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Title

Combined effects of *AHR*, *CYP1A1*, and *XRCC1* genotypes and prenatal maternal smoking on infant birth size: Biomarker assessment in the Hokkaido Study

Authors

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Keywords

Maternal smoking, Aromatic hydrocarbon receptor (AHR), Cytochrome P450 1A1 (CYP1A1), X-ray–complementing gene 1 (XRCC1), Birth size

Highlights

- We examined the association of prenatal smoking and polymorphism with birth size.
- Eight genotypes encoding PAH-metabolizing and DNA-repair genes were investigated.
- Polymorphisms were analyzed in 3,263 healthy pregnant Japanese women.
- We observed adverse birth effects with the combination of *AHR, CYP1A1* and *XRCC1*.
- Prenatal smoking and *AHR/CYP1A1/XRCC1* SNPs are associated with reduced birth size.

Abbreviations

AHR, aromatic hydrocarbon receptor; BP, benzo[a]pyrene; BPDE, benzo[a]pyrene-7,8-diol oxide; CI, confidence interval; CYP, cytochrome P450; dbSNP, database single nucleotide polymorphism; GST, glutathione S-transferase; PAH, polycyclic aromatic hydrocarbon; XRCC1, X-ray complementing repair gene 1.

ABSTRACT

Objectives: We investigated the individual and combined effects of maternal polymorphisms encoding the aromatic hydrocarbon receptor (*AHR*; rs2066853), cytochrome P450 (*CYP*) *1A1* (rs1048963), and the X-ray-complementing gene 1 (*XRCC1*; rs1799782) and prenatal smoking in relation to infant birth size.

Methods: Totally, 3,263 participants (1,998 non-smokers and 1,265 smokers) were included in the study between 2003 and 2007. Two groups of mothers were distinguished by plasma cotinine levels by ELISA measured during the third trimester (cut-off = 11.48 ng/mL). We conducted data analysis using multiple linear regression models.

Results: Infants whose mothers smoked and had *AHR*-GG, *CYP1A1*-AG/GG, and *XRCC1*-CT/TT genotypes weighed, -145 g less than those born of mothers who did not smoke and had the *AHR*-GA/AA, *CYP1A1*-AA, and *XRCC1*-CC genotypes (95%CI: -241, -50).

Conclusions: We demonstrated that infants whose mothers smoked during pregnancy with the combination of *AHR*, *CYP1A1*, and *XRCC1* polymorphisms had lower birth size.

1. INTRODUCTION

In Japan, recent smoking rate of the women is 8.2% [1] and women in Hokkaido prefecture, northernmost of Japan, have the highest that rates (16.4%) in Japan [2]. Although previous studies show adverse effects of maternal smoking during pregnancy, there are few studies examining genetic susceptibility of smokers on birth outcomes. Tobacco smoke is a mixture of more than 4,000 chemicals, e.g., polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, benzene, and acetaldehyde. Many of these compounds are carcinogenic, and their metabolites can form DNA adducts [3-5]. In previous studies, maternal smoking during pregnancy was associated with adverse pregnancy outcomes, i.e., miscarriage, stillbirth, and low birth weight [6-8]. Associations between maternal genetic polymorphisms of PAH- and N-nitrosamine-metabolizing enzymes and/or prenatal smoking and infant birth size have been investigated in epidemiological studies [9-14]. However, only three reports examined combinations of maternal genetic polymorphisms in PAH-metabolizing enzymes and active or passive smoking during pregnancy on birth size [11, 15-16].

Xenobiotic metabolism can be classified as phase I (metabolism) and phase II (conjugation reaction). The aromatic hydrocarbon receptor (AHR), the cytochrome P450s (CYP) 1A1, 1A2, and 1B1, and the glutathione *S*-transferases (GST) M1 and T1 serve as Phase I and Phase II enzymes for such PAHs as benzo[a]pyrene (BP), benzo[a]anthracene, and dioxins contained in tobacco smoke [17-20]. The X-ray-complementing repair 1 protein (*XRCC1*) eliminates the adducts formed by the BP metabolite BP-7.8-diol oxide (BPDE) and DNA [21-22]. Recent studies reported that the *AHR*-GG (G>A, Arg554Lys; dbSNP ID, rs2066853), *CYP1A1*-AG/GG (A>G, Ile462Val; dbSNP ID, rs1048943), *GSTM1*$^{-}$ and *GSTT1*$^{-}$ genotypes are associated with

reduced birth size when the fetus had been exposed to active or passive smoking by the mother [9-11, 13-16]. The *CYP1A2* (A>C, CYP1A2*1F; dbSNP ID, rs762551) genotype has greater enzyme activity in the presence of an inducer, e.g., smoking, caffeine intake, and/or theophylline intake *in vitro* and *in vivo* [23-25]. Smoking has been associated with a decrease in the catalytic activity of *CYP1B1*-CC (C>G, Leu432Val; dbSNP ID, rs1056836) for such substrates as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine [26]. The *XRCC1* (C>T, Arg194Trp; dbSNP ID, rs1799782; G>A, Arg399Gln; dbSNP ID, rs25487) genotypes alter *XRCC1* expression *in vitro* [21, 27]. However, associations between maternal *CYP1A2*, *CYP1B1*, and *XRCC1* polymorphisms and smoking on reduced infant birth size have not been examined. Some studies have shown that associations between maternal genetic polymorphisms of PAH-metabolizing enzymes, prenatal smoking, and infant birth size, but these studies were limited to a small sample size and did not consider various combinations of genotypes. Infant birth size may be further reduced by polymorphic genes encoding metabolizing enzymes and DNA repair proteins. We hypothesized that PAH-metabolizing and DNA repair proteins could affect infant birth size by mediating maternal susceptibility to smoking in pregnant Japanese women. Therefore, the aim of this study was to investigate maternal genetic polymorphisms that might affect passive or active smoking by pregnant Japanese women—with smoking/non-smoking defined by the plasma levels of the biomarker cotinine—on infant birth size. We characterized genetic susceptibility using maternal genotypes of *AHR*, *CYP1A1*, *CYP1A2*, *CYP1B1*, *GSTM1*, *GSTT1*, and *XRCC1* individually and in combination.

2. METHODS

2.1. Questionnaire and medical records

Participants completed a questionnaire during their first trimester of pregnancy that included maternal and paternal information related to age, pre-pregnancy height and weight, previous medical history, educational level, household income, alcohol status, parity, housemates who smoked, job category, and exposure to smoking on the job. We obtained gestational age, infant gender, singleton or twin birth, and the maternal medical history during pregnancy from the subjects' medical records at the time of birth. We asked the mothers to report the birth weight, length, and head circumference in a maternity passbook attached to the questionnaire. In addition, when the child was 4 months old, information about smoking status during the pregnancy was obtained through the following questions: "Did you (or your partner) smoke during your pregnancy?". If the answer was yes, "How many cigarettes did you (or your partner) smoke per day?" If the mother had quit smoking during her pregnancy, we asked when did she quit. We also asked how many cigarettes the mother smoked per week. We defined smoking in the third trimester based on the questionnaire.

2.2. Third-trimester plasma cotinine measurement

Because of strong influence of maternal smoking at the third trimester of pregnancy on reduced birth size [28], we measured the plasma cotinine concentration of the pregnant women during their third trimester (at 8th months of pregnancy) using a very sensitive enzyme-linked immunosorbent assay (ELISA) [29] performed at Cosmic Corporation, Tokyo, Japan. Detection limit of plasma cotinine level was 0.12 ng/mL [29]. A mother was considered to be a non-smoker if her cotinine level was ≤11.48

ng/mL and a smoker if her cotinine level was ≥11.49 ng/mL. In addition, a mother was considered to be a passive smoker if her cotinine level was between 0.22 and 11.48 ng/mL and a passive, non-smoker if her level was <0.21 ng/mL [29]. These cut-off values are based on a previous study [29].

2.3. Genotype measurements

Maternal blood samples were collected on giving birth, and 200-400 μg of the sample was used to extract genomic DNA from 3,263 subjects. The QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) [30] and Maxwell 16 DNA Purification Kit (Promega, Madison, WI, USA) were isolate and purify DNA according to the manufacturer's instruction. *AHR*, *CYP1A1*, *CYP1A2*, *CYP1B1*, *GSTM1* (+/–), and *GSTT1* (+/–) polymorphisms were determined by the TaqMan PCR method as described [11, 31-35]. *XRCC1* genotyping was performed using a 7300/7500 Real-Time PCR system (Applied Biosystems, Foster, CA, USA) and the fluorogenic 5'-nuclease assay with TaqMan Minor Groove Binder probes (Applied Biosystems). Each reaction contained 2 ng/μL genomic DNA, TaqMan 40× Assay-on Demand SNP Genotyping Assay Mix (Applied Biosystems), TaqMan Universal PCR Master Mix, and No AmpErase UNG (Applied Biosystems) in a total volume of 10 μL. Reaction conditions were 10 min at 95°C, followed by 40 cycles at 92°C for 15 s and at 60°C for 1 min. Allelic discrimination was determined by the fluorescence of the two dyes at 60°C. We randomly selected 15 samples (the successfully genotyped samples) and repeated genotyping to check for genotyping quality for each of genetic polymorphisms. The results were 100% concordant.

