Low level maternal smoking and infant birthweight reduction: genetic contributions of GSTT1 and GSTM1 polymorphisms

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Abstract

**Background:** Genetic susceptibility to tobacco smoke might modify the effect of smoking on pregnancy outcomes.

**Methods:** We conducted a case–control study of 543 women who delivered singleton live births in Kaunas (Lithuania), examining the association between low-level tobacco smoke exposure (mean: 4.8 cigarettes/day) during pregnancy, GSTT1 and GSTM1 polymorphisms and birthweight of the infant. Multiple linear-regression analysis was performed adjusting for gestational age, maternal education, family status, body mass index, blood pressure, and parity. Subsequently, we tested for the interaction effect of maternal smoking, GSTT1 and GSTM1 genes polymorphisms with birthweight by adding all the product terms in the regression models.

**Results:** The findings suggested a birthweight reduction among light-smoking with the GSTT1–null genotype (−162.9 g, P = 0.041) and those with the GSTM1–null genotype (−118.7 g, P = 0.069). When a combination of these genotypes was considered, birthweight was significantly lower for infants of smoking women the carriers of the double-null genotypes (−311.2 g, P = 0.008). The interaction effect of maternal smoking, GSTM1 and GSTT1 genotypes was marginally significant on birthweight (−234.5 g, P = 0.078). Among non-smokers, genotype did not independently confer an adverse effect on infant birthweight.

**Conclusions:** The study shows the GSTT1–null genotype, either presents only one or both with GSTM1–null genotype in a single subject, have a modifying effect on birthweight among smoking women even though their smoking is low level. Our data also indicate that identification of the group of susceptible subjects should be based on both environmental exposure and gene polymorphism. Findings of this study add additional evidence on the interplay among two key GST genes and maternal smoking on birth weight of newborns.

Keywords: Birthweight, GST polymorphisms, Smoking, Interaction

Background

Environmental factors contributing to reduced birthweight are a great concern because of the well-known relation of birthweight to infant mortality and adverse health effects in later life. Recent epidemiological studies have linked maternal tobacco-smoking and other environmental exposures to increased risk of low birthweight, preterm delivery, congenital anomalies, pregnancy loss, foetal growth, birthweight [1,2]. Investigators, who have examined the issue, showed dose–response gradients in relation to the number of cigarettes smoked [3] and association between maternal smoking during pregnancy, impaired foetal growth and overweight in childhood [4]. Some studies reported an increased risks of low birth weight (LBW) and small for gestational age with heavier maternal smoking (> 10 cigarettes/day), as well as noting an increased risk for "very preterm" birth (< 35 weeks) [3]. It was reported that smoking even 20 cigarettes/day was not related to risk of preterm birth overall, but cotinine measured at the time of delivery was. A clear association and dose–response gradient was present for risk of foetal growth restriction [5] and...
null and GSTT1−null genotypes have been associated with the effect of maternal smoking on duration of gestation, birth weight, and size [13,14,16]. Several recent studies have reported that genetic susceptibility modulates the risk of adverse pregnancy outcomes from tobacco smoke [15,16,24–31].

Different results have been presented by several authors [16]. In a case–control study, controlling for several confounding factors, the authors have shown that the maternal GSTT1−null genotype had a 1.6-fold reduced risk of infant-growth restriction. However, after adjustment for maternal smoking (categories less than 10 cigarettes per day and more than 10 cigarettes per day), the results are not statistically significant. There is evidence that the effect of cigarette-smoke exposure depends on the population characteristics: among the Japanese, the GSTM1−null genotype decreases foetal growth but this effect is not observed in Caucasians [3,14].

