Environ Int. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Environ Int. 2016 ; 92-93: 220–231. doi:10.1016/j.envint.2016.03.033.

Disinfection by-products exposure and intra-uterine growth restriction: do genetic polymorphisms of *CYP2E1*or deletion of *GSTM1* or *GSTT1* modify the association?

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Abstract

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Competing financial interests
The authors declare they have no financial conflict of interest.

Background—Exposure to disinfection by-products (DBPs) during pregnancy was associated with reduced fetal growth. Genetic susceptibility might play a role, especially for genes encoding for the Cytochrome P450 (CYP2E1) and Glutathione S-Transferase (GST) enzymes, involved in metabolism and activation of DBPs. Few epidemiological studies evaluated these gene-environment interactions and their results were never replicated.

Objective—This study aims to examine interactions between trihalomethanes (THM) or haloacetic acids (HAA) exposure and genetic polymorphisms on small for gestational age (SGA) neonates by investigating single nucleotide polymorphisms (SNPs) in *CYP2E1* gene and *GSTM*1 and *GSTT1* deletions in mothers-children pairs.

Methods—A population-based case-control study of 1549 mothers and 1455 children was conducted on SGA and THM/HAA exposure. DNA was extracted from blood or saliva cells. Targeted SNPs and deletions were genotyped. Statistical interaction between SNPs/deletions and THMs or HAAs *in utero* exposure with regard to SGA occurrence was evaluated by unconditional logistic regression with control of potential confounders.

Results—Previously reported positive modification of the effect of THM uterine exposure by mothers or newborns *CYP2E1* rs3813867 C allele or *GSTM1* deletion was not replicated. However interactions with *CYP2E1* rs117618383 and rs2515641 were observed but were not statistically significant after correction for multiple testing.

Conclusions—Previous positive interactions between THMs exposure and *CYP2E1* and *GSTM1* were not replicated but interactions with other *CYP2E1* polymorphisms are reported.

Keywords

DBPs; intra-uterine-growth-restriction; gene polymorphisms; *CYP2E1*; *GSTM1*; *GSTT1*

1. Introduction

Disinfection of drinking water is an essential component of public health protection. However, chlorine, the main and more widespread disinfectant of drinking water, reacts with organic matter naturally present in water to form numerous “by-product” chemicals (Richardson et al. 2007); the main ones being trihalomethanes (THMs) and haloacetic acids (HAAs), which are omnipresent in chlorinated waters at concentrations easily measurable (10–100 μg/l). These compounds have a well established toxicity at high doses on animals (Amy and International Programme on Chemical Safety 2000). Although data are still limited, there is evidence of a possible effect of THM and HAA exposure during pregnancy on intra-uterine foetal growth (Grellier et al. 2010; Villanueva et al. 2015). Due to the importance of foetal growth restriction on infants and its long term consequences in adult life (Pallotto and Kilbride 2006; Varvarigou 2010), it is necessary to identify factors which might enhance or reduce this risk.

In 2004, in a hospital-based case-control study, Infante-Rivard (Infante-Rivard 2004) found that newborns whose mothers have been exposed at home during their whole pregnancy to an average water supply THM levels above 29.7μg/L (90^{th} percentile of the distribution of concentrations in participants water supply systems) were at higher risk of SGA (<10^{th}

percentile birth weight) if they were carrying one or two C alleles of the *CYP2E1* gene rs3813867 (G1295C) polymorphism (guanidine being replaced by cytosine in the allele). The *CYP2E1* gene represents a target of choice for the study of genetic modification of potential toxic effects of several disinfection by-products because it encodes an isoenzyme which is part of the cytochrome P-450, and therefore might play a major role in phase-1 biological activation of such xenobiotics (Bolt et al. 2003).

More recently (Danileviciute et al. 2012), a population-based case-control study reported that women with the highest exposure to THMs and carrying a deletion of the Glutathione S-Transferase M1*(GSTM1)* gene were at higher risk of delivering low birth weight babies. However, no relationship was found with the SGA outcome, which is a better indicator of intrauterine growth retardation Deletion of the Glutathione S-Transferase Theta 1 (*GSTT1)* in mothers was also studied by Danilevicuite et al. (Danileviciute et al. 2012) but no significant statistical interaction was found. Glutathione S-Transferase (GST) enzymes family play an important role in phase-2 biotransformation of xenobiotics and in cellular detoxification (Hayes and Strange 2000). However, mutations in genes modulating the activity of enzymes such as *GSTT1* and *GSTM1* may also be responsible for enhancing toxic activities of chemicals (Bolt and Thier 2006).

The objective of this study was to revisit previously examined interactions between THM or HAA exposure and genetic polymorphisms with respect to foetal growth restriction by investigating single nucleotide polymorphisms (SNPs) capturing common genetic variation in *CYP2E1* gene as well as *GSTT1* and *GSTM1* deletions in biological samples of mothers-children pairs.

2. Materials and methods

2.1. Study design and population

This is a population-based case-control study conducted prospectively, between August 2006 to April 2008, in the Québec City (Canada) metropolitan area, a region of about 650,000 inhabitants. Participants were from a previous study on the association between exposure to THMs and HAAs during pregnancy and the occurrence of SGA (Levallois et al. 2012). Among the 2647 women (and their child) participating to the original study, 2517 (95%) accepted to be contacted for a follow-up study. A total of 1717 mothers and 1620 children provided DNA samples either from blood (for participants to a previous cohort study (Forest et al. 2014)) or from saliva (for participants recontacted for this study). Details on participants are given on the flow diagram (Figure 1) and in Supplemental material (Methods S1).

To reduce the possibility of a population stratification bias, non Caucasian participants (about 3%) identified from a self-administered questionnaire were removed from the initial sample for this study. Also, because our focus was the effect of the DBPs exposure in the third trimester and in accordance to our previous study (Levallois et al. 2012), only term babies were considered for this study (see Figure 1).

Ethical considerations—The access to the birth certificates for the selection of cases and controls was allowed by the Commission d'accès à l'information of Québec. The initial case-control study and this follow-up gene-environment study were both approved by the Ethics committee of CHU de Québec. For this follow-up study, a consent form was sent by mail to potential participants and returned with signature to the researchers by those who had provided saliva samples. As for the subgroup of participants who had previously consented to the other cohort study, informed written consent had been given during the first perinatal visit, for their own blood sample as well as for cord blood, and included consent for genetic analyses. This study was also approved by the CHU de Québec Ethics Review Board.

2.2. Definition of cases and controls

Cases of SGA were all term singleton newborns with birth weight less than the sex-specific 10^{th} percentile of weight for gestational age, according to the Canadian standards (Kramer et al. 2001). Controls were also term newborns but with birth weight at or above the same sex-specific standard for gestational age. About three controls per case were randomly selected among singletons born the same calendar week in the same geographical study area.

2.3. Interview of mothers

Mothers of cases of SGA and controls had been interviewed by telephone as part of the original study about two months after the birth to gather information on risk factors for SGA as well as socio-economic and lifestyles variables. Usual water consumption (number of glasses per day) and frequency of showers and baths per day or week were asked. Detailed information on the type of water consumed during the whole pregnancy as well as the use of water treatment home-devices and other water handling (boiling or letting stay in the fridge) was also collected.

