*–caffeine subgroups showed significant changes in infant birth head circumference in the smoking group.

Discussion

To our knowledge, this is the first study to consider the effects of maternal caffeine intake during pregnancy and the *CYP1A2* C164A polymorphism on infant birth size. The results show that when caffeine intake is considered independently of genetic factors, its consumption during pregnancy

does not have a significant effect on mean infant birth size after adjustment for confounding factors. However, when both caffeine intake and the *CYP1A2* genotype are considered, there appeared to be a significant effect on infant birth head circumference ($p = 0.024$). The reductions in infant birthweight and birth length associated with caffeine and the *CYP1A2* genotype, however, were eliminated after controlling for smoking and other factors. Previous studies have reported that small head circumference at birth influenced brain development and that low birth length was associated with behavioral problems in childhood (31, 32). Small birth size might adversely affect neurodevelopmental and cognitive outcomes. In a subset of nonsmokers with the *CYP1A2* AA genotype and caffeine consumption of >300 mg/day, there is evidence of decreased infant birthweight and birth head circumference ($p = 0.024$ and 0.027, respectively) as compared with women with the CC/CA genotype who consume <100 mg caffeine/day.

Individuals carrying the *CYP1A2* AA genotype are fast metabolizers of caffeine and have higher levels of paraxanthine than do those carrying the CC and CA genotypes. Women with the high inducible genotype for *CYP1A2* have an increased risk of harmful reproductive outcomes. Signorello *et al*. evaluated the rate of caffeine metabolism as a risk factor for SA (18). Their results indicated that women with high *CYP1A2* activity have an increased risk for SA (OR 2.42, 95% CI 1.01, 5.80 for 100–299 mg caffeine/day; OR 3.17, 95% CI 1.22, 8.22 for ≥300 mg/day), but no increase in SA risk is observed in women with low *CYP1A2* activity [OR 0.46, 95% CI 0.12, 1.73 for ≥300 mg/day] (18). Sata *et al*. investigated how the association between maternal consumption of high levels of caffeine and the risk of RPL is modified by *CYP1A2* activity in a case-control study (19). The RPL risk significantly increased among women with the *CYP1A2* AA genotype [OR 1.94, 95% CI 0.57, 6.66 for 100–299 mg/day; OR 5.23, 95% CI 11.05, 25.9 for >300 mg/day; p for trend = 0.03]. However, no associations were observed between RPL and the *CYP1A2* AC/CC genotypes (19). These findings suggest that there may be adverse effects of the caffeine metabolite paraxanthine, rather than of caffeine itself.

A previous study found that paraxanthine concentrations are higher in women who have SAs than

in the controls, and that the highest levels of paraxanthine had an increased risk of SA [OR 1.9, 95% CI 1.2, 2.8], however, the study reported no associations between SA and caffeine concentrations (33). For fetal growth, high concentrations of paraxanthine are associated with increased risk of IUGR [OR 3.29, 95% CI 1.17, 9.22], and the ratio of paraxanthine to caffeine, which is a marker of *CYP1A2* enzymatic activity, is also associated with an increased risk of IUGR [OR 1.21, 95% CI 1.07, 1.37], whereas higher levels of caffeine are associated with a decreased risk of IUGR (34).

Recent experimental studies in animal models have indicated that prenatal caffeine exposure is associated with developmental toxicities (35). Prenatal caffeine ingestion increases maternal glucocorticoid (GC) levels and inhibits the expression of placental 11β-hydroxysteroid dehydrogenase-2 (11β-HSD-2), an enzyme that prevents fetal exposure to excessive maternal GC. Upon exposure to high levels of maternal GC, the expression of 11β-HSD-1 and glucocorticoid receptor in the fetal hippocampus is enhanced, and then the functional development of the fetal hypothalamic-pituitary-adrenal (HPA) axis is inhibited through negative feedback regulation. The hippocampus is the primary negative feedback regulatory center of the HPA axis (36). Additionally, caffeine can enter the fetus and directly inhibit 11β-HSD2 expression in the hippocampus. The reduced 11β-HSD-2 expression can promote the expression of 11β-HSD-1 and the glucocorticoid receptor in the fetal hippocampus, which also inhibit the activity of the fetal HPA axis as a crucial switch for development in many organs, including the pituitary and brain (37). It has been suggested that these changes are linked to impaired placental and fetal growth, such as decreased fetal body and brain weight (38). Paraxanthine also decreases placental 11β-HSD-2 expression and activity in cultured human trophoblast cells (39). It is possible that paraxanthine may have a greater impact than caffeine on growth and development in fetuses.