Tobacco smoke is a complex mixture of numerous substances that include polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines among them. Recent studies have shown that there are significant associations between exposure to PAHs and reduced foetal growth and preterm birth [1]. Increasing PAH levels during pregnancy may increase the risk of foetal growth restriction [32], lung-function reduction in children, particularly for those whose mothers possessed the polymorphic CYP1A1*2A and GSTM1 deletion [26]. However, there is inconsistency in the relationship between GSTT1 and GSTM1 polymorphisms and smoking effects on foetal development. Variations in the CYP1A1 and GSTM1 genes that encode these enzymes could affect smoking behaviour by altering the levels and duration of tobacco-related PAHs and their metabolites in the body [33].

Although separate GSTM1 and GSTT1 gene deletions are well-studied functional variants, so far, there are only a limited numbers of studies published on the impacts of light cigarette smoking, GST metabolic gene polymorphism, and infant birth weight data.

In this study, we used a case–control design to examine the relationship between maternal smoking, GSTM1, GSTT1 polymorphism, and birth weight. We hypothesized those women with the GSTM1− and GSTT1−null genotype who are exposed to cigarette smoke during pregnancy are at elevated risk for newborn birthweight reduction.

**Methods**

**Participant and outcome assessment**

A prospective cohort study of pregnant women was conducted between 2007 and 2008 in Kaunas, Lithuania (Kaunas HiWATE cohort study). On their first visit to a general practitioner, all pregnant women living in Kaunas were invited to join the cohort and answer to the first...
questionnaire. We recruited these women for the prospective cohort study, enrolling them at 23–35 weeks of gestation at the four prenatal care clinics affiliated to the hospitals of the Kaunas University of Medicine. Participation was on a voluntary basis and the women were enrolled in the study only if they consented to participate in the cohort. The research protocol was approved by the Lithuanian Bioethics Committee and informed consent was obtained from all subjects. A special questionnaire was evolved to interview the women who agreed to participate in the genetic study and blood samples for genetic analysis was collected. Details of the methods and study subjects have been published elsewhere [34]. The subjects of this case–control study were 543 women, who delivered singleton live births at the four hospitals affiliated to the Lithuanian University of Health Science. Multiple births or newborns with major births defects were excluded.

Pregnant women of the cohort were asked to answer second questionnaire provided to them at the clinic before delivery. The interview contained a number of variables including demographics (age, education and family status); reproductive history (miscarriage); job characteristics; self-reported psychosocial stress; health behaviour; diseases; maternal smoking; paternal smoking. The self-reported stress of the respondents was assessed by the following thesis: “My daily activities are very trying and stressful”. Four respondent options were used to define stress: this describes my state (1) very well, (2) fairly well, (3) not very well, (4) not at all. Values 1 and 2 were considered to represent stress; 3 and 4 represented no stress.

The women were followed up with regard to pregnancy outcomes by the research staff. Pregnancy outcomes were ascertained chiefly from computerised hospital admission files and by abstraction of medical records. In this study, infant birthweight was measured in the delivery room by a trained nurse and was accurate to 1 g. The age of gestation was calculated using the data of birth as reported on the birth certificate and the 1st day of the last menstrual period as was ascertained at first interview, and by ultrasound examination.

Smoking exposure

Data regarding smoking behaviour were acquired through face-to-face interviewing. The trained research assistant in person in the hospital setting asked the women to report their daily cigarette consumption both before and during pregnancy. Woman had to answer the questions, “How many cigarettes did you smoke before pregnancy?” and “How many cigarettes did you smoke during pregnancy?” A mother was defined as smokers if she reported smoking at least one cigarette per day during pregnancy. In this study, data on mothers were categorised into two groups with respect to their cigarette smoking habits: those who did not smoke and those women who continue smoking during pregnancy. The parent was defined as a smoker if he smoked at least one cigarette per day. To assess smoking level we calculated the mean number cigarettes smoked per day.

Genotyping

The genomic DNA was extracted according to a standard protocol. The gene GSTM1–null (GenBank accession no. X68676) and GSTT1–null (GenBank accession no. AP000351) genotypes were identified by the multiplex polymerase chain reaction (PCR) in peripheral blood DNA samples. The details of this method for the detection of polymorphism of GSTT1 and GSTM1 can be found elsewhere [35]. This method allows the detection of the presence of the genotype (at least 1 allele present: AA or Aa) or its absence (complete deletion of both alleles: aa).