2.4. DBPs Exposure assessment

The exposure assessment to disinfection by-products of participants was particularly improved over previous studies on this issue. Details are given in the original study (Levallois et al. 2012). In brief, THM and HAA were monitored monthly during the study at 53 sites within the 16 water distribution systems serving the residence of participants. The detection limits for THM species were 0.3 μg/L for chloroform, 0.3 μg/L for bromodichloromethane, 0.4 μg/L for chlorodibromomethane and 0.5 μg/L for bromoform. The detection limits for HAA species were 1.3 μg/L for monochloracetic, 0.9 μg/L for dichloroacetic, 0.4 μg/L for trichloracetic, 1.0 μg/L for monobromoacetic, 0.7 μg/L for dibromoacetic, 0.8 μg/L for bromochloroacetic, 4.6 μg/L for dibromochloroacetic, 4.2 μg/L for bromodichloroacetic and 6.4 μg/L for tribromoacetic.

Exposure assessment of mothers during the last trimester of their pregnancy was based on the estimation of concentrations of these chemicals in the tap water of participants' residence (after correction for home water treatment devices and other handlings) during that period and on the amount of water consumed through ingestion and, for THMs, through the dermal and inhalation routes during home shower and bath during a typical day. (See Supplemental material Methods S1 for details)

2.5.Potential confounders

Variables of interest were collected during the interview of the mothers and considered as potential confounders: maternal age, maternal education, annual household income, working status, marital status, prepregnancy body mass index (BMI), parity, history of chronic disease, medical problems during pregnancy, active smoking during the third trimester and passive smoking throughout the pregnancy, coffee and alcohol consumption, and risky occupational exposure. In addition, since the proportion of subjects with DNA extracted from saliva vs. blood differed between cases and controls (see Figure 1), we included DNA source as potential confounder in our statistical analysis despite genotyping quality rates seem very comparable between the two sources (Abraham et al. 2012).

2.6. Biological samples and DNA extraction

Blood samples of participants to the cohort study (Giguère et al. 2015) were drawn at the first prenatal visit between 10 and 18 weeks, while cord blood was sampled after delivery. Saliva was sampled using the ORAGENE-DNA kits (OG-500 and OG-575; GENOTEK, Kanata, On, Canada) mailed to potential participants with directives for sampling according to the manufacturer, and returned back to the research team by mail. In total, DNA samples from members of 1719 families (1618 mothers-child pairs, 2 children and 99 mothers) were available for DNA extraction. Further details on biological samples and DNA extractions can be found in the Supplemental material (Methods S2).

2.7. Genotyping

2.7.1 SNP selection—Using the Tagger program (Broad Institute, Cambridge, MA), we used a systematic approach to determine the polymorphisms allowing to better capture of the genetic diversity of the locus of interest. To optimize cost-efficiency, we used a r^2 threshold of 0.8 for tag SNPs selection with minor allele frequency of at least 5% (based on the Caucasian samples from Great Britain (GBR), Toscany (TSI) and Utah of Western, and Northern European origin (CEU) of the 1000 Genomes project (1000 Genomes Project).

In addition to the SNPs rs3813867 (G1295C) and rs2031920 (G1055C) analyzed in previous studies (Cantor et al. 2010; Infante-Rivard 2004), additional SNPs were selected for genotyping of common variants in CYP2E1 gene as described above. Fifteen SNPs within CYP2E1 (including 5 kb at both ends of the gene) were selected in total and twelve of them were successfully genotyped (see Table 1).

2.7.2 SNPs and deletions genotyping—SNPs were genotyped by Sequenom Technology at the Plate-forme de génotypage du CHU de Québec-Université Laval. For DNA quantification, double stranded DNA concentration was assessed using the QuantiFluor dsDNA system (Promega Corporation, Madison, USA). Genotyping of *GSTT1* and *GSTM1* deletions was performed by a multiplex PCR approach (adapted from (Bauer et al. 2006)G. Details on both methods are given in Supplemental materials (Methods S2).

2.8. Statistical analysis

Multivariable unconditional logistic regression was used to calculate odds ratios (ORs) and their 95% confidence interval (CI). The concentrations in tap water and exposure doses of

THMs and HAAs were categorized by quartiles based on the control group exposure, and associations with SGA were determined by comparing the fourth quartile (the exposed category) with the first three quartiles of exposure (the reference category). SNP genotypes were coded as variant allele counts (0, 1 or 2) or as variant allele carrier status (yes/no) when the variant allele had a frequency of less than 5%. Both mother and child genotypes were included in the same model, as well as product terms with the indicator variable for the exposed category. Statistical interaction on the multiplicative scale was assessed by Wald tests of each product term. ORs of SGA between high and low exposure levels were then computed for child carriers, mother carriers, and mother or child non-carriers (wild type) from the regression coefficients (note that the exposure OR was the same whether the mother or the child was non carrier, due to our assumption of no interaction between mother and child genotypes). Known SGA risk factors associated in univariate analysis with SGA (with $p< 0.2$) were added to the regression model: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection (due to the small number of cases and matched controls per week, dates of selection within the same month were combined to insure sufficient size of all selection strata). Missing values of prepregnancy BMI were imputed to the reference category.

In addition, in order to maximize the power to replicate the statistical interaction with total THM concentration in newborns carriers of the C allele of the *CYP2E1* gene rs3813867 polymorphism (Infante-Rivard 2004), we re-estimated logistic regression coefficients using the semiparametric likelihood of Chen et al. (Chen et al. 2012) assuming that exposure and child genotypes are conditionally independent given mother's genotype. A further gain in precision was obtained by including mother-child pairs with missing child genotypes, where maternal genotype provides partial information on child genotype (Nguile-Makao and Bureau 2015). We selected prepregnancy BMI and parity as covariates with the largest confounding effect, since the semiparametric estimation procedure converges only with a limited number of covariates.

Multiplicative interaction involving previously studied polymorphisms was declared statistically significant when p for interaction was <0.05. Multiple testing was considered for polymorphisms not previously studied using Bonferroni correction, i.e. dividing the significance level by the number of SNPs investigated (n=10).

3. Results

3.1. Characteristics of participants and exposure to DBPs

As expected, characteristics of mothers of cases were different from those of controls (Table 2). Mean concentrations of DBPs were slightly higher in drinking water serving the homes of cases than those of controls but these differences are very slight (Table 3). Mean and 75 percentiles of the distribution of DBPs concentration among controls (on which are based our highest exposure category for statistical analysis) are presented in supplemental materials (Table S1).

3.2 Multiple statistical interactions

We present successively the results of each gene with exposure assessed first by concentration of DBPs in participant's residence followed by results for internal doses of DBPs in participant mothers. Also, only previously studied polymorphisms and polymorphisms with a nominal interactions p-value < 0.05 in CYP2E1 are presented in the full paper (all p-values are for interaction tests). Results of the association with DBPs exposure without considering genes polymorphisms and the complete results for CYP2E1 are presented in supplemental materials (Table S2 and S3 respectively).

3.2.1 CYP2E1

3.2.1.1. Tap water concentration of THMs: The modifying effect of newborns and mothers genotypes on the association of DBPs with SGA was analyzed for 12 CYP2E1 SNPs. No statistically significant interaction was found for either rs3813867 or rs2031920 for THMs concentration. However, for both SNPs, an OR above 3 was found for total THMs exposure above the 3rd quartile: OR=3.7 (95%CI: 0.7–19.1) and 3.4 (95%CI: 0.7–16.5) in newborn carriers of the C allele (variant with cytosine) respectively compared to about 1 for the wild types (Table 4a).