The question of why adverse effects are more pronounced in nonsmokers with the AA genotype is of particular interest. Rodenburg *et al.* examined the modifying effects of gender, age, smoking status, and *CYP1A2* genotype on caffeine intake and showed that smoking status and gender were

responsible for the largest differences in caffeine intake: men who smoked metabolized caffeine more rapidly, and the genetic effect explained only a portion of this effect (40). A previous study in a Turkish population also found that smoking status and gender explained most of the variation in the metabolite ratio of caffeine after coffee intake (24 and 10%, respectively), whereas genotype explained <1% (41). Thus, the lack of a significant association between caffeine intake, genotype and birth measures among smokers in our study may be due to the stronger influence of smoking than the *CYP1A2* genotype on caffeine metabolism.

Two strengths of the present study are that possible recall bias due to retrospective data collection has been eliminated and that caffeine intake was assessed using a format fitted to the dietary habits of the study population. First, because women in this study assessed their average caffeine intake prospectively at enrollment, before birth outcomes were known, there was no possibility for women with reduced birth size infants to overestimate their caffeine consumption, or for women with heavier or healthier babies to underestimate intake. Second, a questionnaire based on a survey by Nagata *et al*. was used to accurately assess caffeine consumption (22). However, although subjects chose from nine possible responses for frequency of caffeine intake, information on exact portion size and preparation method was not obtained. To ensure more accurate caffeine estimations, several classifications for canned coffee, instant coffee, and several subcategories for tea, as well as standard size notations for each category, were added for this study.

It has been suggested that the *CYP1A2* activity may differ between ethnicities. Denden *et al*. conducted a meta-analysis to assess an association between the *CYP1A2* rs762551 polymorphism and caffeine consumption. Although Asians showed a significantly increased coffee intake compared to Caucasians, significant relationship between the AA genotype and coffee intake was found only in Caucasian ethnicity, not in Asians (42). Schliep *et al*. investigated whether caffeine intake was associated with reproductive hormone levels and its effect was modified by race. They reported that caffeine intake ≥200 mg/day was associated with reduced free estradiol (E2) levels among Caucasian women and elevated E2 levels among Asian women; however, these differences

by race may be due to limited numbers of Asians with high exposure (43). Although the significant effect of caffeine on birth size modified by maternal gene polymorphisms was found, further study with a larger population may help support the results of this study, since our study sample consisted of relatively small number of women who consumed high levels of caffeine and who smoked during pregnancy. Moreover, interpretation of our results is limited by the following points: average maternal age of 1796 candidate women was 29.7±4.9 years and it was younger than that of 476 women. It is possible that women who took an interest in our study would have participated more frequently and this may limit generalizability of the enrolled study population, as the possibility of selection bias exists; levels of caffeine in urine or serum were not measured in this study. However, a recent study determined that reported caffeine intake estimates were strongly correlated with urinary and cord blood biomarkers throughout pregnancy, suggesting that self-reported caffeine intake is a satisfactory estimate of actual caffeine intake (44); the analyses may have been affected by possible uncontrolled or inadequately controlled confounding variables. Other limitations of this study are that nausea, thought to affect caffeine-seeking behavior and indicate overall health of the pregnancy (45), was not controlled for in the model. In addition, separate measures of caffeine intake during all three trimesters of pregnancy would serve to clarify both changes in caffeine-seeking behavior, as well as determine a critical window for adverse effects. Further research is needed to determine adverse effects of paraxanthine on reproductive outcomes in both experimental and epidemiological studies.

In conclusion, after consideration of *CYP1A2* C164A genotype, nonsmokers who are fast metabolizers of caffeine and who consume ≥300 mg caffeine/day have an increased risk of having infants with decreased birthweight and birth head circumference. The absence of these associations among smokers may be due to the stronger influence of smoking compared to *CYP1A2* phenotypic effects on caffeine metabolism.