2.4. Selection of participants

The study participants were Japanese mother-child pairs drawn from the birth cohort of The Hokkaido Study on Environmental and Children's Health begun in February 2003 [36-37]. The study protocol has been described [36-37]. In brief, this is a prospective ongoing birth cohort study. All Japanese pregnant women who presented at any of the participating healthcare facilities for prenatal care during the first trimester were considered eligible for the study. Those who agreed to participate in the study were contacted and recruited at 37 gynecological hospitals and clinics within Hokkaido, the northernmost prefecture in Japan. Data were generated from these participants by means of baseline questionnaires, biochemical assays, birth records, and four-month ~~4-month~~ postpartum questionnaires. To date, there have been only three reports on the association between prenatal smoking, maternal genetic polymorphisms, and infant birth size [8, 12, 38]. Figure 1 shows the flow diagram for the participants in this study. A total of 10,731 expectant mothers were initially enrolled between February 2003 and December 2007. We acquired the medical records of the mothers at birth, third-trimester plasma cotinine levels, and genomic DNA for 8,256 potential candidates. Of these candidates, 8,015 remained after excluding 92 for birthing twins, 120 for hypertension during pregnancy, and 39 for gestational diabetes. We divided the 8,015 participants into two categories using plasma cotinine values of ≤11.48 ng/mL (6,730 subjects, non-smoking) and ≥11.49 ng/mL (1,285 subjects, smoking). The number of subjects with cotinine levels of ≤11.48 ng/mL was randomly decreased to 2,010 because 6,730 participants would have been too large a population to timely and cost effectively perform genetic analyses. To detect significant birth weight reduction (using α = P (type I error; false positive) = 0.05, β = P (type II error; false negative) = 0.20, and

standardized effect size = 0.10) we calculated that a minimum of 1,598 participants would be needed in sample size calculation based on t-test [39]. Only 1,598 subjects were statistically required [39] with 2,000 necessary to compensate for losses post-genetic analysis. Infant birth weight, length, and head circumference were obtained for 1,998 of the 2,010 subjects considered as non-smokers and 1,265 of the 1,285 subjects considered as smokers.

2.5. Statistical methods

When we compared data acquired for non-smokers versus data for smokers, the Mann-Whitney *U*-test was used as a non-parametric test for two continuous variables, and the χ^2 test was used as the statistical test for two categorical variables. The response variables were infant birth weight, birth length, and birth head circumference. The independent variables were (i) maternal genotype, (ii) maternal smoking status, (iii) one categorical variable that was the combination of maternal genotype and smoking status, and (iv) one categorical variable that was the combination of two or three genotypes and smoking status. Multiple linear regression models were used to evaluate the interrelationship between independent variables and response variables, while adjusting for the following covariates: maternal age (years), height (cm), weight before pregnancy (kg), parity (primiparous or multiparous), alcohol status during the first trimester (yes or no), educational level (junior high school, senior high school, college, or university), annual household income (<3, 3 to <5, 5 to <8, or ≥8 million yen), infant gender, and gestational age (weeks) [40-43]. The data were examined in the following steps. We used a multiple linear regression model to examine the association of each maternal demographical variable (infant gender, gestational age, maternal age, height, weight

before pregnancy, parity, alcohol intake during the first trimester, education level and annual household income) and genotype and infant birth size adjusted for potential confounders identified in a previous study. Next, we used a multiple linear regression model to examine the association of combined maternal smoking and genotype and infant birth size for nine potential confounders. Third, we used a multiple linear regression model to examine the association of combined maternal smoking and all combinations of three genotypes with infant birth size. We treated "low-risk" genotypes [*AHR*-GA/AA, *CYP1A1*-AA, *CYP1A2*-AA, *CYP1B1*-CC, $GSTM1^{+}$, $GSTT1^{+}$, *XRCC1*-CC (C>T, Arg194Trp) and *XRCC1*-GG (G>A, Arg399Gln)] as references for the multiple linear regression analyses. We tested the effects caused by the gene and smoking interaction terms (e.g., *AHR* × smoking; *AHR* × *CYP1A1* × smoking; *AHR* × *CYP1A1* × *XRCC1* × smoking). The interaction *P*-value (P_{int}) was calculated using a post-estimation combined *F*-test for the two interaction variables, namely maternal genotypes and smoking. A *P*-value < 0.05 was considered significant, and Bonferroni corrections were used for multiple comparisons.

2.6. Ethics

This study was conducted with the written, informed consent of all participants. The Institutional Ethical Board for Human Gene and Genome Studies of Hokkaido University Graduate School of Medicine approved the study protocol. The guidelines of the Declaration of Helsinki were followed.

3. RESULTS

Table 1 shows the characteristics in relation to their prenatal smoking status. Compared with infants to born to non-smokers, infants born to smokers had a mean lower birth weight (3,081 g versus 3,002 g; $P < 0.001$), higher proportion of female babies (52.0% versus 48.5%; $P = 0.046$), and shorter mean gestational age (39.0 weeks versus 38.9 weeks; $P = 0.009$). Compared with non-smokers, smokers were younger (mean 30.3 years versus 29.4 years; $P < 0.001$), more multiparous (59.6% versus 64.2%; $P < 0.001$), were less educated (highest education level Junior High School, 2.5% versus 13.9%; $P < 0.001$), and had lower annual household income (< 3 million yen, 21.0% versus 24.5%; $P < 0.001$). During their first trimester, smokers held fewer specialist and technical jobs (17.1% versus 10.6%) and more clerical jobs (26.8% versus 32.4%) than non-smokers ($P < 0.001$). For both groups of mothers, no biases for *AHR*, *CYP1A1*, *CYP1A2*, *CYP1B1*, *GSTM1*, *GSTT1*, or *XRCC1* genotypes were found (data not shown). In comparison with the partners of non-smokers, the partners of smokers were also more likely to be smokers (46.0% versus 56.8%; $P < 0.001$), and smoked more cigarettes (≥ 20 cigarettes/day, 33.6% versus 42.6%; $P < 0.001$). For non-smokers, 44.9% of the cigarettes (calculated as: 46.0% (smoking partners of non-smokers) × (30.2% + 15.8% + 51.7%; indoor smoking frequency more than once per day/week)) were smoked by partners more than one per week, and 63.7% of the cigarettes (calculated as: 57.9% + 5.8%; more than one cohabitant who smoked during the mother's first trimester) were smoked by cohabitants.

Table 2 shows the self-reported smoking habits and the third-trimester plasma cotinine levels of the mothers. Of the self-described non-smokers, 1,602 (91.1%) had plasma cotinine levels ≤11.48 ng/mL, whereas 157 (8.9%) had concentrations ≥11.49

ng/mL. Of those mothers who self-reported as smokers and reported that they smoked 1–4 cigarettes/day, 97 of 112 (86.6%) had cotinine concentrations ≥11.49 ng/mL, which is 33.8 times that of the median concentration of mothers who did not smoke. The true number of pregnant women exposed to smoking can be given, so 649 (94.6%) of the 686 women who reported themselves to be smokers, seemed to have greater accuracy.

Table 3 reports the estimated effects of demographic characteristics and maternal genotypes on infant birth size. Compared with infant males, infant females had significantly lower birth weight (-110 g; 95% confidence intervals (CI): -143, -78), birth length (-0.71 cm; 95% CI: -0.89, -0.53), and birth head circumference (-0.42 cm; 95% CI: -0.56, -0.28). Each week of gestational age correlated with an increase in the mean birth weight (136 g; 95% CI: 123, 150), birth length (0.65 cm; 95% CI; 0.58, 0.73), and birth head circumference (0.28 cm; 95% CI: 0.22, 0.34). Each year of maternal age correlated with an increase in the mean birth head circumference (0.02 cm; 95% CI: 0.01, 0.04). Each centimeter of maternal height correlated with an increase in mean birth weight (6 g; 95% CI: 2, 9), birth length (0.06 cm; 95% CI: 0.04, 0.07), and birth head circumference (0.02 cm; 95% CI: 0.01, 0.04). Each kilogram of maternal weight before pregnancy correlated with an increase in the mean birth weight (7 g; 95% CI: 5, 9), birth length (0.02 cm; 95%CI: 0.01, 0.03), and birth head circumference (0.01 cm; 95% CI: 0.00, 0.02). Compared with primiparous births, infants from multiparous births had significantly greater mean birth weight (81 g; 95% CI: 47, 116), slight increase in birth length (0.05 cm; 95% CI: -0.14, 0.24).