Only one SNP (rs117618383) gave a statistically significant positive interaction between the variant T allele in children and concentration of total THMs above the third quartile: OR=4.6 (95%CI: 1.2–17.6) for 1 or 2 alleles compared to 1.0 (95%CI: 0.7–1.5) for the wild type ($p_{interaction}$: 0.03). Inversely, a negative interaction barely below the nominal 0.05 significance level ($p_{interaction}$: 0.049) was found in presence of this allele and exposure to brominated THMs above the third quartile, but did not result in a nominally significant negative association in the allele carriers (Table 4a). A negative interaction was also present for mothers carrying one or two T alleles of the SNP rs2515641 and exposed to higher levels of brominated THM: OR=0.3 (95% CI: 0.1–0.8) compared to 1.1 (95%CI: 0.7–1.6) for the wild type ($p_{interaction}$: 0.01) (Table 4.a). However, none of these interactions remained significant after correction for multiple testing.

3.2.1.2. Tap water concentration of HAAs: The presence of the rs117618383 T allele in children also resulted in a significant interaction with the exposure to total HAAs above the third quartile: OR=5.3 for HAA5 (95%CI: 1.5–18.6) compared to 1.4 (95%CI: 1.0–1.9) for the wild type (Table 4b, $p_{interaction}$: 0.04) but it was not statistically significant after correction for multiple testing.

3.2.1.3. Internal dose of DBPs: When the internal doses of THMs were taken into account, no interaction was found with either rs3813867 or rs2031920 for THM concentration. The interaction found previously for rs117618383 with TTHMs exposure persists but with a p-value > 0.05 (Table 4c). However, the negative interaction found in children for the same rs117618383 SNP with exposure to brominated THMs remained just below the nominal 0.05 significance level ($p_{Interaction}$: 0.042) while the negative association to brominated THMs in T allele carriers remained non significant (Table 4c). The negative interaction found with mother carriers of the rs2515641 T allele (Table 4c) was reduced compared to the result with tap water concentration. When the internal dose of HAAs was taken into

account, the association with exposure to total HAAs in newborn carriers of the rs117618383 T allele was slightly weaker and non significant (OR=4.2 for HAA5 (95%CI: 1.2–14.5), $p_{interaction}$: 0.08) (Table 4d) than that observed with tap water concentration. A negative interaction was found for mothers with one or two alleles of the two target SNPs rs3813867 and rs2031920, especially for HAA9: OR=0.1 (95%CI: 0.0–1.0) and 0.1 (95%CI: 0.0–0.9) respectively compare to 1.7 (95%:1.2–2.3) for wild types, $p_{interaction}$: 0.01 for both interactions (Table 4d). None of these interactions remained significant after correction for multiple testing.

3.2.2. GST deletions—Deletion of *GSTT1* and *GSTM1* were analyzed. Neither deletion was associated with a modification effect of DBPs (Table 5a, 5b, 5c, 5d).

3.2.3. Semiparametric likelihood analysis of total THMs and rs3813867 SNP of the CYP2E1 gene—First, we confirmed that adjusting for prepregnancy BMI and parity only produced estimates of OR of SGA for exposure to total THMs in newborns carrier of the C allele roughly similar to the estimate from the fully adjusted logistic regression on the same sample (Supplemental materials, Table S4). We then reintroduced 106 mother-child pairs with missing child genotype but otherwise complete observations, for a total sample size of 1535 (321 cases and 1214 controls) and estimated the smaller model by maximizing the semiparametric model of Chen (Chen et al. 2012). The interaction p-values in mothers were lower but not significant at the 0.05 level (Supplemental materials, Table S4).

4. Discussion

Foetal growth restriction is both an environmental and a genetic disease (Infante-Rivard 2007; Johnston et al. 2002). The study of genetic polymorphisms is expected to shed light on the causal nature of previously found environmental associations. Because of the possibility of biased results and type 1 error, replication is considered a gold rule in genetic epidemiology (Ioannidis 2013). Our study aimed to replicate the results of the two previous gene-interaction studies on foetal growth restriction and DBP exposure (Danileviciute et al. 2012; Infante-Rivard 2004). However, despite efforts to use cutting edge environmental epidemiologic methods, we were not able to replicate the interactions previously reported. Effect modifications by other gene polymorphisms were observed with a nominal p-value < 0.05, but none of these interactions remained statistically significant after correction for multiple testing.

4.1 Replication of previous studies

The effect modification found by Infante-Rivard (Infante-Rivard 2004) was very striking with an OR of 13.2 for children carriers of one or two C alleles of *CYP2E1* rs3813867 and exposed *in utero* to total THMs ≥30 μg/L, compared to 0.82 for carrier of the wild type (p : 0.027). However results were very imprecise (95%CI of the OR: 1.2–146.7) and the author stressed the need of confirmation. Our study evaluated effect modification with an exposure to total THMs≥58 μg/L (75^{th} percentile of distribution of THMs in controls residence), and found a non-statistically interaction: OR=3.7 (95%CI: 0.7–19.1) for children with one or two C alleles compared to 1.2 (95%CI: 0.8–1.8) for carriers of the wild type (p interaction=0.36).

Moreover, the association was weaker with the internal dose of THMs. Infante-Rivard also found a non-statistically significant association with SGA for mothers carriers of one or two alleles exposed to THMs (OR=6.5; 95%CI:0.6–71.5), while we found a non statistically significant protective effect for such mothers (OR=0.4; 95%CI:0.1–7.1). However, our understanding is that Infante-Rivard analyzed the two genotypes in separate logistic regression models instead of the same logistic regression model as we did, such that the association with SGA in mothers may be confounded by the effect of the newborn genotype.

CYP2E1 gene is known to encode part of the cytochromes P-450 which play an important role in phase-1 biological activation of chemicals (Bolt et al. 2003) and particularly chloroform (Gemma et al. 2003)(Meek et al. 2002). Because of the limited statistical power of our study and possible random misclassification of exposure, we cannot exclude a possible weak effect. Danileviciute et al. (Danileviciute et al. 2012) found an OR of low birth weight of 4.4 (95%CI:1.4–14.1) in women with a *GSTM1* double deletion exposed to higher internal doses of THMs (levels in drinking water was reported to be 21.9μg/L in the highest exposed area), compared to an OR of 0.34 in women with the GSTM1 gene ($p < 0.05$). The association was reduced to 1.8 (95%CI: 0.9–3.6) when SGA was considered as the issue under study, compared to an OR of 0.86 for non deletion ($p > 0.05$). In our study, either using the concentrations of THMs at the residence or the internal dose estimated by our models, no interactions were found for mothers with deleted *GSTM1*. Moreover, we confirmed the absence of interaction with the deletion of *GSTT1* that was reported in the same study (Danileviciute et al. 2012).

The rationale to study of *GSTM1* and *GSTT1* refers to the possible interaction with phase 2 metabolism of chemicals such as brominated THMs and HAAs. As a matter of fact, the GSTT1 enzyme is responsible for increasing toxic properties of some chemicals (Nakajima and Aoyama 2000) and essential in the activation of the mutagen activity of brominated THMs (Landi et al. 1999). Moreover, *GSTT1* polymorphisms were involved in the increased associations between THM and bladder cancer in Spain (Cantor et al. 2010). The levels of HAAs found in our drinking water networks were important but the levels of our brominated THMs were low (Table 3). This could have reduced our capacity to study such interaction.