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References

1. 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.
2. Benowitz NL, Jacob P 3rd, Mayan H, Denaro C. Sympathomimetic effects of paraxanthine and caffeine in humans. Clin Pharmacol Ther 1995;58:684-91.
3. Food Standards Agency (2008). Food Standards Agency publishes new caffeine advice for pregnant women. http://www.food.gov.uk/news/pressreleases/2008/nov/caffeineadvice. (Accessed December 2016).
4. Yamada M, Sasaki S, Murakami K, et al. Estimation of caffeine intake in Japanese adults using 16 d weighed diet records based on a food composition database newly developed for Japanese populations. Public Health Nutr. 2010;13:663-72.
5. Derbyshire E, Abdula S. Habitual caffeine intake in women of childbearing age. J Hum Nutr Diet. 2008;21:159-64.
6. Kalow W, Tang BK. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. Clin Pharmacol Ther 1991;50:508-19.
7. Syme MR, Paxton JW, and Keelan JA. Drug transfer and metabolism by the human placenta. Clin Pharmacokinet, 2004;43:487-514.
8. Bracken MB, Triche EW, Belanger K, Hellenbrand K, Leaderer BP. Association of maternal caffeine consumption with decrements in fetal growth. Am J Epidemiol 2003;57:456-66.
9. Bakker R, Steegers EA, Obradov A, Raat H, Hofman A, Jaddoe VW. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: the Generation R Study. Am J Clin Nutr 2010;91:1691-8.
10. CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ 2010;340:c2331.

11. Grosso LM, Rosenberg KD, Belanger K, Saftlas AF, Leaderer B, Bracken MB. Maternal caffeine intake and intrauterine growth retardation. Epidemiology 2001;2:447-55.

12. Clausson B, Granath F, Ekbom A, et al. Effect of caffeine exposure during pregnancy on birth weight and gestational age. Am J Epidemiol 2002;155:429-36.

13. Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol 2008;198:279.e1-8.

14. Maconochie N, Doyle P, Prior S, Simmons R. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. BJOG 2007;114:170-86.

15. Klonoff-Cohen H, Bleha J, Lam-Kruglick P. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer. Hum Reprod 2002;17:1746-54.

16. Greenwood DC, Thatcher NJ, Ye J, Garrard L, et al. Caffeine intake during pregnancy and adverse birth outcomes: a systematic review and dose-response meta-analysis. Eur J Epidemiol 2014;29:725-34.

17. Chen LW, Wu Y, Neelakantan N, Chong MF, Pan A, van Dam RM. Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose-response meta-analysis. BMC Med 2014;12:174.

18. Signorello LB, Nordmark A, Granath F, et al. Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses. Obstet Gynecol 2001;98:1059-66.

19. Sata F, Yamada H, Suzuki K, et al. Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. Mol Hum Reprod 2005;11:357-60.

20. Schmidt RJ, Romitti PA, Burns TL, et al. National Birth Defects Prevention Study Caffeine, selected metabolic gene variants, and risk for neural tube defects. Birth Defects Res A Clin Mol Teratol 2010;88:560-69.

21. Kishi R, Kobayashi S, Ikeno T, et al. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile--updated 2013. Environ Health Prev Med 2013;18:429-50.

22. Nagata C, Kabuto M, Shimizu H. Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women. Nutr Cancer 1998;30:21-4.

23. Washino N, Saijo Y, Sasaki S, et al. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect 2009;117:660-7.

24. Santos IS, Victora CG, Huttly S, Carvalhal JB. Caffeine intake and low birth weight: a population-based case-control study. Am J Epidemiol 1998;147:620-7.

25. Ohmi H, Hirooka K, Mochizuki Y Fetal growth and the timing of exposure to maternal smoking. Pediatr Int 2002;44:55-9.

26. Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262-8.

27. Kobayashi S, Sata F, Sasaki S, et al. Genetic association of aromatic hydrocarbon receptor (AHR) and cytochromeP450, family 1, subfamily A, polypeptide 1 (CYP1A1) polymorphisms with dioxin blood concentrations among pregnant Japanese women. Toxicol Lett 2013;219:269-78.