The associations of maternal smoking during the third trimester of pregnancy with infant birth weight (Figure 2), infant birth length (Figure 3), and infant birth head circumference (Figure 4) were then examined with respect to the eight maternal eight

genotypes. Compared with infants to born to non-smokers, infants born to smokers had lower ~~a~~ mean birth weight (-71 g; 95% CI, -103, -40). Compared with infants to born to non-smokers having one of reference (low-risk) genotypes, infants born to smokers with the *AHR*-GG (-56 g; 95% CI, -91, -20), *CYP1A1*-AG/GG (-62 g; 95% CI, -95, -30), *CYP1A2*-AC/CC (-36 g; 95% CI, -63, -9), *GSTM1*$^{-}$ (-43 g; 95% CI, -71, -14), *XRCC1* (C>T)-CT/TT genotype (-59 g; 95% CI, -87, -30), or *XRCC1* (G>A)-GA/AA (-46 g; 95% CI, -76, -15) genotypes had a significantly lower mean birth weight (Figure 2). We did not find any significant P_{int} (data not shown).

Compared with infants to born to non-smokers, infants born to smokers had shorter mean birth length (-0.23 cm; 95% CI, -0.41, -0.06). Compared with infants to born to non-smokers having one of reference (low-risk) genotypes, infants born to smokers with the *AHR*-GG (-0.35 cm; 95% CI, -0.55, -0.16), *CYP1A1*-AG/GG (-0.27 cm; 95% CI, -0.45, -0.09), or *XRCC1* (C>T)-CT/TT (-0.18 cm; 95% CI, -0.34, -0.02) genotypes had a significantly shorter mean birth length (Figure 3). We did not find any significant P_{int} (data not shown).

Compared with infants to born to non-smokers, infants born to smokers had smaller mean birth head circumference (-0.28 cm; 95% CI, -0.41, -0.15). Compared with infants to born to non-smokers having one of reference (low-risk) genotypes, infants born to smokers with the *AHR*-GG (-0.12 cm; 95% CI, -0.27, 0.03), *CYP1A1*-AG/GG (-0.21 cm; 95% CI, -0.35, -0.08), or *XRCC1* (C>T)-CT/TT (-0.18 cm; 95% CI, -0.30, -0.06) genotype had significantly smaller mean birth head circumference (Figure 4). We did not find any significant P_{int} (data not shown)

Furthermore, when we examined the association between maternal smoking and infant birth size in combination with the eight maternal genetic polymorphisms, we

observed an association with only three genetic polymorphisms, namely *AHR*, *CYP1A1* and *XRCC1* (C>T), among the 56 possible patterns. Compared with infants born to non-smokers carrying one of the "low-risk" genotypes *AHR*-GA/AA, *CYP1A1*-AA, and *XRCC1* (C>T)-CC (N = 144, 4.4%), infants born to non-smokers carrying one of the "high-risk" genotypes *AHR*-GG, *CYP1A1*-AG/GG, and *XRCC1* (C>T)-CT/TT genotypes (N = 48, 1.5%) had significantly greater mean birth weight (97 g; 95% CI: 32, 162), non-significantly longer mean birth length (0.35 cm; 95% CI: -0.01, 0.71), and significantly larger birth head circumference (0.33 cm; 95% CI, 0.05, 0.60). However, infants born to smokers carrying one of the "high-risk" genotypes (N = 68, 2.1%) had significantly lower mean birth weight (-145 g; 95% CI: -241, -50), shorter mean birth length (-0.70 cm; 95% CI: -1.24, -0.17), and smaller birth head circumference (-0.53 cm; 95% CI, -0.94, -0.13). We did not find any significant P_{int} (data not shown).

4. DISCUSSION

To the best of our knowledge, our study is the first to clearly show an association with *XRCC1* (C>T, Arg194Trp) of DNA repair genotypes and third-trimester maternal smoking—as defined by the maternal cotinine plasma levels—and infant birth size. Our most important finding is that infants born to smokers combined with *AHR*-GG, *CYP1A1*-AG/GG, and *XRCC1*-CT/TT genotypes are significantly more likely to have a reduced birth size compared with infants born to non-smokers carrying one of the *AHR*-GA/AA, *CYP1A1*-AA and *XRCC1*-CC genotypes. This result is consistent with those of previous studies, which suggested that infants born to ~~mothers~~ smokers with *AHR*-GG and *CYP1A1*-AG/GG compared to infants born to non-smokers with *AHR*-GA/AA and *CYP1A1*-AA genotypes suffered a greater adverse effect on their growth *in utero* [9, 11]. The genotypes *AHR*-GG, *CYP1A1*-AG/GG, *CYP1A2*-AC/CC, and *CYP1B1*-CG/GG correlate with an increase in the metabolic activities and expression of their protein products, and *XRCC1*-CT/TT correlates with a decrease in its protein product's DNA repair activity in comparison with their referent genotypes [23, 44-48]. GSTM1 and GSTT1 are detoxifying enzymes [49]. Infants born to smokers during pregnancy and had specific *AHR*, *CYP1A1*, *CYP1A2*, *GSTM1*, and *XRCC1* genotypes, but not those with *CYP1B1* and *GSTT1*, had a reduced birth weight of 56–62 g in comparison with infants born to non-smokers and had the referent genotypes (Figure 2). Mothers with *AHR*-GG, *CYP1A1*-AG/GG, and *XRCC1*-CT/TT are expected to produce BPDE metabolites and BPDE-DNA adducts to a greater extent than are mothers with the three referent genotypes [17-22, 50-52]. Compared with the birth weights of infants born to non-smokers with the low-risk genotypes of *AHR*-GA/AA, *CYP1A1*-AA, and *XRCC1*-CC, those of infants born to non-smokers with *AHR*-GG,

CYP1A1-AG/GG, and *XRCC1*-CT/TT were 97 g heavier. However, infants born to smokers with “high-risk” genotypes of the same genes were 145 g lighter than those from non-smokers carrying the “low-risk” genotypes. Our first hypothesis is that the “high-risk” genotypes *AHR*-GG and *CYP1A1*-AG/GG produce more BPDE metabolites and thus require more detoxification of BPDE compared with the reference genotypes. Non-smokers carrying the “high-risk” genotypes promote this route to detoxify BPDE, and thus their infants might have greater birth weight than non-smokers carrying the “low-risk” genotypes. Our secondary hypothesis is that the “high-risk” genotype of *XRCC1* (C>T)-CT/TT produces more was BPDE-DNA adducts and compromised DNA repair than the reference genotype. Smoking combined with “high-risk” genotypes promotes conversion of PAH to BPDE-DNA adducts and therefore produces infants with lower birth weight than non-smoking combined with “low-risk” genotypes. We cannot ignore that third-trimester maternal smoking may be more damaging to infant birth size when mothers have *AHR*-GG, *CYP1A1*-AG/GG, and *XRCC1*-CT/TT.

Compared with infants born to mothers with *AHR*-GA/AA who did not smoke, infants born to mothers with *AHR*-GA/AA who smoked were shown in our previous study to have a 211 g decrease in birth weight [11], whereas in this study we found 56 g decrease in birth weight. The key difference between the results of the two studies may be the definition of third-trimester maternal smoking. The different maternal smoking definitions and different numbers of female infants born to mothers who smoked (61.6% [11] vs. 48.5% (this study)) may have affected the birth weight results.

In this study, 1,759 mothers reported no smoking during the third trimester of pregnancy, however 157 of them had cotinine levels ≥11.49 ng/mL and considered as smokers. Given that 1,265 mothers had cotinine levels ≥11.49 ng/mL, the percentage of

mothers who claimed that they did not smoke yet had cotinine levels ≥11.49 ng/mL was 12.4% (= 157 out of 1,265). Mothers who smoked during the third trimester more often had partners who smoked than did mothers who did not smoke (Table 1). Possibly, mothers who claimed to be non-smokers yet had high cotinine levels were not truthfully reporting their smoking status as shown in previous studies [53-54] or were strongly exposed to the smoke of partners, housemates, and/or co-workers. A total of 692 mothers reported smoking during their third trimester; of those 37 mothers had cotinine levels ≤11.48 ng/mL and categorized as non-smokers in this study (Table 2). A total of 1,998 mothers had cotinine levels ≤11.48 ng/mL. Therefore, the percentage of mothers who smoked but considered non-smokers based on their cotinine levels was 1.9% (= 37 out of 1,998). Possible, the reason for the unexpectedly low plasma cotinine levels in mothers who claimed to smoke was that they smoked only occasionally, which caused their typical cotinine levels to be underestimated at the time of measurement as found in previous studies [55-56]. We conclude that measurement of cotinine levels is a more accurate assessment than allowing the mothers to self-report their third-trimester smoking status.