4.2. Systematic assessment of genetic variation in CYP2E1

We observed a positive interaction between THM exposure and rs117618383 but it was not statistically significant after correction for multiple testing. This is the first time to our knowledge such interaction is reported with this SNP. It was also found to interact with the exposure to HAAs, this has never been reported either. A negative interaction with brominated THMs was found for newborn carriers of rs117618383 with some consistency between concentration and internal dose exposure assessment. A negative interaction with brominated THM concentration was also found for mother carriers of rs2515641 without consistency when considering internal dose. These interactions also disappeared after correction for multiple testing

Examination of the squared correlation between SNP alleles in gene *CYP2E1* showed little linkage disequilibrium, with the only exception of rs3813867 and rs2031920 which are strongly correlated (see Supplemental materials Figure S1), confirming that the selection

procedure selected non-redundant polymorphisms. Therefore, the possible interactions involving rs117618383 and rs2515641 represent independent signals from the previously reported interactions in *CYP2E1*. In the 1000 Genomes samples of European ancestry, rs117618383 tags no other SNP with a $r^2 > 0.8$, but exhibits weaker correlation with several SNPs (www.1000genomes.org).

Of note, the biological function of the SNPs selected using the Tagger program to capture as much genetic diversity as possible is unknown, and a positive association would suggest that the SNP is in linkage disequilibrium with another, yet to be identified SNP, biologically responsible for the interaction. However, we note that rs117618383 is in the upstream regulatory area, and that rs2515641 is a synonymous coding variant. Variant rs2515641 was recently associated with kidney transplant rejection in a Korean study (Kim et al. 2014). The direct causality of this synonymous substitution remains to be shown. Most likely, it is in linkage disequilibrium with another SNP biologically responsible for the association.

4.3. Strengths and limitations of the study

Our study has several strengths compared to previous studies. We conducted a population-based study which is less prone to selection bias (Rothman et al. 2008) than a previous hospital-based study (Infante-Rivard 2004). Our exposure assessment was optimized with monthly sampling of DBPs in the drinking water systems serving the residences of participants, high quality DBPs laboratory analysis and detailed description of water consumption as well as correction for the effectiveness of home treatment devices and other handling for DBP removal. All routes of exposure were also considered for THMs which are volatiles and lipophilic and internal dose was estimated using a PBPK model taking into consideration personal anthropometric characteristics of each participant (Levallois et al. 2012). Also, it is important to consider that our higher exposure levels to THMs (nearly the double than the levels evaluated in previous studies) are normally more favourable to detect an association. We also studied specifically the exposure to DBPs during the third trimester, the most important for foetal growth, and thus the optimal window for detecting an effect of growth parameters.

Moreover, an important strength of our study is the statistical analysis of the maternal and newborn genotypes which was done in the same regression model, mutually adjusting the effects involving one for the effects involving the other, as previously recommended to avoid confounding bias (Shi et al. 2008). Our sample size was comparable to the Infante-Rivard sample and larger than the Danileviciute study which evaluated only genotyped mothers. We were also able to minimize population stratification bias, limiting our study to Caucasians living in an area of Quebec with a large majority of French-Canadian ancestry.

Despite important strengths, our study has also some limitations. Even if the global sample size was important, our study has limited statistical power. For instance, the *CYP2E1* rs3813867 was found in 9% of Infante-Rivard 412 controls (Infante-Rivard 2004) but it was only found in 6% of our 1137 controls for which this SNP was successfully genotyped. Also, because of the need for recontacting 73% of our 2500 potential participants with a subsequent moderate participation rate, our sample size was reduced to nearly 60% of the original sample. Even if it is unlikely that this has led to a selection bias, it has reduced our

statistical power. As a matter of fact, a post-hoc statistical power analysis found that our power to detect an interaction relative risk (RR) of 3 was only 54% for *CYP2E1* rs3813867. However, *GSTM1* and *GSTT1* deletions were more frequent in our sample which gave a better statistical power for these genetic polymorphisms (to detect an interaction RR of 3 our statistical power was 80% for *GSTM1*). Also, we studied only the effect of last trimester exposure and did not consider the exposure occurring in the first trimester of pregnancy. Since this exposure might differ from the last trimester, we were not able to evaluate the possible interaction of these polymorphism and deletions with THMs and HAAs on the early stage of development.

5. Conclusion

With an improved methodology and quite a large sample size, we did not replicate the previously reported positive interactions found with *CYP2E1* rs3813867 and *GSTT1* deletion with THM exposure, suggesting that any effect of these polymorphisms in our population must be small. However, we report a new positive gene-environment interaction with *CYP2E1* rs117618383 and exposure to THMs and HAAs, on the occurrence of SGA, which needs to be replicated. Other reported negative interactions might also to be explored further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by the Canadian Institutes for Health Research (MOP 102708). We would like to thank Sylvie Marcoux, co-investigator of the initial study, Marion Bustamante for help in choosing the selected SNPs, Catherine Gonthier, coordinator of the data collection, Christelle Legay for help in drinking water sampling and DBPs analysis, Nathalie Bernard for extracting DNA and performing deletion analysis, Sylvie Desjardins of the genetic platform of CHU de Quebec, and research technicians and nurses who worked for the recruitment of participants and retrieval of data from the medical records.

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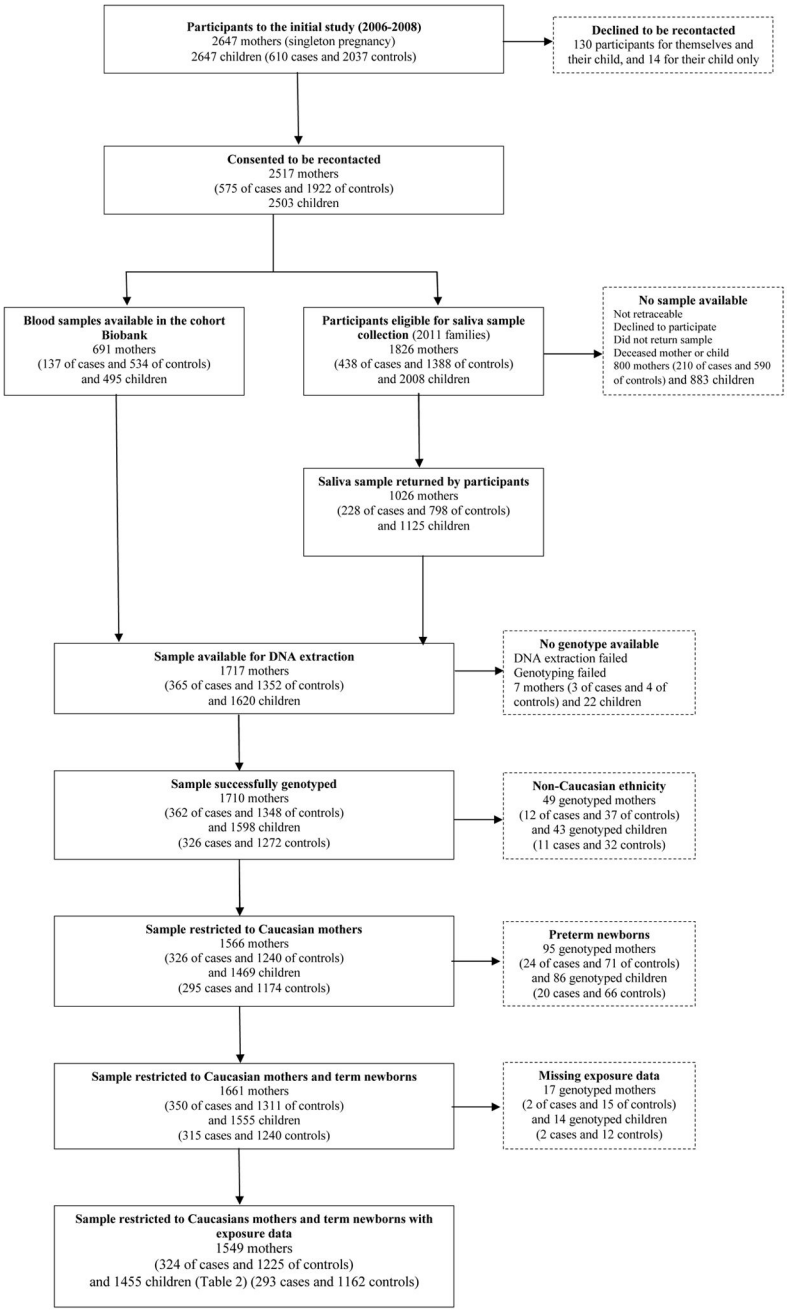

Figure 1.