Maternal blood samples were collected in vials containing EDTA and stored at a temperature of −20°C. DNA was purified from the peripheral blood using DNA purification kits (MBI “Fermentas”, Vilnius, Lithuania). DNA concentrations were quantified with a spectrophotometer (Eppendorff BioPhotometer, 61310488, Hamburg, Germany). A PCR-based study of GSTM1 and GSTT1 polymorphism was carried out according to the method described previously [24]. The research staffs were blinded to outcome. The primers used for PCR were as follows: GSTM1 forward 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ and reverse 5′-GTT GGG CTC AAA TAT ACG GTG G-3′; GSTT1 forward 5′-TTC CTT ACT CCT CAC ATC TC-3′ and reverse 5′-TCA CCG GAT CAT GGC CAG CA-3′. As internal control, a 268-bp fragment of the human β-globin gene (GenBank accession no. U01317) was coamplified with a second set of primers (5′-CAA CTT CAT CCA CGT TCA CC-3′) and (5′-GAG CCA AGG ACA GGT AC-3′) (Biomers.net – the biopolymer factory, Germany). PCR was carried out in a final volume of 25 μl. The procedure followed for PCR was: primary denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, 30 cycles were conducted. Final extension was at 72°C for 10 min. The PCR products were electrophoresed in 2% agarose gels and stained in ethidium bromide. The DNA bands were visualised by UV transillumination (EASY Win32, Herolab, Germany). GSTM1 and GSTT1 polymorphisms were coded as present (GSTM1–1 and GSTT1–1) or null (GSTM1–0 and GSTT1–0). To confirm the analyses we repeated genotyping for GSTM1 and for GSTT1 in 150 subjects. The genotyping consistency rates were 100% for both GSTM1 and GSTT1.

Statistical methods

We first examined the associations between the maternal characteristics and smoking status during pregnancy,
in addition to the birthweight of the infants, by the Student’s t-test. We then used multiple linear-regression models to estimate the association of maternal cigarette smoking during pregnancy and the maternal genetic polymorphism in relation to birthweight of the newborn, with adjustment for major covariates. These included prepregnancy body mass index (BMI = weight/height^2), blood pressure, parity, gestational age, education and family status. The Mantel – Haenszel test was used to test the interaction between GSTT1 and GSTM1 on maternal smoking. Results from these association analyses were further verified using regression models to test the associations of GSTT1 GSTM1 and maternal smoking, including the effect from the interaction between the two genes. The subgroups were defined for maternal smoking status during pregnancy (no vs. yes) and genotypes for GSTT1 (null vs. present) and GSTM1 (null vs. present). We tested the gene–cigarette smoke interaction effect for birthweight reduction by adding all the product term (both 2-way and 3-way terms) in the model adjusting for potential effect modifiers. In the analyses, beta (β) represents the difference in mean birthweight (continuous variable) for cigarette smoking between the variant genotype after adjustment for the selected effect modifiers. Statistical significance was defined as P < 0.05. All statistical analyses were carried out using the SPSS software for Windows version 12.0.1.

**Results**

The analysis included 543 pregnant women: 460 non-smokers and 83 continuous smokers during pregnancy. The mean number cigarettes smoked per day were 4.8. Before pregnancy smoked 140 (25.7%) study subjects, among them 42.9% continuous smokers during pregnancy and 57.1% non-smokers during pregnancy. Prevalence of passive smoking at home (husband smoking) among continuous smokers during pregnancy was 91% and among non-smokers during pregnancy it was 50.4%. A total of 95.9% women were Lithuanian and the 2 groups did not differ in ethnicity.