The strengths of this study are as follows. First, we accurately defined third-trimester maternal smoking status by measuring plasma cotinine levels. There may be a risk of circle conclusion, and it is unsure what the use of plasma cotinine level, instead of self-reported smoking status. Second, we correlated reduction in birth size for infants born to mothers who smoked with polymorphisms in genes encoding a PAH receptor, PAH metabolic and detoxification enzymes, and a DNA repair enzyme. In particular, we assessed the effects of combinations of these polymorphisms. However, we only measured the cotinine levels of the mothers a single time in the limitation of

this study. Cotinine has a short biological half-life of 17.9 hours [57]. However, by categorizing mothers as smokers or non-smokers based on cotinine level, we seem to have accurately identified the mothers (94.6% accuracy, with 692 mothers self-reporting as smokers with 655 having cotinine levels ≥11.49 ng/mL, and 91.1% accuracy, with 1,759 mothers self-reporting as non-smokers with 1,602 having cotinine levels ≤11.48 ng/mL (Table 2)). A second limitation is that the comparison of birth size of infants born to non-smokers and smokers was not adjusted for the other maternal genetic polymorphisms, such as those of secretor gene fucosyltransferase (*FUT*) *2*, maternally expressed imprinted gene *H19*, and epidermal growth factor (*EFG*) genes, that are known to be associated with birth weight [58-60]. Therefore, the results obtained here may be affected by individual genetic factors, such as genetic polymorphisms, not examined in this study.

5. CONCLUSIONS

We show that the correlation between third-trimester maternal smoking—as defined by the maternal plasma cotinine levels—and a reduced size of their infants is affected by specific combinations of *AHR*, *CYP1A1*, and *XRCC1* polymorphisms. We must still evaluate the exposure of infants to toxic substances in cigarette smoke after birth. Animal studies have found that active and/or passive smoking during pregnancy affects numerous immune functions and endocrine systems [61-64]. Little research has been conducted on the effects of passive maternal smoking in conjunction with maternal or infant genetic factors. In the future, we will investigate if passive maternal smoking—as affected by maternal genetic factors such as a combination of *AHR*, *CYP1A1*, and *XRCC1* genotypes—correlates with infant birth size.

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Conflicts of interests

None.

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Figure legends

Figure 1. Participant flow diagram.

Figure 2. Associations of maternal smoking during the third trimester of pregnancy with infant birth weight by eight maternal genotypes.

Multiple linear regression models adjusted for maternal age, height, weight before pregnancy, parity, alcohol intake during pregnancy, maternal education level, annual household income, gestational age and infant gender.
*; Statistically significant decreased birth weight for infants of smokers compared with reference ($P < 0.05$).

Figure 3. Associations of maternal smoking during the third trimester of pregnancy with infant birth length by eight maternal genotypes.

Multiple linear regression models adjusted for maternal age, height, weight before pregnancy, parity, alcohol intake during pregnancy, maternal education level, annual household income, gestational age and infant gender.
*; Statistically significant decreased birth length for infants of smokers compared with reference ($P < 0.05$).

Figure 4. Associations of maternal smoking during the third trimester of pregnancy with infant birth head circumference by eight maternal genotypes.

Multiple linear regression models adjusted for maternal age, height, weight before pregnancy, parity, alcohol intake during pregnancy, maternal education level, annual household income, gestational age and infant gender.
*; Statistically significant decreased birth head circumference for infants of smokers compared with reference ($P < 0.05$).

Figure 1.

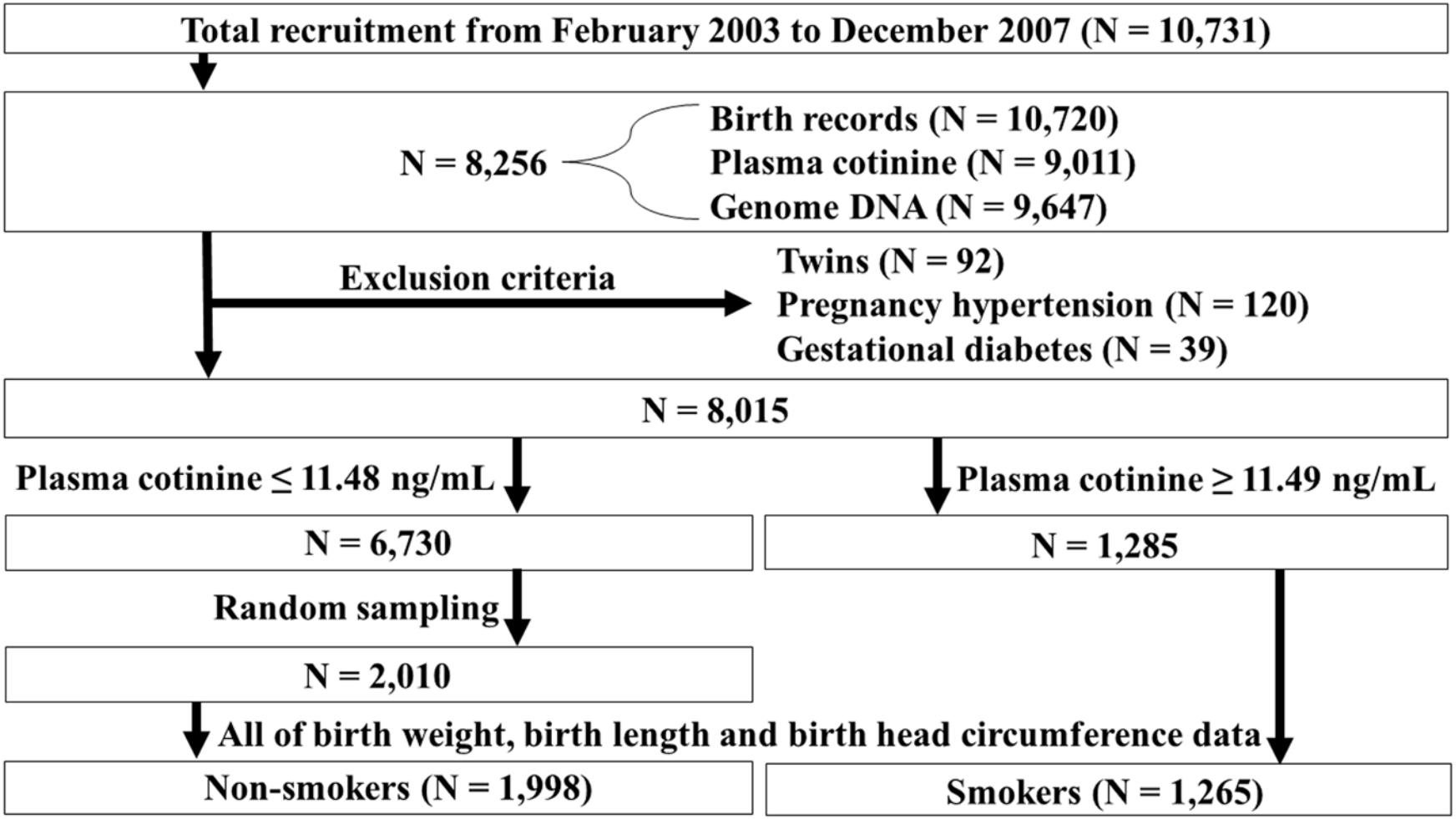
Total recruitment from February 2003 to December 2007 (N = 10,731)
N = 8,256
Birth records (N = 10,720)
Plasma cotinine (N = 9,011)
Genome DNA (N = 9,647)
Exclusion criteria
Twins (N = 92)
Pregnancy hypertension (N = 120)
Gestational diabetes (N = 39)
N = 8,015
Plasma cotinine ≤ 11.48 ng/mL
Plasma cotinine ≥ 11.49 ng/mL
N = 6,730
N = 1,285
Random sampling
N = 2,010
All of birth weight, birth length and birth head circumference data
Non-smokers (N = 1,998)
Smokers (N = 1,265)

Figure 2.