Diagram Flow Chart starting from the participants to the initial study (610 cases and 2037 controls) to the participants to this study (293 cases and 1162 controls)

Table 1

Frequency of SNPs and deletions genotypes in comparison with the European sub-populations of the (1000 Genomes Project)

Chrom	Gene	Number rs	Position[a]	Molecular consequence[b]	Clinical significance[b]	Allele	MA[c]	MAFC[d]	MAF[e]
10	*CYP2E1*								
		rs10857730	135326822	intron	-	T/G	G	0.13	0.17
		rs11101800	135327325	intron	-	C/T	T	0.06	0.08
		rs117618383	135328727	2KB upstream	-	A/T	T	0.04	0.04
		rs11101807	135335647	unknown	-	T/C	C	-	0.09
		rs6537612	135336717	unknown	-	A/T	T	0.08	0.10
		rs41299398	135339122	2KB upstream	-	C/T	T	0.09	0.09
		rs3813866	135339334	2KB upstream	-	T/A	A	0.05	0.06
		rs3813867	135339605	2KB upstream	pathogenic	G/C	C	0.03	0.04
		rs2031920	135339845	2KB upstream	pathogenic	C/T	T	0.03	0.04
		rs6413421	135345811	intron	-	T/C	C	0.06	0.06
		rs915907	135346927	intron	-	C/A	A	0.16	0.18
		rs2011661	135348786	Intron	-	C/T	T	-	0.06
		rs2515641	135351362	synonymous	-[f]	T/C	T	0.11	0.13
		rs2480259	135352076	intron	-	A/G	A	0.19	0.23
		rs11101815	135355927	unknown	-	A/G	G	-	0.10
1	*GSTM1*	–	110230230-110251661	deletion		A[g] mu1	Null[h]	0.56	–
22	*GSTT1*	–	24376133-24384680	deletion		A[g] theta1	Null[h]	0.21	–

[a] Position in genome assembly GRCh37.p13

[b] Terminology and data from the National Center for Biotechnology Information Variation Viewer, retrieved on March 9th, 2016 (www.ncbi.nlm.nih.gov)

[c] MA: minor allele

[d] MAFC: minor allele frequency computed in the sample of mothers of controls. A missing value indicates that genotyping of the SNP failed.

[e] MAF: minor allele frequency of 1000 Genomes EUR sub-populations (1000 Genomes Project).

[f] rs2515641 was recently associated with kidney transplant rejection (Kim et al. 2014)

[g] A: Glutathione S-transferase

[h] Null: double deletion.

Table 2

Distribution of maternal characteristics of cases (n=293) and controls (n=1162)

	Cases n (%)	Controls n (%)
Maternal age (yrs)		
< 25	25 (9)	95 (8)
25–29	112 (38)	509 (44)
30–34	122 (42)	416 (36)
≥ 35	34 (12)	142 (12)
Highest education level obtained (yrs)		
≤ 12	55 (19)	167 (15)
> 12	237 (81)	983 (85)
Annual household income ($ Can)		
<35,000	50 (17)	111 (10)
35,000–69,999	110 (38)	502 (43)
≥70,000	133 (45)	549 (47)
Prepregnancy Body mass index (kg/m^2)		
<19.8	87 (29)	191 (17)
19.8–25.9	152 (52)	699 (60)
26.0–29.9	28 (9)	143 (12)
>29.9	23 (8)	120 (10)
Missing	5 (2)	9 (1)
Parity		
Nulliparous	201 (69)	555 (48)
Parous	91 (31)	595 (51)
Maternal smoking *		
Never or only before the 3rd trimester	253 (86)	1086 (93)
Ever in the 3rd trimester	40 (14)	76 (7)
Passive smoking at home *		
Yes	36 (12)	78 (7)
No	256 (88)	1072 (93)
Coffee consumption *		
Yes	153 (52)	526 (46)
No	139 (48)	624 (54)
Alcohol consumption *		
Yes	130 (45)	431 (37)
No	162 (55)	719 (63)
History of chronic disease		
Yes	29 (10)	84 (7)
No	263 (90)	1066 (93)
Preeclampsia *		
Yes	25 (9)	52 (5)

	Cases n (%)	Controls n (%)
No	267 (91)	1098 (95)

* During pregnancy

Table 3

Exposure to DBPs of cases and controls during the last trimester of pregnancy

	Cases Mean (SD)	Controls Mean (SD)	p
DBPs water concentration			
Trihalomethanes (THMs), µg/L			
Chloroform	43.6 (40.6)	41.9 (40.3)	0.26
Brominated THM	6.2 (4.4)	6.0 (3.9)	0.47
Total THMs	49.8 (39.9)	47.9 (39.4)	0.20
Haloacetic acids (HAAs), µg/L			
Dichloroacetic acid	15.7 (15.1)	15.3 (15.0)	0.59
Trichloroacetic acid	17.8 (21.2)	17.0 (21.1)	0.47
Total AAH (5 species)[a]	36.6 (36.8)	35.3 (36.7)	0.47
Total AAH (9 species)[b]	44.8 (37.4)	43.5 (37.1)	0.47
DBPs internal dose			
Trihalomethanes (THMs) multiexposure dose, µg/day			
Chloroform	134.9 (149.8)	132.7 (151.6)	0.66
Brominated THM	18.2 (16.9)	18.4 (16.5)	0.91
Total THM	153.1 (152.5)	151.0 (153.1)	0.72
Haloacetic acids (HAAs) internal dose, µg/day			
Dichloroacetic acid	14.5 (20.6)	12.1 (19.2)	0.007
Trichloroacetic acid	16.2 (26.3)	13.2 (25.6)	0.005
Total AAHs (5 species)[a]	34.0 (50.0)	28.0 (46.7)	0.005
Total AAHs (9 species)[b]	41.2 (54.1)	34.5 (50.7)	0.006

[a]HAA(5)=sum of the main five HAA species (DCAA, TCAA, monochloracetic acid, monobromacetic acid, dibromoacetic acid)

[b]HAA(9) =sum of all HAA species

SD : Standard deviation

Table 4a

Association between exposure to THMs concentration (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers carriers of *CYP2E1* SNP genotypes of wild type or with 1 or 2 variant alleles