28. England LJ, Kendrick JS,Wilson HG, et al. Effects of smoking reduction during pregnancy on the birth weight of term infants. Am J Epidemiol 2001;154:694–701.

29. Chaiton M, Cohen JE, Rehm J, Abdulle M, O'Loughlin J. Confounders or intermediate variables? Testing mechanisms for the relationship between depression and smoking in a longitudinal cohort study. Addict Behav. 2015;42:154-61.

30. Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T. Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human CYP1A2 in the Japanese population. Jpn J Cancer Res 1999;90:899-902.

31. Broekman BF , Chan YH, Chong YS, et al. The influence of birth size on intelligence in healthy children. Pediatrics. 2009;123:e1011-6.

32. Wiles NJ, Peters TJ, Heron J, et al. Fetal growth and childhood behavioral problems: results from the ALSPAC cohort. Am J Epidemiol 2006;163:829–37.

33. Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG. Maternal serum paraxanthine, a caffeine metabolite, and the risk of spontaneous abortion. N Engl J Med 1999;341:1639-44.

34. Grosso LM, Triche EW, Belanger K, Benowitz NL, Holford TR, Bracken MB. Caffeine metabolites in umbilical cord blood, cytochrome P-450 1A2 activity, and intrauterine growth restriction. Am J Epidemiol 2006;163:1035-41.

35. Huang J, Zhou S, Ping J, et al. Role of p53- dependent placental apoptosis in the reproductive and developmentaltoxicities of caffeine in rodents. Clin Exp Pharmacol Physiol 2012;39:357-63.

36. Xu D, Zhang B, Liang G, et al. Caffeine-induced activated glucocorticoid metabolism in the hippocampus causes hypothalamic-pituitary-adrenal axis inhibition in fetal rats. PLoS One 2012;7:e44497.

37. Moisiadis VG, Matthews SG Glucocorticoids and fetal programming part 1: outcomes. Nat. Rev. Endocrinol 2014;10:391-402.

38. Xu D, Wu Y, Liu F, et al. A hypothalamic-pituitary-adrenal axis-associated neuroendocrine metabolic programmed alteration in offspring rats of IUGR induced by prenatal caffeine ingestion. Toxicol Appl Pharmacol 2012;264:395-403.

39. Sharmin S, Guan H, Williams AS, Yang K. Caffeine reduces 11β-hydroxysteroid dehydrogenase type 2 expression in human trophoblast cells through the adenosine A(2B) receptor. PLoS One 2012;7:e38082.

40. Rodenburg EM, Eijgelsheim M, Geleijnse JM, et al. CYP1A2 and coffee intake and the modifying effect of sex, age, and smoking. Am J Clin Nutr 2012;96:182-7.

41. Gunes A, Ozbey G, Vural EH, et al. Influence of genetic polymorphisms, smoking, gender and age on CYP1A2 activity in a Turkish population. Pharmacogenomics 2009;10:769-78.

42. 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:gmr.15027487.

43. Schliep KC, Schisterman EF, Mumford SL, et al. Caffeinated beverage intake and reproductive hormones among premenopausal women in the BioCycle Study. Am J Clin Nutr. 2012;95:488-97.

44. Grosso LM, Triche E, Benowitz NL, Bracken MB. Prenatal caffeine assessment: fetal and maternal biomarkers or self-reported intake? Ann Epidemiol 2008;18:172-8.

45. Signorello LB, McLaughlin J. Maternal caffeine consumption and spontaneous abortion. A review of epidemiologic evidence. Epidemiology. 2004;15:229-39.

Table 1. Characteristics of mother–infant pairs in relation to maternal caffeine intake levels during pregnancy (n = 476)