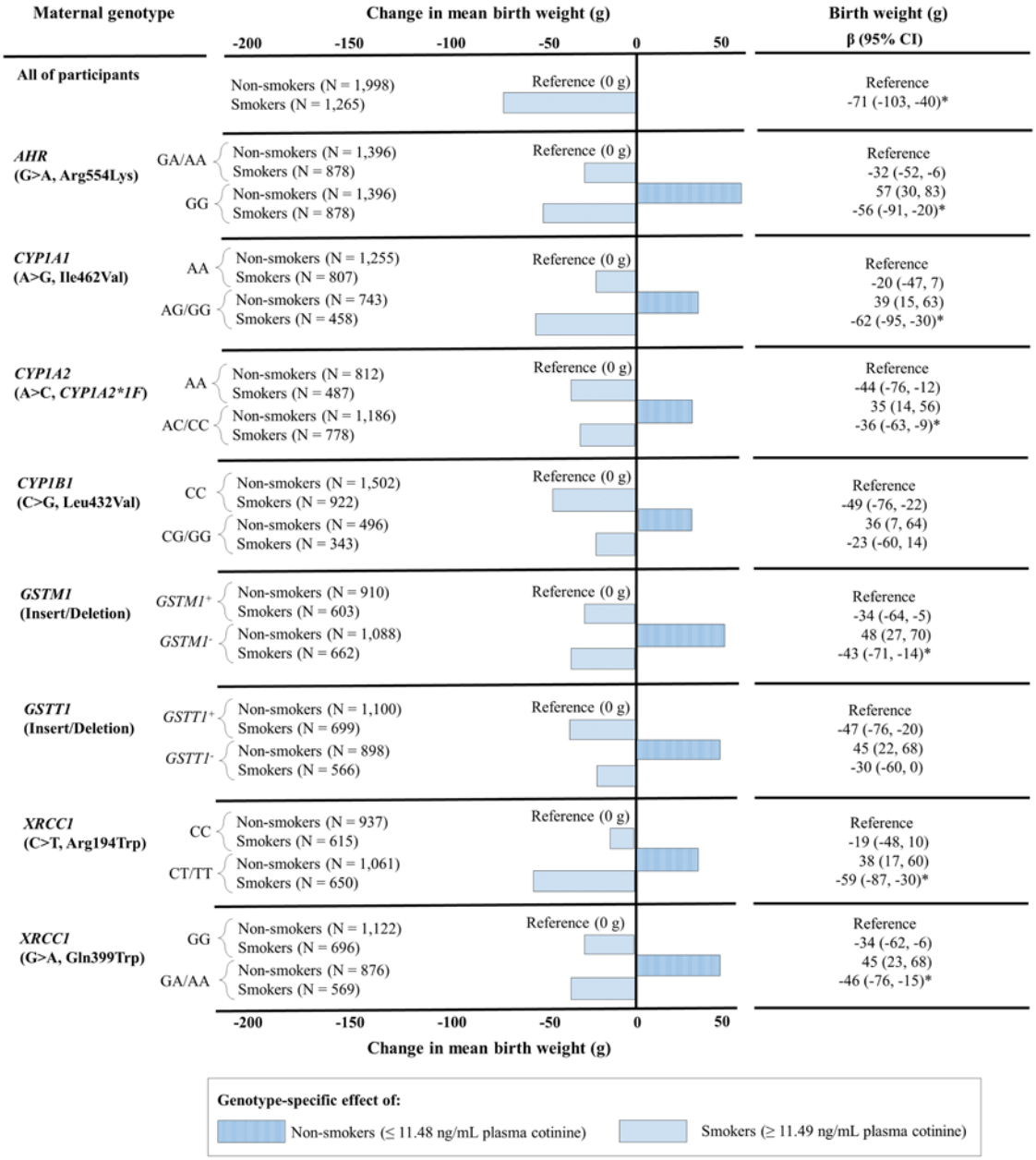
Maternal genotype
Change in mean birth weight (g)
Birth weight (g)
β (95% CI)
-200 -150 -100 -50 0 50
All of participants
Non-smokers (N = 1,998)
Smokers (N = 1,265)
Reference (0 g)
Reference
-71 (-103, -40)*
AHR
(G>A, Arg554Lys)
GA/AA
Non-smokers (N = 1,396)
Smokers (N = 878)
Reference (0 g)
Reference
-32 (-52, -6)
GG
Non-smokers (N = 1,396)
Smokers (N = 878)
57 (30, 83)
-56 (-91, -20)*
CYP1A1
(A>G, Ile462Val)
AA
Non-smokers (N = 1,255)
Smokers (N = 807)
Reference (0 g)
Reference
-20 (-47, 7)
AG/GG
Non-smokers (N = 743)
Smokers (N = 458)
39 (15, 63)
-62 (-95, -30)*
CYP1A2
(A>C, CYP1A2*1F)
AA
Non-smokers (N = 812)
Smokers (N = 487)
Reference (0 g)
Reference
-44 (-76, -12)
AC/CC
Non-smokers (N = 1,186)
Smokers (N = 778)
35 (14, 56)
-36 (-63, -9)*
CYP1B1
(C>G, Leu432Val)
CC
Non-smokers (N = 1,502)
Smokers (N = 922)
Reference (0 g)
Reference
-49 (-76, -22)
CG/GG
Non-smokers (N = 496)
Smokers (N = 343)
36 (7, 64)
-23 (-60, 14)
GSTM1
(Insert/Deletion)
GSTM1+
Non-smokers (N = 910)
Smokers (N = 603)
Reference (0 g)
Reference
-34 (-64, -5)
GSTM1-
Non-smokers (N = 1,088)
Smokers (N = 662)
48 (27, 70)
-43 (-71, -14)*
GSTT1
(Insert/Deletion)
GSTT1+
Non-smokers (N = 1,100)
Smokers (N = 699)
Reference (0 g)
Reference
-47 (-76, -20)
GSTT1-
Non-smokers (N = 898)
Smokers (N = 566)
45 (22, 68)
-30 (-60, 0)
XRCC1
(C>T, Arg194Trp)
CC
Non-smokers (N = 937)
Smokers (N = 615)
Reference (0 g)
Reference
-19 (-48, 10)
CT/TT
Non-smokers (N = 1,061)
Smokers (N = 650)
38 (17, 60)
-59 (-87, -30)*
XRCC1
(G>A, Gln399Trp)
GG
Non-smokers (N = 1,122)
Smokers (N = 696)
Reference (0 g)
Reference
-34 (-62, -6)
GA/AA
Non-smokers (N = 876)
Smokers (N = 569)
45 (23, 68)
-46 (-76, -15)*
-200 -150 -100 -50 0 50
Change in mean birth weight (g)
Genotype-specific effect of:
Non-smokers (≤ 11.48 ng/mL plasma cotinine)
Smokers (≥ 11.49 ng/mL plasma cotinine)

Figure 3.