CYP2E1/SNPs	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns								
rs3813867								
Wild type	275	1071	1.1 (0.8 – 1.6)	0.56	1.1 (0.8 – 1.6)	0.26	1.2 (0.8 – 1.8)	0.36
1 or 2 var.al[c]	15	66	1.8 (0.4 – 8.9)		0.4 (0.1 – 2.5)		3.7 (0.7 – 19.1)	
rs2031920								
Wild type	267	1042	1.1 (0.8 – 1.6)	0.59	1.1 (0.8 – 1.6)	0.15	1.1 (0.8 – 1.7)	0.43
1 or 2 var.al	17	65	1.7 (0.4 – 7.8)		0.3 (0.0 – 1.9)		3.4 (0.7 – 16.5)	
rs117618383								
Wild type	258	1061	1.0 (0.7 – 1.5)	0.03[d]	1.2 (0.8 – 1.6)	0.05[d]	1.0 (0.7 – 1.5)	0.03[d]
1 or 2 var.al	27	77	4.0 (1.2 – 13.7)		0.3 (0.1 – 1.1)		4.6 (1.2 – 17.6)	
rs2515641								
Wild type	210	905	1.2 (0.8 – 1.7)	0.85[d]	1.1 (0.7 – 1.6)	0.05[d]	1.2 (0.8 – 1.9)	0.98[d]
1 or 2 var.al	71	231	1.3 (0.6 – 2.7)		2.5 (1.1 – 5.5)		1.7 (0.7 – 4.0)	
Mothers								
rs3813867								
Wild type	273	1061	1.1 (0.8 – 1.6)	0.32	1.1 (0.8 – 1.6)	0.84	1.2 (0.8 – 1.6)	0.23
1 or 2 var.al	17	76	0.5 (0.1 – 2.4)		1.3 (0.3 – 7.1)		0.4 (0.1 – 7.1)	
rs2031920								
Wild type	267	1034	1.1 (0.8 – 1.6)	0.31	1.1 (0.8 – 1.6)	0.69	1.1 (0.8 – 1.6)	0.26
1 or 2 var.al	17	73	0.5 (0.1 – 2.4)		1.6 (0.3 – 8.8)		0.4 (0.1 – 8.8)	
rs117618383								
Wild type	262	1054	1.0 (0.7 – 1.5)	0.95[d]	1.2 (0.8 – 1.6)	0.58[d]	1.0 (0.7 – 1.6)	0.81[d]
1 or 2 var.al	23	84	1.1 (0.3 – 4.0)		1.7 (0.5 – 6.1)		1.0 (0.2 – 6.1)	
rs2515641								

CYP2E1/SNPs	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Wild type	216	910	1.2 (0.8 – 1.7)	0.93[d]	1.1 (0.7 – 1.6)	0.01[d]	1.2 (0.8 – 1.6)	0.98[d]
1 or 2 var.al	65	226	1.2 (0.6 – 2.6)		0.3 (0.1 – 0.8)		1.0 (0.4 – 0.8)	

[a] CHCl3=Chloroform, THMBr= Brominated THM, TTHM= Total Trihalomethanes

[b] OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

[c] 1 or var. al = 1 or 2 variant alleles.

[d] The Bonferroni corrected significance level of 0.005 applies to these interaction tests.

Table 4b

Association between exposure to HAAs concentration (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers carriers of *CYP2E1* SNP genotypes of wild type or with 1 or 2 variant alleles

CYP2E1/SNPs	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns										
rs3813867										
Wild type	275	1071	1.3 (0.9 – 1.7)	0.38	1.4 (1.0 – 1.9)	0.14	1.4 (1.0 – 2.0)	0.15	1.5 (1.1 – 2.0)	0.16
1 or 2 var.al[c]	15	66	2.5 (0.6 – 11.2)		4.5 (1.0 – 20.7)		4.5 (1.0 –20.8)		4.5 (1.0 – 21.0)	
rs2031920										
Wild type	267	1042	1.2 (0.9 – 1.7)	0.30	1.3 (1.0 – 1.9)	0.16	1.4 (1.0 – 1.9)	0.17	1.4 (1.0 – 2.0)	0.18
1 or 2 var.al	17	65	2.7 (0.6 – 11.9)		3.9 (0.9 – 17.2)		3.9 (0.9 –17.3)		3.9 (0.9 –17.4)	
rs117618383										
Wild type	258	1061	1.1 (0.8 – 1.6)	0.03[d]	1.4 (1.0 – 1.9)	0.10[d]	1.4 (1.0 – 1.9)	0.04[d]	1.4 (1.0 – 1.9)	0.03[d]
1 or 2 var.al	27	77	4.9 (1.3 – 17.9)		3.9 (1.1 – 13.5)		5.3 (1.5 –18.6)		5.8 (1.6 –20.7)	
rs2515641										
Wild type	210	905	1.2 (0.8 – 1.7)	0.96[d]	1.3 (0.9 – 1.9)	0.63[d]	1.5 (1.0 – 2.2)	0.81[d]	1.5 (1.1 – 2.2)	0.84[d]
1 or 2 var.al	71	231	1.2 (0.6 – 2.6)		1.6 (0.8 – 3.4)		1.4 (0.7 – 2.9)		1.4 (0.7 – 3.0)	
Mothers										
rs3813867										
Wild type	273	1061	1.3 (0.9 – 1.7)	0.19	1.4 (1.0 – 1.9)	0.06	1.4 (1.0 – 1.9)	0.05	1.5 (1.1 – 2.0)	0.051
1 or 2 var.al	17	76	0.5 (0.1 – 2.0)		0.3 (0.1 – 1.5)		0.3 (0.1 – 1.5)		0.3 (0.1 – 1.5)	
rs2031920										
Wild type	267	1034	1.2 (0.9 – 1.7)	0.15	1.3 (1.0 – 1.9)	0.06	1.4 (1.0 – 1.9)	0.06	1.4 (1.0 – 2.0)	0.057
1 or 2 var.al	17	73	0.4 (0.1 – 1.9)		0.3 (0.1 – 1.4)		0.3 (0.1 – 1.4)		0.3 (0.1 – 1.5)	
rs117618383										
Wild type	262	1054	1.1 (0.8 – 1.6)	0.84[d]	1.4 (1.0 – 1.9)	0.95[d]	1.4 (1.0 – 1.9)	0.77[d]	1.4 (1.0 – 1.9)	0.70[d]
1 or 2 var.al	23	84	1.0 (0.2 – 3.9)		1.3 (0.4 – 4.8)		1.1 (0.3 – 4.8)		1.0 (0.3 – 4.1)	
rs2515641										

CYP2E1/SNPs	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Wild type	216	910	1.2 (0.8 – 1.7)	0.40[d]	1.3 (0.9 – 1.9)	0.48[d]	1.5 (1.0 – 1.9)	0.69[d]	1.5 (1.1 – 2.2)	0.77[d]
1 or 2 var.al	65	226	1.6 (0.8 – 3.5)		1.7 (0.8 – 3.6)		1.7 (0.8 – 3.6)		1.7 (0.8 – 3.6)	

[a]DCAA=Dichloracetic acid, TCAA=Trichloroacetic acid, HAA(5)=sum of the main five HAA species (DCAA, TCAA, monochloracetic acid, monobromacetic acid, dibromoacetic acid), HAA(9) =sum of all HAA species.

[b]OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

[c]1 or var. al = 1 or 2 variant alleles.

[d]The Bonferroni corrected significance level of 0.005 applies to these interaction tests.