We also conducted analyses comparing questionnaire data and birth certificate data on various characteristics among participants and non-participants. The mean birthweight and gestational duration were similar among the two groups. These two groups did not differed by ethnic group, however, non-participating mothers were younger, less educated (did not graduate from university, 46.6% vs. 54.3%), more often smokers (smokers, 9.6% vs. 6.9%), and did have fewer prior births (no child, 64.1% vs. 45.1%), than that of participants.

We found that among 543 participant, 450 (82.9%) possessed at least one copy of the functional gene, GSTT1–plus genotype and the remaining 93 (17.1%) had the GSTT1–null genotype. The GSTM1 gene among 543 study subjects, 293 (54%) possessed at least one copy of the functional gene, GSTM1–plus genotype and the remaining 250 (46.0%) had the GSTM1–null genotype. The carriers of the double-null genotypes comprised 8.7% of the total population studied. For the GSTM1 and GSTT1 polymorphisms, we were unable to determine whether they were in Hardy-Weinberg equilibrium because heterozygous individuals could not be distinguished from homozygous wild type.

Maternal characteristics with reference to the tobacco smoke exposure status are presented in Table 1. The nonexposed and exposed groups were similar in terms of the maternal prepregnancy BMI, hypertension, perceived stress, history of miscarriage, parity, sex of infant, and ethnic group, whereas the two groups differed with reference to maternal age, education and family status (P < 0.001). The mean birthweight of the infants was 3399.0 g for the nonexposed group and that for the exposed group was 3284.1 g, but there was no statistically significant difference (P = 0.132). The mean gestational age was 38.9 weeks for the both groups. In terms of the frequency of the GSTM1–null genotype, women in the group exposed to tobacco smoke and the group non-exposed were similar (41.0% and 47.0%, P = 0.340), whereas the GSTT1–null genotype was found in 26.5% of the smokers and in 15.4% of the non-smokers (P = 0.018).

Table 2 presents the influence of maternal characteristics on the birthweight of the infants as the difference in mean birthweight in relation to the maternal characteristics listed in each row.

The characteristics that positively affected the crude mean birthweight were increased BMI, hypertension and gestational age. Low levels of education, not married status and previous preterm were associated with reduction in the mean birthweight. In terms of the GSTM1– and GSTT1– genotype frequencies, there was no significant influence on the crude birthweight of infants. After adjustment for the BMI, gestational age and loss outliers, the maternal characteristics that affected the reduction in birthweight were as follows: low education levels (−155.3 g), maternal smoking during pregnancy (−137.0 g), parental smoking (−93.3 g) and GSTT1–null genotype in smokers (−211.8 g, P = 0.036). When both GSTT1– and GSTM1– null genotypes were considered, continuous maternal smoking during pregnancy was associated with a mean reduction of 340.4 g (P = 0.016) in birthweight of infants.

Table 3 presents the crude and adjusted combined associations of continuous maternal smoking during pregnancy and maternal GSTT1 and GSTM1 genotypes with reference to infant birthweight, where β represents the difference in mean birthweight between each subgroup and the reference group.

After complete adjustment for gestational age, BMI, education, family status, parity and hypertension, the
Table 1 Characteristics of pregnant women and their newborn infants according to exposure to cigarette smoke during pregnancy