Characteristics		Maternal caffeine intake during pregnancy			p-value
		<100mg/day n=180	100-299mg/day n=265	≥300mg/day n=31	
Maternal characteristics					
Age (years)	30.7 (4.9)	30.1 (4.9)	31.2 (4.9)	30.7 (4.9)	0.063
Pre pregnancy BMI (kg/m^2)	21.1 (3.0)	21.3 (3.2)	21.0 (2.8)	21.0 (3.3)	0.692
Annual household income (million yen)					
<5	324 (68.5)	124 (69.3)	179 (68.1)	21 (67.7)	
≥5	149 (31.5)	55 (30.7)	84 (31.9)	10 (32.3)	0.960
Education level (years)					
≤12	210 (44.1)	73 (40.6)	122 (46.0)	15 (48.4)	
≥13	266 (55.9)	107 (59.4)	143 (54.0)	16 (51.6)	0.460
Smoking status during pregnancy					
Nonsmoker	374 (78.6)	160 (88.9)	194 (73.2)	20 (64.5)	
Smoker	102 (21.4)	20 (11.1)	71 (26.8)	11 (35.5)	<0.001
Alcohol intake during pregnancy					
No	328 (68.9)	136 (75.6)	169 (63.8)	23 (74.2)	
Yes	148 (31.1)	44 (24.4)	96 (36.2)	8 (25.8)	0.025
Alcohol intake among drinkers during pregnancy (g/day)	1.4(0.3-152.0) [a]	1.2 (0.3-14.0) [a]	1.9 (0.4-98.2) [a]	1.6 (0.4-152.0) [a]	0.094
Parity					
0	226 (47.5)	93 (51.7)	120 (45.3)	13 (41.9)	
≥1	250 (52.5)	87 (48.3)	145 (54.7)	18 (58.1)	0.340
Mode of delivery					
Vaginal	378 (79.4)	143 (79.4)	212 (80.0)	23 (74.2)	
Cesarean section	98 (20.6)	37 (20.6)	53 (20.0)	8 (25.8)	0.751
CYP1A2 genotype					
CC	64 (13.4)	26 (14.4)	33 (12.5)	5 (16.2)	
CA	225 (47.3)	85 (47.2)	127 (47.9)	13 (41.9)	
AA	187 (39.3)	69 (38.4)	105 (39.6)	13 (41.9)	0.937
CC/CA	289 (60.7)	111 (61.6)	160 (60.4)	18 (58.1)	0.917
Infant characteristics					
Gender					
Male	226 (47.5)	81 (45.0)	129 (48.7)	16 (51.6)	
Female	250 (52.5)	99 (55.0)	136 (51.3)	15 (48.4)	0.667
Gestational age (wks)	39.0 (1.4)	39.1 (1.4)	39.0 (1.4)	38.4 (1.8)	0.080
Birth weight (g)	3067 (374)	3087 (363)	3066 (368)	2962 (473)	0.228
Birth length (cm)	48.1 (1.9)	48.1 (2.1)	48.1 (1.8)	47.5 (1.9)	0.198
Birth head circumference (cm)	33.3 (1.3)	33.3 (1.3)	33.3 (1.3)	33.0 (1.5)	0.383

Mean (SD) or n (%)

[a] Median (minimum–maximum)

Table 2. Individual associations of maternal caffeine intake during pregnancy, maternal smoking status, and maternal *CYP1A2* genotype with infant birth size (n = 476)

(A) Infant birthweight

	Infant birthweight						
		Crude			Adjusted [a, b]		
	n	Δbw (g)	95% CI	p-value	Δbw (g)	95% CI	p-value
Caffeine Intake							
<100 mg/day	180	Reference			Reference		
100–299mg/day	265	-21	-92, 50	0.558	-2	-64, 61	0.956
≥300 mg/day	31	-125	-268, 18	0.086	-34	-159, 91	0.594
Smoking Status							
Nonsmoker	374	Reference			Reference		
Smoker	102	-90	-172, -8	0.032	-85	-157, -12	0.022
CYP1A2 Genotype							
CC/CA	289	Reference			Reference		
AA	187	15	-54, 84	0.671	26	-34, 85	0.398

Δbw: mean difference in birthweight in the multiple linear regression model

[a] Caffeine intake and *CYP1A2* genotype were adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Smoking status was adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