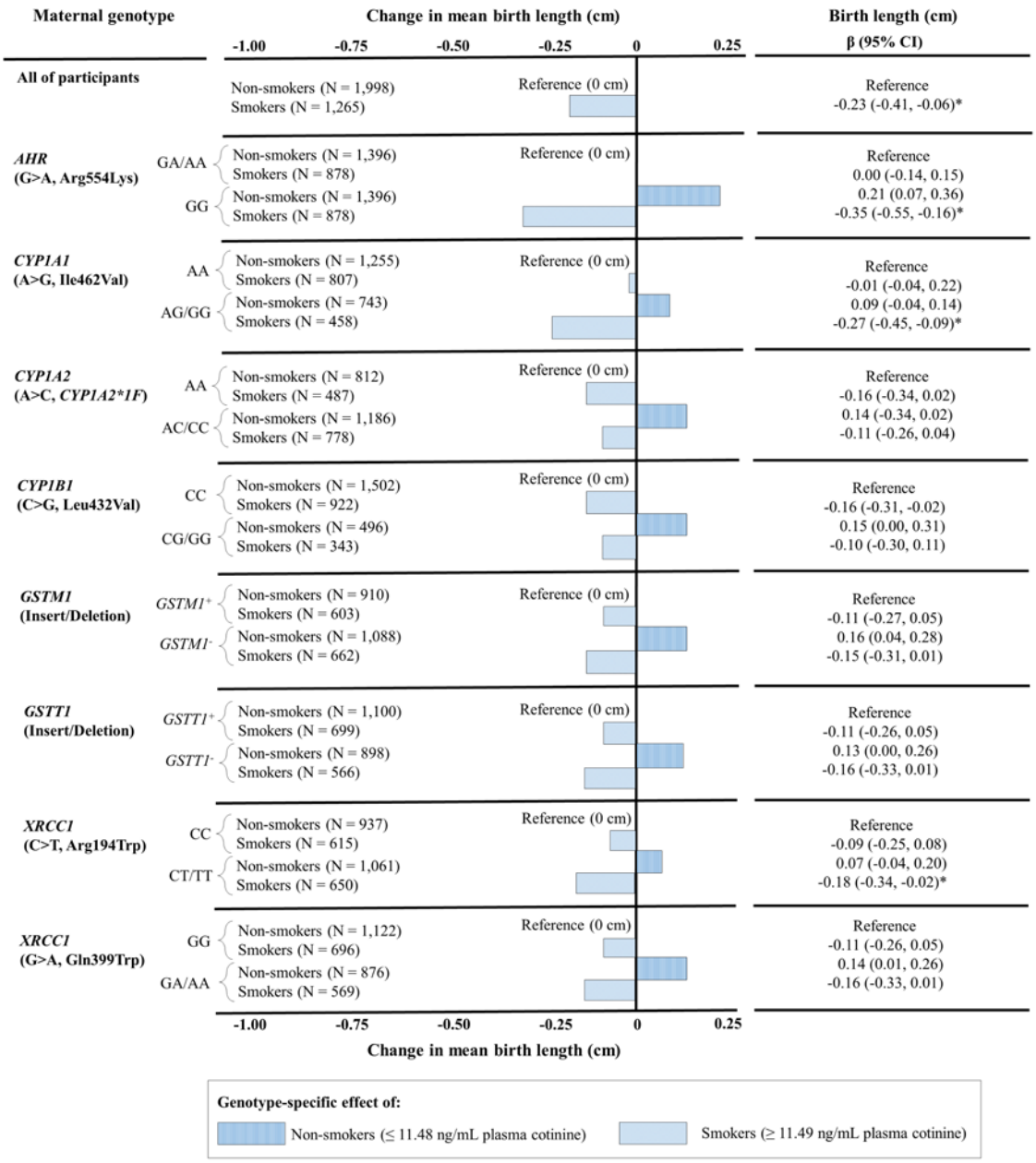
Maternal genotype
Change in mean birth length (cm)
Birth length (cm)
β (95% CI)
-1.00
-0.75
-0.50
-0.25
0
0.25
All of participants
Non-smokers (N = 1,998)
Smokers (N = 1,265)
Reference (0 cm)
Reference
-0.23 (-0.41, -0.06)*
AHR
(G>A, Arg554Lys)
GA/AA
Non-smokers (N = 1,396)
Smokers (N = 878)
GG
Non-smokers (N = 1,396)
Smokers (N = 878)
Reference (0 cm)
Reference
0.00 (-0.14, 0.15)
0.21 (0.07, 0.36)
-0.35 (-0.55, -0.16)*
CYP1A1
(A>G, Ile462Val)
AA
Non-smokers (N = 1,255)
Smokers (N = 807)
AG/GG
Non-smokers (N = 743)
Smokers (N = 458)
Reference (0 cm)
Reference
-0.01 (-0.04, 0.22)
0.09 (-0.04, 0.14)
-0.27 (-0.45, -0.09)*
CYP1A2
(A>C, CYP1A2*1F)
AA
Non-smokers (N = 812)
Smokers (N = 487)
AC/CC
Non-smokers (N = 1,186)
Smokers (N = 778)
Reference (0 cm)
Reference
-0.16 (-0.34, 0.02)
0.14 (-0.34, 0.02)
-0.11 (-0.26, 0.04)
CYP1B1
(C>G, Leu432Val)
CC
Non-smokers (N = 1,502)
Smokers (N = 922)
CG/GG
Non-smokers (N = 496)
Smokers (N = 343)
Reference (0 cm)
Reference
-0.16 (-0.31, -0.02)
0.15 (0.00, 0.31)
-0.10 (-0.30, 0.11)
GSTM1
(Insert/Deletion)
GSTM1+
Non-smokers (N = 910)
Smokers (N = 603)
GSTM1-
Non-smokers (N = 1,088)
Smokers (N = 662)
Reference (0 cm)
Reference
-0.11 (-0.27, 0.05)
0.16 (0.04, 0.28)
-0.15 (-0.31, 0.01)
GSTT1
(Insert/Deletion)
GSTT1+
Non-smokers (N = 1,100)
Smokers (N = 699)
GSTT1-
Non-smokers (N = 898)
Smokers (N = 566)
Reference (0 cm)
Reference
-0.11 (-0.26, 0.05)
0.13 (0.00, 0.26)
-0.16 (-0.33, 0.01)
XRCC1
(C>T, Arg194Trp)
CC
Non-smokers (N = 937)
Smokers (N = 615)
CT/TT
Non-smokers (N = 1,061)
Smokers (N = 650)
Reference (0 cm)
Reference
-0.09 (-0.25, 0.08)
0.07 (-0.04, 0.20)
-0.18 (-0.34, -0.02)*
XRCC1
(G>A, Gln399Trp)
GG
Non-smokers (N = 1,122)
Smokers (N = 696)
GA/AA
Non-smokers (N = 876)
Smokers (N = 569)
Reference (0 cm)
Reference
-0.11 (-0.26, 0.05)
0.14 (0.01, 0.26)
-0.16 (-0.33, 0.01)
-1.00
-0.75
-0.50
-0.25
0
0.25
Change in mean birth length (cm)
Genotype-specific effect of:
Non-smokers (≤ 11.48 ng/mL plasma cotinine)
Smokers (≥ 11.49 ng/mL plasma cotinine)

Figure 4.

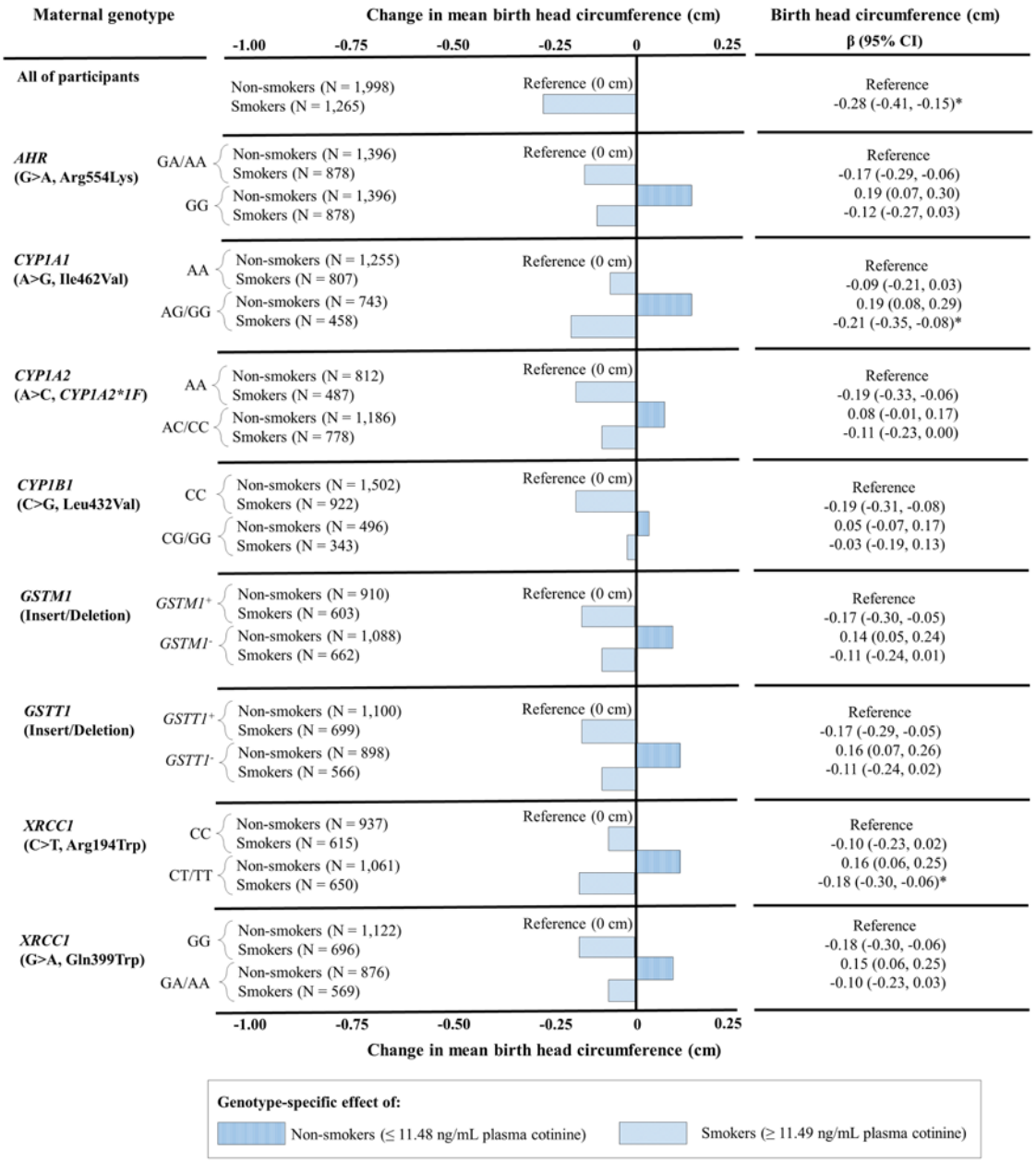
Maternal genotype
Change in mean birth head circumference (cm)
Birth head circumference (cm)
β (95% CI)
-1.00
-0.75
-0.50
-0.25
0
0.25
All of participants
Non-smokers (N = 1,998)
Smokers (N = 1,265)
Reference (0 cm)
Reference
-0.28 (-0.41, -0.15)*
AHR
(G>A, Arg554Lys)
GA/AA
Non-smokers (N = 1,396)
Smokers (N = 878)
GG
Non-smokers (N = 1,396)
Smokers (N = 878)
Reference (0 cm)
Reference
-0.17 (-0.29, -0.06)
0.19 (0.07, 0.30)
-0.12 (-0.27, 0.03)
CYP1A1
(A>G, Ile462Val)
AA
Non-smokers (N = 1,255)
Smokers (N = 807)
AG/GG
Non-smokers (N = 743)
Smokers (N = 458)
Reference (0 cm)
Reference
-0.09 (-0.21, 0.03)
0.19 (0.08, 0.29)
-0.21 (-0.35, -0.08)*
CYP1A2
(A>C, CYP1A2*1F)
AA
Non-smokers (N = 812)
Smokers (N = 487)
AC/CC
Non-smokers (N = 1,186)
Smokers (N = 778)
Reference (0 cm)
Reference
-0.19 (-0.33, -0.06)
0.08 (-0.01, 0.17)
-0.11 (-0.23, 0.00)
CYP1B1
(C>G, Leu432Val)
CC
Non-smokers (N = 1,502)
Smokers (N = 922)
CG/GG
Non-smokers (N = 496)
Smokers (N = 343)
Reference (0 cm)
Reference
-0.19 (-0.31, -0.08)
0.05 (-0.07, 0.17)
-0.03 (-0.19, 0.13)
GSTM1
(Insert/Deletion)
GSTM1+
Non-smokers (N = 910)
Smokers (N = 603)
GSTM1-
Non-smokers (N = 1,088)
Smokers (N = 662)
Reference (0 cm)
Reference
-0.17 (-0.30, -0.05)
0.14 (0.05, 0.24)
-0.11 (-0.24, 0.01)
GSTT1
(Insert/Deletion)
GSTT1+
Non-smokers (N = 1,100)
Smokers (N = 699)
GSTT1-
Non-smokers (N = 898)
Smokers (N = 566)
Reference (0 cm)
Reference
-0.17 (-0.29, -0.05)
0.16 (0.07, 0.26)
-0.11 (-0.24, 0.02)
XRCC1
(C>T, Arg194Trp)
CC
Non-smokers (N = 937)
Smokers (N = 615)
CT/TT
Non-smokers (N = 1,061)
Smokers (N = 650)
Reference (0 cm)
Reference
-0.10 (-0.23, 0.02)
0.16 (0.06, 0.25)
-0.18 (-0.30, -0.06)*
XRCC1
(G>A, Gln399Trp)
GG
Non-smokers (N = 1,122)
Smokers (N = 696)
GA/AA
Non-smokers (N = 876)
Smokers (N = 569)
Reference (0 cm)
Reference
-0.18 (-0.30, -0.06)
0.15 (0.06, 0.25)
-0.10 (-0.23, 0.03)
-1.00
-0.75
-0.50
-0.25
0
0.25
Change in mean birth head circumference (cm)
Genotype-specific effect of:
Non-smokers (≤ 11.48 ng/mL plasma cotinine)
Smokers (≥ 11.49 ng/mL plasma cotinine)