| Maternal characteristics continuous and binary | Non-smoking (n=460) | Smoking (n=83) | P-value |
|------------------------------------------------|--------------------|---------------|---------|
| Maternal age, mean (SD), years                 | 29.0 (5.0)         | 26.4 (5.7)    | < 0.001 |
| Maternal height, mean (SD), cm                 | 167.6 (5.7)        | 167.3 (7.2)   | 0.703   |
| Maternal weight, mean (SD), kg                 | 75.3 (12.5)        | 77.4 (15.2)   | 0.250   |
| BMI, mean (SD)                                  | 26.8 (4.3)         | 27.6 (4.8)    | 0.136   |
| Gestational age, mean (SD), weeks              | 38.9 (2.2)         | 38.9 (2.2)    | 0.979   |
| Birthweight, mean (SD), g                       | 3399.0 (639.2)     | 3284.1 (632.5)| 0.132   |
| Birth length, mean (SD), cm                     | 51.3 (3.1)         | 50.8 (3.5)    | 0.150   |
| Infant sex, n (%)                               |                    |               |         |
| male                                           | 245 (53.3)         | 47 (56.6)     | 0.737   |
| female                                         | 213 (46.3)         | 36 (43.4)     |         |
| Education, n (%)                                |                    |               |         |
| university, college                             | 437 (95.0)         | 53 (63.9)     | <0.001  |
| ≤ 12 years                                     | 23 (5.0)           | 30 (36.1)     |         |
| Marital status, n (%)                           |                    |               |         |
| married                                        | 377 (82.0)         | 40 (48.2)     | <0.001  |
| not married                                     | 83 (18.0)          | 43 (51.8)     |         |
| Parity, n (%)                                   |                    |               |         |
| 1º                                             | 217 (47.2)         | 38 (45.8)     | 0.905   |
| 2º and more                                     | 243 (52.8)         | 45 (54.2)     |         |
| Miscarriage, n (%)                              |                    |               |         |
| no prior                                       | 369 (80.2)         | 70 (84.3)     | 0.450   |
| yes                                            | 91 (19.8)          | 13 (15.7)     |         |
| Blood pressure, n (%)                           |                    |               |         |
| < 120/80 mm/Hg                                  | 324 (70.4)         | 56 (67.5)     | 0.604   |
| > 120/80 mm/Hg                                  | 136 (29.6)         | 27 (32.5)     |         |
| Stress, n (%)                                   |                    |               |         |
| no                                             | 379 (82.4)         | 64 (77.1)     | 0.281   |
| yes                                            | 81 (17.6)          | 19 (22.9)     |         |
| Ethnic group, n (%)                             |                    |               |         |
| Lithuanian                                      | 441 (95.9)         | 80 (96.4)     | 0.571   |
| other                                          | 19 (4.1)           | 3 (3.6)       |         |
| Passive smoking                                 |                    |               |         |
| Yes                                            | 235 (50.4)         | 75 (91.0)     | <0.001  |
| No                                             | 225 (49.6)         | 8 (9.0)       |         |
| GSTT1, n (%)                                    |                    |               |         |
| present                                        | 389 (84.6)         | 61 (73.5)     | 0.018   |
| null                                           | 71 (15.4)          | 22 (26.5)     |         |
| GSTM1, n (%)                                    |                    |               |         |
| present                                        | 244 (53.0)         | 49 (59.0)     | 0.340   |
| null                                           | 216 (47.0)         | 34 (41.0)     |         |

SD standard deviation of the variability of individual observations.
reduction in birthweight (analysed as a continuous variable) for continuous smokers was 83.4 g (P = 0.073). Among non-smoking mothers, the GSTT1–null genotype alone did not confer a significant adverse effect on birthweight (−22.6 g, P = 0.345). The findings suggested a birthweight reduction among low-level smoking mothers with the GSTT1–null genotype (−162.9 g, P = 0.041). Maternal smoking was associated with a mean reduction of 58.8 g in birthweight for the GSTM1–plus and 118.7 g, P = 0.069 for the GSTM1–null genotypes; nevertheless, there was no statistically significant difference. When a combination of these genotypes was considered, a modifying effect was revealed and birthweight was significantly lower for infants of smoking women carriers of the double-null genotypes (−311.2 g; P = 0.008). The interaction effect of maternal smoking, GSTM1 and GSTT1 genotypes was marginally significant on birthweight (−234.5 g; P = 0.078).