(B) Infant birth length

	Infant birth length						
		Crude			Adjusted [a, b]		
	n	Δbl (cm)	95% CI	p-value	Δbl (cm)	95% CI	p-value
Caffeine Intake							
<100 mg/day	180	Reference			Reference		
100–299 mg/day	265	0.1	-0.3, 0.5	0.609	0.2	-0.2, 0.5	0.318
≥300 mg/day	31	-0.6	-1.3, 0.2	0.134	-0.2	-0.8, 0.5	0.595
Smoking Status							
Nonsmoker	374	Reference			Reference		
Smoker	102	-0.3	-0.7, 0.1	0.142	-0.3	-0.6, 0.1	0.193
CYP1A2 Genotype							
CC/CA	289	Reference			Reference		
AA	187	0.1	-0.3, 0.4	0.663	0.1	-0.2, 0.4	0.461

Δbl: mean difference in birth length in the multiple linear regression model

[a] Caffeine intake and *CYP1A2* genotype were adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Smoking status was adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

(C) Infant birth head circumference

	Infant birth head circumference						
		Crude			Adjusted [a, b]		
	n	Δbhc (cm)	95% CI	p-value	Δbhc (cm)	95% CI	p-value
Caffeine Intake							
<100 mg/day	180	Reference			Reference		
100–299 mg/day	265	-0.002	-0.3, 0.3	0.987	0.02	-0.2, 0.3	0.852
≥300 mg/day	31	-0.3	-0.8, 0.2	0.184	-0.2	-0.7, 0.3	0.390
Smoking Status							
Nonsmoker	374	Reference			Reference		
Smoker	102	-0.3	-0.6, 0.03	0.078	-0.2	-0.5, 0.1	0.149
CYP1A2 Genotype							
CC/CA	289	Reference			Reference		
AA	187	-0.1	-0.3, 0.1	0.384	-0.05	-0.3, 0.2	0.673

Δbhc: mean difference in birth head circumference in the multiple linear regression model

[a] Caffeine intake and *CYP1A2* genotype were adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Smoking status was adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

Table 3. Combined association of maternal caffeine intake during pregnancy and maternal *CYP1A2* genotype with infant birthweight by maternal smoking status

		All (n = 476)							Nonsmokers (n = 374)				Smokers (n = 102)			
			Crude			Adjusted [a]				Adjusted [b]				Adjusted [b]		
		n	Δbw (g)	95% CI	p-value	Δbw (g)	95% CI	p-value	n	Δbw (g)	95% CI	p-value	n	Δbw (g)	95% CI	p-value
CYP1A2 Genotype	Caffeine Intake															
	<100 mg/day	111		Reference			Reference		100		Reference		11		Reference	
CC/CA	100–299 mg/day	160	-37	-128, 53	0.416	-23	-102, 55	0.560	115	-25	-114, 64	0.577	45	-20	-215, 175	0.837
	≥300 mg/day	18	25	-161, 210	0.795	56	-105, 218	0.493	12	104	-94, 302	0.301	6	-116	-427, 196	0.462
	<100 mg/day	69	17	-95, 129	0.762	11	-85, 108	0.817	60	4	-106, 106	0.994	9	107	-165, 379	0.438
AA	100–299 mg/day	105	20	-79, 120	0.689	40	-46, 127	0.360	79	53	-46, 152	0.293	26	-11	-221, 200	0.919
	≥300 mg/day	13	-316	-531, -102	0.004	-149	-335, 37	0.116	8	-277	-516, -37	0.024	5	49	-263, 361	0.756
Interaction [c]			-358	-647, -70	0.015	-217	-467, 34	0.090		-381	-699, -64	0.019		58	-393, 510	0.799

Δbw: mean difference in birthweight in the multiple linear regression model

[a] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[c] Test of interaction: tests the null hypothesis that Δbw = 0 in the multiple linear regression model for the product term maternal *CYP1A2* AA genotype × caffeine intake of ≥300 mg/day.