Table 1. Participant characteristics in relation to prenatal smoking status based on plasma cotinine

Characteristics	Non-smokers (N = 1,998) N (%)	Smokers (N = 1,265) N (%)	*P* value
Infants			
Birth weight (g)[a]	3,081 ± 384[c]	3,002 ± 379[c]	<0.001***
Birth length (cm)[a]	49.1 ± 2.1[c]	48.8 ± 2.6[c]	0.001**
Birth head circumference (cm)[a]	33.3 ± 1.5[c]	33.0 ± 1.5[c]	<0.001***
Gender[b]			
Male	959 (48.0)	652 (51.5)	0.046*
Female	1,039 (52.0)	613 (48.5)	
Missing data	0 (0.0)	1 (0.1)	
Gestational age (weeks)[a]	39.0 ± 1.3[c]	38.9 ± 1.3[c]	0.009**
Mothers			
Age (years)[a]	30.3 ± 4.5[c]	29.4 ± 4.7[c]	<0.001***
Height (cm)[a]	158 ± 5[c]	158 ± 6[c]	0.171
Weight before pregnancy (kg)[a]	53 ± 8[c]	53 ± 11[c]	0.601
Parity[b]			
Primiparous	895 (44.8)	431 (34.1)	<0.001***
Multiparous	1,103 (59.6)	784 (62.0)	
Missing data	0 (0.0)	50 (4.0)	
Alcohol intake during the first trimester[b]			
No	807 (40.4)	431 (34.1)	<0.001***
Yes	1,191 (59.6)	812 (64.2)	
Missing data	0 (0.0)	22 (1.7)	
Educational level[b]			
Junior High School	49 (2.5)	176 (13.9)	<0.001***
Senior High School	833 (41.7)	726 (57.4)	
Junior College	880 (44.0)	316 (25.0)	
University	235 (11.8)	23 (1.8)	
Missing data	1 (0.1)	24 (1.9)	
Annual household intake (million yen)[b]			
<3	419 (21.0)	310 (24.5)	<0.001***
3 to <5	900 (45.0)	499 (39.4)	
5 to <8	527 (26.4)	186 (14.7)	
≥8	152 (7.6)	57 (4.5)	
Missing data	0 (0.0)	213 (16.8)	
Number of cigarettes smoked per day during the third trimester	-		
0 to <5	15 (0.8)	97 (7.7)	-
5 to <10	14 (0.7)	196 (15.5)	
10 to <15	5 (0.3)	247 (19.5)	
15 to <20	3 (0.2)	51 (4.0)	
≥20	0 (0.0)	58 (4.6)	
Missing data	-	616 (48.7)	
Number of cohabitants who smoked during the mothers' first trimester[b]			
0	700 (35.5)	96 (7.6)	<0.001***
1	1,156 (57.9)	947 (74.9)	
≥2	116 (5.8)	190 (15.0)	
Missing data	26 (1.3)	32 (2.5)	
Mothers' job category during their first trimester[b]			
Manager	2 (0.1)	2 (0.2)	<0.001***
Specialist and technical personnel	341 (17.1)	134 (10.6)	
Clerical personnel	536 (26.8)	410 (32.4)	
Sales	3 (0.2)	2 (0.2)	
Services	58 (2.9)	53 (4.2)	
Security service	3 (0.2)	0 (0.0)	
Agriculture and forestry	60 (3.0)	44 (3.5)	
Production	19 (1.0)	5 (0.4)	
Transit and driver service	0 (0.0)	1 (0.1)	
Builder and driller	1 (0.1)	0 (0.0)	
Traffic, janitorial service and packer	9 (0.5)	5 (0.4)	
Housewife, white hands and unclear job category	924 (46.2)	554 (43.8)	
Housewife and white hands	912 (45.6)	544 (43.0)	
Missing data	42 (2.1)	55 (4.3)	
Exposure to smoking during the first trimester on the job[b]	(N = 1,086)	(N = 721)	
Non-exposure	537 (49.4)	148 (20.5)	<0.001***
1–2 days/week	158 (14.5)	69 (9.6)	
3–4 days/week	109 (10.0)	73 (10.1)	
Almost every day	265 (24.4)	396 (54.9)	
Missing data	17 (1.6)	35 (4.9)	