**Discussion**

In this molecular epidemiological study on maternal cigarette smoking and genetic determinants of xenobiotic metabolism, we found some evidence that the effects of maternal smoking on infant birthweight were modified by the maternal GSTT1 and GSTM1 genotypes. This study shows that even light maternal smoking (mean: 4.8 cigarettes/day) has an increased risk for infant birthweight reduction among genetically susceptible women. Smokers with the variant GSTT1–null genotype had babies with lower mean birthweight (162.9 g) than non-smokers with the same genotype (P = 0.041), while smokers with the variant GSTM1–null genotype had babies with lower mean birthweight (118.7 g) than non-smokers with the same genotype (P = 0.069). We also found a gene–gene interaction among smokers. A combination of the GSTM1–null and the GSTT1–null genotypes has been found to exacerbate the effect of maternal exposure to tobacco-smoking on the birthweight of infants more than the presence of either genotype alone: −311.2 g, P = 0.008 in smokers vs. 10.1 g, P = 0.447 in non-smokers. An interaction effect of maternal smoking, GSTM1 and GSTT1 genotypes was marginally significant on birthweight (−234.5 g, P = 0.078). All associations were assessed after a number of relevant covariates were statistically controlled. These data and previous studies reported findings [18] suggest that the observed reductions in infants’ birthweight from this sample could be related to the main effects of prenatal exposure to tobacco.

Consistent with earlier studies, we found that maternal cigarette-smoking reduced the birthweight of infants [8,36] and that infant birthweight may vary in relation to gestational age, BMI, parity, and other variables of the population considered in the corresponding study [5,37].
### Table 3 Associations between maternal smoking during pregnancy and infant birthweight by maternal GSTT1 and GSTM1 genotype assessed by the crude and adjusted coefficient \( \beta \) in linear regression

| Genotype | Smoking status during pregnancy | Birthweight, g | Birthweight, g \( \beta^{+} \) crude (SE) P | Birthweight, g \( \beta^{++} \) adjusted (SE) P | Birthweight, g \( \beta^{+++} \) adjusted (SE) P |
|----------|--------------------------------|----------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| **Total sample** | Non-smoking (n = 456) | 3390.3 | Referent | Referent | Referent |
| | Smoking (n = 83) | 3284.1 | \(-86.5 (57.5) 0.066\) | \(-83.4 (57.1) 0.073\) | \(-83.4 (57.1) 0.073\) |
| **GSTT1** | Present | Non-smoking (n = 385) | 3401.4 | Referent | Referent | Referent |
| | Present | Smoking (n = 61) | 3320.7 | \(-70.4 (65.7) 0.143\) | \(-72.3 (65.1) 0.134\) | \(-38.8 (57.6) 0.250\) |
| | Null | Non-smoking (n = 71) | 3330.0 | Referent | Referent | Referent |
| | Null | Smoking (n = 22) | 3182.9 | \(-115.9 (129) 0.186\) | \(-123.7 (131) 0.175\) | \(-162.9 (93.0) 0.041\) |
| *Interaction: smoking \( \times \) GSTT1-null |  |  | \(-111.9 (123) 0.182\) | \(-96.8 (122.5) 0.215\) |  |
| **GSTM1** | Present | Non-smoking (n = 242) | 3413.6 | Referent | Referent | Referent |
| | Present | Smoking (n = 49) | 3255.9 | \(-87.2 (73.9) 0.119\) | \(-85.1 (74.2) 0.126\) | \(-58.8 (66.1) 0.187\) |
| | Null | Non-smoking (n = 214) | 3363.9 | Referent | Referent | Referent |
| | Null | Smoking (n = 34) | 3324.8 | \(-97.1 (92.0) 0.146\) | \(-100.6 (90.2) 0.133\) | \(-118.7 (79.6) 0.069\) |
| *Interaction: smoking \( \times \) GSTM1-null |  |  | \(-170 (106.1) 0.437\) | \(-26.6 (105.6) 0.400\) |  |
| **GSTT1 & GSTM1** | Present | Non-smoking (n = 207) | 3429.4 | Referent | Referent | Referent |
| | Present | Smoking (n = 38) | 3251.2 | \(-137.9 (82.3) 0.048\) | \(-135.5 (82.6) 0.051\) | \(-84.7 (71.2) 0.118\) |
| | Null | Non-smoking (n = 36) | 3339.7 | Referent | Referent | Referent |
| | Null | Smoking (n = 11) | 3093.5 | \(-318.0 (198) 0.058\) | \(-320.8 (203) 0.061\) | \(-311.2 (128) 0.008\) |
| *Interaction: smoking \( \times \) GSTT1-null \( \times \) GSTM1-null |  |  | \(-240.3 (164) 0.072\) | \(-234.5 (164.3) 0.078\) |  |