Table 4c

Association between internal dose of THMs (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers carriers of *CYP2E1* SNP genotypes of wild type or 1 or 2 variant alleles

CYP2E1/SNPs	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns								
rs3813867								
Wild type	275	1071	1.2 (0.9 – 1.7)	0.69	1.1 (0.8 – 1.6)	0.27	1.2 (0.9 – 1.7)	0.52
1 or 2 var.al[c]	15	66	1.7 (0.3 – 8.7)		0.3 (0.0 – 3.2)		2.1 (0.4 – 11.2)	
rs2031920								
Wild type	267	1042	1.1 (0.8 – 1.6)	0.47	1.1 (0.8 – 1.6)	0.19	1.2 (0.9 – 1.7)	0.52
1 or 2 var.al	17	65	2.0 (0.4 – 9.8)		0.2 (0.0 – 2.5)		2.1 (0.4 – 10)	
rs117618383								
Wild type	258	1061	1.1 (0.8 – 1.6)	0.09[d]	1.2 (0.8 – 1.6)	0.04[d]	1.1 (0.8 – 1.5)	0.14[d]
1 or 2 var.al	27	77	3.4 (0.9 – 11.9)		0.2 (0.0 – 1.1)		2.8 (0.8 – 9.6)	
rs2515641								
Wild type	210	905	1.2 (0.8 – 1.7)	0.29[d]	1.0 (0.7 – 1.5)	0.12[d]	1.2 (0.8 – 1.7)	0.22[d]
1 or 2 var.al	71	231	1.8 (0.8 – 4.0)		1.9 (0.9 – 4.3)		1.9 (0.9 – 4.2)	
Mothers								
rs3813867								
Wild type	273	1061	1.2 (0.9 – 1.7)	0.16	1.1 (0.8 – 1.6)	0.53	1.2 (0.9 – 1.6)	0.13
1 or 2 var.al	17	76	0.3 (0.1 – 2.0)		0.7 (0.1 – 3.4)		0.3 (0.1 – 3.4)	
rs2031920								
Wild type	267	1034	1.1 (0.8 – 1.6)	0.14	1.1 (0.8 – 1.6)	0.66	1.2 (0.9 – 1.6)	0.13
1 or 2 var.al	17	73	0.3 (0.0 – 1.8)		0.8 (0.1 – 4.0)		0.3 (0.0 – 4.0)	
rs117618383								
Wild type	262	1054	1.1 (0.8 – 1.6)	0.67[d]	1.2 (0.8 – 1.6)	0.71[d]	1.1 (0.8 – 1.6)	0.97[d]
1 or 2 var.al	23	84	0.8 (0.2 – 3.2)		0.9 (0.2 – 3.6)		1.1 (0.3 – 3.6)	
rs2515641								

CYP2E1/SNPs	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Wild type	216	910	1.2 (0.8 – 1.7)	0.39[d]	1.0 (0.7 – 1.5)	0.10[d]	1.2 (0.8 – 1.5)	0.20[d]
1 or 2 var.al	65	226	0.8 (0.4 – 1.8)		0.5 (0.2 – 1.2)		0.8 (0.4 – 1.2)	

[a] CHCl3=Chloroform, THMBr= Brominated THM, TTHM= Total Trihalomethanes

[b] OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

[c] 1 or var. al = 1 or 2 variant alleles.

[d] The Bonferroni corrected significance level of 0.005 applies to these interaction tests.

Table 4d

Association between internal dose of HAAs (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers carriers of *CYP2E1* SNP genotypes of wild type or with 1 or 2 variant alleles

CYP2E1/SNPs	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction
Newborns										
rs3813867										
Wild type	275	1071	1.7 (1.2 – 2.3)	0.51	1.7 (1.2 – 2.3)	0.55	1.6 (1.2 – 2.2)	0.45	1.7 (1.2 – 2.3)	0.23
1 or 2 var.al[c]	15	66	2.9 (0.6 – 14.8)		2.9 (0.5 – 15.1)		3.0 (0.6 – 14.9)		4.7 (0.9 – 23.7)	
rs2031920										
Wild type	267	1042	1.6 (1.2 – 2.2)	0.46	1.6 (1.2 – 2.2)	0.51	1.6 (1.1 – 2.1)	0.44	1.7 (1.2 – 2.3)	0.23
1 or 2 var.al	17	65	2.9 (0.6 – 13.4)		2.7 (0.6 – 13.2)		2.9 (0.6 – 13.5)		4.4 (0.9 – 20.8)	
rs117618383										
Wild type	258	1061	1.5 (1.0 – 2.0)	0.07[d]	1.5 (1.1 – 2.0)	0.12[d]	1.3 (1.0 – 1.9)	0.08[d]	1.4 (1.0 – 2.0)	0.12[d]
1 or 2 var.al	27	77	4.7 (1.3 – 16.8)		4.1 (1.1 – 14.8)		4.2 (1.2 – 14.5)		3.8 (1.1 – 13.2)	
rs2515641										
Wild type	210	905	1.6 (1.1 – 2.2)	0.52[d]	1.5 (1.1 – 2.2)	0.66[d]	1.4 (1.0 – 2.0)	0.71[d]	1.6 (1.1 – 2.3)	0.60[d]
1 or 2 var.al	71	231	1.2 (0.6 – 2.6)		1.8 (0.9 – 3.8)		1.2 (0.6 – 2.6)		1.3 (0.6 – 2.8)	
Mothers										
rs3813867										
Wild type	273	1061	1.7 (1.2 – 2.3)	0.098	1.7 (1.2 – 2.3)	0.050	1.6 (1.2 – 2.3)	0.086	1.7 (1.2 – 2.3)	0.011
1 or 2 var.al	17	76	0.4 (0.1 – 2.2)		0.3 (0.1 – 1.7)		0.4 (0.1 – 1.7)		0.1 (0.0 – 1.0)	
rs2031920										
Wild type	267	1034	1.6 (1.2 – 2.2)	0.12	1.6 (1.2 – 2.2)	0.054	1.6 (1.1 – 2.2)	0.089	1.7 (1.2 – 2.3)	0.010
1 or 2 var.al	17	73	0.4 (0.1 – 2.2)		0.3 (0 – 1.6)		0.4 (0.1 – 1.6)		0.1 (0.0 – 0.9)	
rs117618383										
Wild type	262	1054	1.5 (1.0 – 2.0)	0.97[d]	1.5 (1.1 – 2)	0.67[d]	1.3 (1.0 – 2.0)	0.79[d]	1.4 (1.0 – 2.0)	0.75[d]
1 or 2 var.al	23	84	1.5 (0.4 – 5.6)		2 (0.5 – 7.4)		1.6 (0.4 – 7.4)		1.8 (0.5 – 6.5)	
rs2515641										

CYP2E1/SNPs	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction	Adjusted OR [b] (95% CI)	p interaction
Wild type	216	910	1.6 (1.1 – 2.2)	0.45[d]	1.5 (1.1 – 2.2)	0.76[d]	1.4 (1.0 – 2.2)	0.39[d]	1.6 (1.1 – 2.3)	0.48[d]
1 or 2 var.al	65	226	2.1 (1.0 – 4.3)		1.7 (0.8 – 3.5)		1.9 (0.9 – 3.5)		2.1 (1.0 – 4.3)	

[a]DCAA=Dichloracetic acid, TCAA=Trichloroacetic acid, HAA(5)=sum of the main five HAA species (DCAA, TCAA, monochloracetic acid, monobromacetic acid, dibromoacetic acid), HAA(9) =sum of all HAA species.

[b]OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

[c]1 or var. al = 1 or 2 variant alleles.

[d]The Bonferroni corrected significance level of 0.005 applies to these interaction tests.