Partners			
Smoking status during the third trimester[b]			
No	721 (36.1)	90 (7.1)	<0.001***
Yes	920 (46.0)	719 (56.8)	
Missing data	357 (17.9)	456 (36.0)	
Indoor smoking frequency during the third trimester (only smokers) (days/week)[b]	(N = 920)	(N = 719)	
≤1	278 (30.2)	66 (9.2)	<0.001***
2–5	145 (15.8)	109 (15.2)	
≥6	476 (51.7)	520 (72.3)	
Missing data	21 (2.3)	24 (3.3)	
Smoked cigarettes during the mothers' third trimester (per day)[b]	(N = 920)	(N = 719)	
0 to <5	115 (12.5)	34 (4.7)	<0.001***
5 to <10	119 (12.9)	68 (9.5)	
10 to <15	205 (22.3)	161 (22.4)	
15 to <20	79 (8.6)	86 (12.0)	
≥20	309 (33.6)	306 (42.6)	
Missing data	93 (10.1)	64 (8.9)	
Alcohol intake during the first trimester[b]			
No	508 (25.4)	368 (29.0)	0.015*
Yes	1,457 (72.9)	852 (67.4)	
Missing data	33 (1.7)	45 (3.6)	
Job category during the first trimester[b]			
Manager	47 (2.4)	25 (2.0)	<0.001***
Specialist and technical personnel	354 (17.7)	127 (10.0)	
Clerical personnel	254 (12.7)	130 (10.3)	
Sales	1 (0.1)	1 (0.1)	
Services	51 (2.6)	30 (2.4)	
Security service	55 (2.8)	25 (2.0)	
Agriculture and forestry	123 (6.2)	104 (8.2)	
Production	75 (3.8)	36 (2.8)	
Transit and driver service	113 (5.7)	127 (10.0)	
Builder and driller	75 (3.8)	78 (6.2)	
Traffic, janitorial service, and packers	12 (0.6)	8 (0.6)	
Housewife, white hands and job category unclear	833 (41.7)	561 (44.3)	
Missing data	5 (0.3)	13 (1.0)	
Educational level[b]			
Junior High School	100 (5.0)	230 (18.1)	<0.001***
Senior High School	817 (40.9)	606 (47.9)	
Junior College	485 (24.3)	260 (20.6)	
University	567 (28.4)	121 (9.6)	
Missing data	29 (1.5)	48 (3.8)	

[a] Mann-Whitney *U*-test. [b] χ^2-test. [c] Mean ± SD (Standard deviation).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2. Self-reported smoking habits and plasma cotinine levels in 3,263 pregnant women

Self-reported smoking habits (Answered when the infants were 4 months of age)		Plasma cotinine level at the third trimester (ng/mL)			
Third trimester	No. (%)	Median (inter-quartile range)	No. (%)		
			≤0.21	0.22–11.48	≥11.49
Non-smoking mothers	1,759 (53.9)	0.34 (0.15–0.91)	607 (34.5)	995 (56.6)	157 (8.9)
Smoking mothers	686 (21.0)	(-)	2 (0.3)	35 (5.1)	649 (94.6)
1–4 cigarettes/day	112 (3.4)	55.1 (26.6–95.4)	0 (0.0)	15 (13.4)	97 (86.6)
5–9 cigarettes/day	210 (6.4)	88.9 (52.4–128.9)	2 (1.0)	12 (5.7)	196 (93.3)
10–14 cigarettes/day	252 (7.7)	117.3 (79.5–155.9)	0 (0.0)	5 (2.0)	247 (98.0)
15–19 cigarettes/day	54 (1.7)	140.0 (104.2–190.9)	0 (0.0)	3 (5.6)	51 (94.4)
≥20 cigarettes/day	58 (1.8)	128.5 (101.6–175.6)	0 (0.0)	0 (0.0)	58 (100.0)
Missing data	6 (0.2)	76.0 (58.0–92.7)	0 (0.0)	0 (0.0)	6 (100.0)
Dropped out when the infant was 4 months of age	812 (24.9)	62.5 (0.41–111.0)	124 (15.3)	235 (28.9)	453 (55.8)
Total	3,263 (100.0)	1.01 (0.25–79.2)	733 (22.5)	1,265 (38.8)	1,265 (38.8)

Table 3. Estimated effects of demographic characteristics and maternal genotypes on infant birth size (N = 3,263).

	Categories/unit/genotypes	N (%)	Birth weight (g) β (95% CI)	Birth length (cm) β (95% CI)	Birth head circumference (cm) β (95% CI)
Infants					
Gender	Male	1,611 (49.4)	Reference	Reference	Reference
	Female	1,651 (50.6)	-110 (-143, -78)	-0.71 (-0.89, -0.53)	-0.42 (-0.56, -0.28)
	Missing data	1 (0.0)	(-)	(-)	(-)
Gestational age (weeks)	Each week	39.0 ± 1.3[a]	136 (123, 150)	0.65 (0.58, 0.73)	0.28 (0.22, 0.34)
Mothers					
Age (years)	Each year	30.0 ± 4.6[a]	0 (-4, 4)	-0.01 (-0.03, 0.02)	0.02 (0.01, 0.04)
Height (cm)	Each centimeter	158.1 ± 5.4[a]	6 (2, 9)	0.06 (0.04, 0.07)	0.02 (0.01, 0.04)
Weight before pregnancy (kg)	Each kilogram	53.0 ± 9.3[a]	7 (5, 9)	0.02 (0.01, 0.03)	0.01 (0.00, 0.02)
Parity	Primiparous	1,341 (41.1)	Reference	Reference	Reference
	Multiparous	1,887 (57.8)	81 (47, 116)	0.05 (-0.14, 0.24)	0.36 (0.21, 0.51)
	Missing data	35 (1.1)	(-)	(-)	(-)
Alcohol intake during the first trimester	No	1,238 (37.9)	Reference	Reference	Reference
	Yes	2,003 (61.4)	-18 (-34, -3)	-0.10 (-0.19, -0.02)	-0.07 (-0.14, 0.00)
	Missing data	22 (0.3)	(-)	(-)	(-)
Education level	Junior High school	225 (6.9)	Reference	Reference	Reference
	Senior High school	1,559 (47.8)	22 (-59, 104)	0.20 (-0.25, 0.65)	-0.14 (-0.49, 0.22)
	Junior College	1,196 (36.7)	38 (-45, 121)	0.14 (-0.32, 0.60)	-0.03 (-0.40, 0.33)
	University	258 (7.9)	71 (-24, 166)	0.40 (-0.13, 0.92)	0.12 (-0.30, 0.53)
	Missing data	25 (0.8)	(-)	(-)	(-)
Annual household income (million yen)	<3	729 (22.3)	Reference	Reference	Reference
	3 to <5	1,399 (42.9)	-33 (-77, 11)	-0.07 (-0.31, 0.17)	-0.11 (-0.30, 0.08)
	5 to <8	713 (21.9)	-3 (-53, 48)	0.02 (-0.27, 0.30)	0.06 (-0.16, 0.28)
	≥8	209 (6.4)	-71 (-144, 2)	-0.34 (-0.74, 0.06)	-0.01 (-0.33, 0.31)
	Missing data	213 (6.4)	(-)	(-)	(-)
AHR (G>A, Arg554Lys)	GA/AA	2,274 (69.7)	Reference	Reference	Reference
	GG	989 (30.3)	22 (-14, 58)	-0.02 (-0.22, 0.18)	0.13 (-0.03, 0.29)
CYP1A1 (A>G, Ile462Val)	AA	2,062 (63.2)	Reference	Reference	Reference
	AG/GG	1,201 (36.8)	-17 (-50, 17)	-0.19 (-0.38, -0.01)	0.01 (-0.13, 0.16)
CYP1A2 (A>C, *CYP1A2*1F*)	AA	1,299 (39.8)	Reference	Reference	Reference
	AC/CC	1,964 (60.2)	-15 (-49, 18)	0.08 (-0.11, 0.26)	-0.12 (-0.27, 0.03)
CYP1B1 (C>G, Leu432Val)	CC	2,424 (74.3)	Reference	Reference	Reference
	CG/GG	839 (25.7)	6 (-31, 43)	0.06 (-0.15, 0.26)	-0.04 (-0.20, 0.13)
GSTM1 (Insert/Deletion)	*GSTM1*$^{+}$	1,513 (46.4)	Reference	Reference	Reference
	GSTM1^{-}	1,750 (53.6)	13 (-19, 46)	0.09 (-0.09, 0.27)	0.04 (-0.11, 0.18)
GSTT1 (Insert/Deletion)	*GSTT1*$^{+}$	1,799 (55.1)	Reference	Reference	Reference
	GSTT1^{-}	1,464 (44.9)	11 (-22, 44)	-0.05 (-0.23, 0.13)	0.03 (-0.12, 0.17)
XRCC1 (C>T, Arg194Trp)	CC	1,552 (47.6)	Reference	Reference	Reference
	CT/TT	1,711 (52.4)	-18 (-51, 14)	-0.14 (-0.32, 0.04)	0.04 (-0.10, 0.18)
XRCC1 (G>A, Gln399Trp)	GG	1,818 (55.7)	Reference	Reference	Reference
	GA/AA	1,445 (44.3)	-5 (-38, 28)	-0.09 (-0.27, 0.09)	0.04 (-0.10, 0.19)

Multiple linear regression models adjusted for maternal age, height, weight before pregnancy, parity, alcohol intake during pregnancy, maternal education

level, annual household income, gestational age and infant gender.
[a] Mean $\pm$ SD (Standard deviation).