\( \beta \) represent the difference in mean birth weight for cigarette smoking between the variant genotype.
+ \( \beta \) crude.
++ \( \beta \) after adjustment for the covariates: gestational age, body mass index, education, family status, parity and blood pressure.
*Test of interaction: a P value is presented for testing the null hypothesis, \( \beta = 0 \) in multiple linear regression models for the product term, smoking \( \times \) genotypes.
Women reporting three or more stressful life events were significantly more likely to have a low birthweight infant after controlling for smoking and other socio-demographic covariates [38].

Findings of this study provide additional data supporting the conclusion that light maternal smoking during pregnancy may lead to reduced birthweight in infants. Our results corroborate the results of other studies that identification of the group of susceptible subjects should be based on both environmental exposure and gene polymorphism and that the individual differences in metabolic activation and detoxification of xenobiotics partly depends on the genetic polymorphisms associated with the GST enzymes [13-15,18,24,34]. When the GSTT1 genotype is considered in smoking pregnant women, the three different studies estimated reduction in birthweight among the GSTT1–plus and GSTT1–null groups was as follows: 43 g (P = 0.48) [14], 222 g (P < 0.05) [15], and 642 g (P < 0.001) [13]. When the GSTM1 genotype is considered, the estimated reduction in birthweight between GSTM1–plus and GSTM1–null groups is 171 g (P = 0.04) [14] and 222 g (P < 0.05), respectively [15]. The effects on the reduction of birthweight are not observed among women with GSTM1–null or GSTT1–null genotypes who had never smoked and the data have been adjusted to the main confounding factors.

A combination of the GSTM1–null and the GSTT1–null genotypes has been found to exacerbate the effect of maternal exposure to environmental tobacco-smoking on the birthweight of infants more than the presence of either genotype alone. Our previous publicised study have shown that when both GSTM1 and GSTT1 genotypes were considered, the greater increase in low birthweight and intra-uterine growth restriction risk was found among smoking mothers with the GSTM1 genotype absent, OR 3.31 [95% CI 0.60, 18.4] and OR 2.47 [95% CI 0.31, 13.1], correspondingly [34].

We can postulate that the significant differences between the publicised studies, which are devoted to the effects of tobacco-smoke exposure on birthweight, could be attributed to the diverse ethnic composition of the populations considered in the studies, resulting in different distributions of the GST allelic frequency and different levels of cigarette-smoke exposure, because dose–response gradients in relation to the number of cigarettes smoked do exist [19]. Furthermore, these results may be affected by the residual uncontrolled confounding variables, such as prepregnancy BMI, hypertension, stress level, gestational age and others, which are negatively or positively associated with birthweight.

The main factors influencing birth-weight reduction are gestational age and the organism’s response to toxicity from environmental xenobiotics, such as tobacco PAHs. Tobacco smoke toxins impair placental vasculature function and subsequent transplacental transport of oxygen and nutrients, and may lead to changes in vascular resistance [39,40]. Reduction in blood flow increase apoptosis and it is possible that this could be one of the mechanisms playing a role in the growth restriction [41].