Table 5a

Association between exposure to THMs concentration (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers *GSTT1* or *GSTM1* deletion status

Gene/deletion	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns								
GSTT1								
No or single deletion	198	793	1.1 (0.8 – 1.7)	0.86	0.9 (0.6 – 1.4)	0.54	1.3 (0.9 – 2.0)	0.47
Double deletion	73	300	1.2 (0.6 – 2.4)		1.2 (0.6 – 2.3)		1.7 (0.9 – 3.3)	
GSTM1								
No or single deletion	112	491	1.6 (0.9 – 2.7)	0.18	1.1 (0.6 – 2.0)	0.77	1.6 (1.0 – 2.8)	0.31
Double deletion	159	602	1.0 (0.5 – 2.0)		1.3 (0.7 – 2.5)		1.1 (0.6 – 2.3)	
Mothers								
GSTT1								
No or single deletion	59	228	1.1 (0.8 – 1.7)	0.57	0.9 (0.6 – 1.4)	0.28	1.3 (0.9 – 1.4)	0.77
Double deletion	212	865	1.4 (0.7 – 3.0)		1.4 (0.7 – 3.0)		1.5 (0.7 – 3.0)	
GSTM1								
No or single deletion	146	610	1.6 (0.9 – 2.7)	0.91	1.1 (0.6 – 2.0)	0.53	1.6 (1.0 – 2.0)	0.61
Double deletion	125	483	1.7 (0.8 – 3.4)		0.9 (0.4 – 1.9)		2.0 (1.0 – 1.9)	

[a] CHCl3=Chloroform, THMBr= Brominated THM, TTHM= Total Trihalomethanes

[b] OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

Table 5b

Association between exposure to HAAs concentration (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers *GSTT1* or *GSTM1* deletion status

Gene/deletion	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns										
GSTT1										
No or single deletion	198	793	1.3 (0.9 – 2.0)	0.87	1.6 (1.0 – 2.3)	0.61	1.6 (1.1 – 2.3)	0.80	1.6 (1.1 – 2.4)	0.61
Double deletion	73	300	1.4 (0.7 – 2.7)		1.3 (0.7 – 2.5)		1.4 (0.8 – 2.7)		1.3 (0.7 – 2.6)	
GSTM1										
No or single deletion	112	491	1.4 (0.8 – 2.3)	0.90	1.5 (0.9 – 2.5)	0.69	1.5 (0.9 – 2.5)	0.57	1.5 (0.9 – 2.5)	0.57
Double deletion	159	602	1.4 (0.7 – 2.8)		1.3 (0.7 – 2.5)		1.2 (0.6 – 2.3)		1.2 (0.6 – 2.4)	
Mothers										
GSTT1										
No or single deletion	212	865	1.3 (0.9 – 2.0)	0.51	1.6 (1.0 – 2.3)	0.99	1.6 (1.1 – 2.3)	0.63	1.6 (1.1 – 2.4)	0.91
Double deletion	59	228	1.0 (0.5 – 2.2)		1.6 (0.8 – 3.3)		1.3 (0.6 – 3.3)		1.6 (0.7 – 3.2)	
GSTM1										
No or single deletion	125	483	1.4 (0.8 – 2.3)	0.61	1.5 (0.9 – 2.5)	0.70	1.5 (0.9 – 2.5)	0.53	1.5 (0.9 – 2.5)	0.49
Double deletion	146	610	1.2 (0.6 – 2.3)		1.7 (0.9 – 3.4)		1.8 (0.9 – 3.4)		1.9 (1.0 – 3.8)	

[a]DCAA=Dichloracetic acid, TCAA=Trichloroacetic acid, HAA(5)=sum of the main five HAA species (DCAA, TCAA, monochloracetic acid, monobromacetic acid, dibromoacetic acid), HAA(9) =sum of all HAA species.

[b]OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

Table 5c

Association between internal dose of THMs (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers *GSTT1* or *GSTM1* deletion status

Gene/deletion	Cases	Controls	CHCL3[a]		THMBr[a]		TTHM[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns								
GSTT1								
No or single deletion	198	793	1.1 (0.7 – 1.7)	0.61	0.9 (0.6 – 1.4)	0.86	1.1 (0.7 – 1.6)	0.44
Double deletion	73	300	1.4 (0.7 – 2.7)		1.0 (0.5 – 2.0)		1.4 (0.7 – 2.7)	
GSTM1								
No or single deletion	112	491	1.4 (0.8 – 2.4)	0.96	1.1 (0.6 – 1.8)	0.67	1.4 (0.8 – 2.4)	0.95
Double deletion	159	602	1.4 (0.7 – 2.8)		1.2 (0.6 – 2.4)		1.4 (0.7 – 2.8)	
Mothers								
GSTT1								
No or single deletion	59	228	1.1 (0.7 – 1.7)	0.48	0.9 (0.6 – 1.4)	0.65	1.1 (0.7 – 1.4)	0.18
Double deletion	212	865	1.5 (0.7 – 3.2)		1.1 (0.5 – 2.3)		1.8 (0.9 – 2.3)	
GSTM1								
No or single deletion	146	610	1.4 (0.8 – 2.4)	0.67	1.1 (0.6 – 1.8)	0.38	1.4 (0.8 – 1.8)	0.64
Double deletion	125	483	1.2 (0.6 – 2.4)		0.8 (0.4 – 1.6)		1.2 (0.6 – 1.6)	

[a] CHCl3=Chloroform, THMBr= Brominated THM, TTHM= Total Trihalomethanes

[b] OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.

Table 5d

Association between internal dose of HAAs (within the fourth quartile in comparison to the first three quartiles) and SGA for newborns and mothers *GSTT1* or *GSTM1* deletion status

Gene/deletion	Cases	Controls	DCAA[a]		TCAA[a]		HAA5[a]		HAA9[a]	
	n	n	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction	Adjusted OR[b] (95% CI)	p interaction
Newborns										
GSTT1										
No or single deletion	198	793	1.6 (1.1 – 2.3)	0.35	1.7 (1.1 – 2.5)	0.40	1.7 (1.1 – 2.5)	0.41	1.7 (1.2 – 2.5)	0.95
Double deletion	73	300	2.2 (1.2 – 4.1)		2.3 (1.2 – 4.3)		2.2 (1.2 – 4.2)		1.7 (0.9 – 3.3)	
GSTM1										
No or single deletion	112	491	1.6 (1.0 – 2.6)	0.91	1.9 (1.1 – 3.1)	0.84	1.5 (0.9 – 2.5)	0.88	1.5 (0.9 – 2.5)	0.42
Double deletion	159	602	1.5 (0.8 – 3.0)		1.8 (0.9 – 3.4)		1.6 (0.8 – 3.0)		2.0 (1.0 – 3.9)	
Mothers										
GSTT1										
No or single deletion	212	865	1.6 (1.1 – 2.3)	0.26	1.7 (1.1 – 2.5)	0.14	1.7 (1.1 – 2.5)	0.061	1.7 (1.2 – 2.5)	0.27
Double deletion	59	228	1.0 (0.5 – 2.1)		0.9 (0.4 – 2.0)		0.8 (0.4 – 2.0)		1.1 (0.5 – 2.3)	
GSTM1										
No or single deletion	125	483	1.6 (1.0 – 2.6)	0.95	1.9 (1.1 – 3.1)	0.50	1.5 (0.9 – 3.1)	0.94	1.5 (0.9 – 2.5)	0.51
Double deletion	146	610	1.6 (0.8 – 3.3)		1.5 (0.7 – 3.0)		1.5 (0.8 –3.0)		1.2 (0.6 – 2.4)	

[a]DCAA=Dichloracetic acid, TCAA=Trichloroacetic acid, HAA(5)=sum of the main five HAA species (DCAA, TCAA, monochloracetic acid, monobromacetic acid, dibromoacetic acid), HAA(9) =sum of all HAA species.

[b]OR adjusted for the following variables: maternal age, maternal education, annual household income, prepregnancy BMI, parity, history of chronic disease, preeclampsia, active smoking during the third trimester, passive smoking throughout pregnancy, coffee and alcohol consumption during pregnancy and month of selection.