Table 4. Combined association of maternal caffeine intake during pregnancy and maternal *CYP1A2* genotype with infant birth length by maternal smoking status

		All (n = 476)							Nonsmokers (n = 374)				Smokers (n = 102)			
			Crude			Adjusted [a]				Adjusted [b]				Adjusted [b]		
		n	Δbl (cm)	95% CI	p-value	Δbl (cm)	95% CI	p-value	n	Δbl (cm)	95% CI	p-value	n	Δbl (cm)	95% CI	p-value
CYP1A2 Genotype	Caffeine Intake															
	<100 mg/day	111		Reference			Reference		100		Reference		11		Reference	
CC/CA	100–299 mg/day	160	0.1	-0.4, 0.5	0.716	0.2	-0.3, 0.6	0.467	115	0.2	-0.3, 0.7	0.424	45	0.1	-0.9, 1.1	0.833
	≥300 mg/day	18	0.1	-0.9, 1.0	0.883	0.2	-0.7, 1.0	0.732	12	0.5	-0.6, 1.5	0.404	6	-0.9	-2.4, 0.6	0.244
	<100 mg/day	69	0.2	-0.4, 0.7	0.554	0.2	-0.4, 0.7	0.536	60	0.1	-0.5, 0.7	0.773	9	0.8	-0.5, 2.1	0.243
AA	100–299 mg/day	105	0.3	-0.2, 0.8	0.290	0.3	-0.1, 0.8	0.150	79	0.4	-0.2, 0.9	0.203	26	0.4	-0.7, 1.4	0.474
	≥300 mg/day	13	-1.3	-2.4, -0.2	0.024	-0.5	-1.5, 0.5	0.331	8	-0.7	-2.0, 0.6	0.293	5	-0.2	-1.8, 1.3	0.759
Interaction [c]			-1.5	-3.0, -0.03	0.045	-0.8	-2.2, 0.5	0.237		-1.2	-3.0, 0.5	0.159		-0.1	-2.3, 2.1	0.911

Δbl: mean difference in birth length in the multiple linear regression model

[a] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[c] Test of interaction: tests the null hypothesis that Δbl = 0 in the multiple linear regression model for the product term maternal *CYP1A2* AA genotype × caffeine intake of ≥300 mg/day.

Table 5. Combined association of maternal caffeine intake during pregnancy and maternal *CYP1A2* genotype with infant birth head circumference by maternal smoking status

		All (n = 476)							Nonsmokers (n = 374)				Smokers (n = 102)			
			Crude			Adjusted [a]				Adjusted [b]				Adjusted [b]		
		n	Δbhc (cm)	95% CI	p-value	Δbhc (cm)	95% CI	p-value	n	Δbhc (cm)	95% CI	p-value	n	Δbhc (cm)	95% CI	p-value
CYP1A2 Genotype	Caffeine Intake															
	<100 mg/day	111		Reference			Reference		100		Reference		11		Reference	
CC/CA	100–299 mg/day	160	-0.1	-0.4, 0.2	0.489	-0.1	-0.4, 0.2	0.665	115	-0.1	-0.4, 0.3	0.647	45	0.02	-0.8, 0.8	0.952
	≥300 mg/day	18	0.1	-0.5, 0.8	0.671	0.2	-0.5, 0.8	0.610	12	-0.01	-0.8, 0.7	0.993	6	0.5	-0.8, 1.8	0.424
	<100 mg/day	69	-0.2	-0.6, 0.2	0.348	-0.1	-0.5, 0.3	0.552	60	-0.1	-0.5, 0.3	0.538	9	0.2	-0.9, 1.3	0.759
AA	100–299 mg/day	105	-0.02	-0.4, 0.3	0.925	0.04	-0.3, 0.4	0.795	79	0.01	-0.4, 0.4	0.975	26	0.1	-0.7, 1.0	0.778
	≥300 mg/day	13	-1.2	-2.0, -0.4	0.002	-0.8	-1.5, -0.1	0.024	8	-1.0	-1.9, -0.1	0.027	5	-0.4	-1.6, 0.9	0.566
Interaction [c]			-1.1	-2.0, -0.1	0.029	-0.9	-1.8, 0.1	0.076		-0.9	-2.1, 0.3	0.143		-1.1	-2.9, 0.8	0.258

Δbhc: mean difference in birth head circumference in the multiple linear regression model

[a] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), maternal smoking status, alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[b] Adjusted for maternal age, pre-pregnancy BMI, education (≤12, ≥13), alcohol intake during pregnancy, parity (0, ≥1), mode of delivery, infant gender, and gestational age.

[c] Test of interaction: tests the null hypothesis that Δbhc = 0 in the multiple linear regression model for the product term maternal *CYP1A2* AA genotype × caffeine intake of ≥300 mg/day.