Several recent studies have investigating how genetic susceptibility modulates risk of adverse pregnancy outcomes from environmental exposures such as cigarette smoke. Toxic chemicals could disturb foetal and placental cellular regulation via elevated DNA adducts and DNA damage [42]. Oxidative damage to placental DNA and increased levels of 8-oxodG in placental DNA can result in intrauterine growth restriction and low birthweight [43]. Maternal tobacco smoke exposure at an epigenome-wide level is associated with placental gene expression and DNA differential methylation and smoking-mediated birthweight reduction [44].

It is likely that smoking mothers with high-risk genotypes may have higher levels of PAH-DNA adducts and DNA strand breakage due to the increased activity of enzymes that metabolize cigarette toxins (e.g., CYP1A1 Aa and aa) and lower or absent activity of enzymes that detoxify these compounds (e.g. GSTT1–null, GSTM1–null genotypes) [45]. Moreover, such gene–smoking interactions may exert their synergistic effects on birthweight through maternal and foetal inflammatory responses and immune responses [46]. As reported by some authors, maternal exposure to tobacco smoke induces oxidative stress. Furthermore, maternal genetic polymorphisms related to GSTM1 and GSTT1 may modify the oxidative stress caused by maternal exposure to tobacco smoke [47].

We have investigated the genetic effects and the gene-environment interaction by controlling for major confounding variables. This study has the advantage of being the first to show that even light maternal smoking, in association with double-null GSTT1 and GSTM1 genotypes, might significantly decrease the infant birthweight. In this study, we estimated that the percentage of GSTT1–null genotype was 17.2% and that of GSTM1 was 46.0%. The carriers of double-null genotypes were 8.7% of the total population studied.

When the results of this study are interpreted, a few conditions should be considered. This is a low-risk population with low-level tobacco smoke exposure (4.8 cig./day) and low prevalence of GSTT1–null genotype; these factors may limit the extrapolation of these results to other populations. One of the limitations of the study is the relatively small sample size with the GSTT1–null genotype. The evaluation of exposure to tobacco smoke was indirect; we used self-reported information on smoking during pregnancy, and thus the possibility of bias in both reporting and exposure classification exists. We also examined phase-II metabolic genes without study genes expressed in phase-1. However, in this study, we controlled for the main
variables that might confound the association between maternal smoking, genetic polymorphism and birthweight; therefore, the residual confounding of the results by smoking is expected to be small. Despite these limitations, findings of this study provide additional data supporting the conclusion that maternal smoking during pregnancy may lead to reduced birth weight in newborns.

Conclusion

The study shows the modifying effect of the GSTT1 and GSTM1 genotypes on birthweight among smoking women and presents evidence that carriers of the null genotypes should be treated as an increased susceptibility group for infant birthweight decrease. Our findings provide additional insight into the biological determinants of response to environmental exposure based on the combination of genes and individual characteristics. Genotyping for the GSTT1 and GSTM1 polymorphisms, simple and inexpensive assays, could be suitable biomarkers identifying genetically susceptible pregnant women. These risk stratification markers could provide a valuable approach to estimate the "causal" effects of risk behaviours with genetic-predisposing factors (such as smoking) and could lead to targeted smoking cessation interventions during pregnancy as prevention for infants with low birthweight. The GSTT1—null genotype, either presents only one or both with GSTM1—null genotype in a single subject, may have a modifying effect on birthweight among smoking women even though their smoking is low level. Our data also show that identification of a susceptible-subject group should be based on both environmental exposure and gene polymorphism.

Competing interest

We confirm that all authors have no actual or potential competing interests regarding the submitted article and the nature of those interests.

Authors’ contributions

AD was involved in primary data collection as well as the coding and analysis of data, and the preparation of the manuscript. RG was involved in the conceptualization of the research, the preparation of the manuscript. AP contributed to genetic analysis. RD contributed to the development of the survey instrument, contributed to data acquisition. MJN contributed to qualitative data analysis and contributed to revisions of the manuscript. All authors read and approved the final manuscript.

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