Prenatal exposure to drinking-water chlorination by-products, cytochrome P450 gene polymorphisms and small-for-gestational-age neonates

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Abstract

Genetic susceptibility may modulate chlorination by-products (CBPs) effects on fetal growth, especially genes coding for the cytochrome P450 involved in the metabolism of CBPs and steroidogenesis.

In a case-control study of 1432 mother-child pairs, we assessed the association between maternal and child single nucleotide polymorphisms (SNPs) within *CYP1A2*, *CYP2A6*, *CYP2D6* and *CYP17A1* genes and small-for-gestational-age neonates (SGA <10th percentile) as well as interaction between these SNPs and maternal exposure to trihalomethanes or haloacetic acids (HAAs) during the third trimester of pregnancy.

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Authors' Roles

S.B. analysed and interpreted the data and wrote the paper, P.L. designed, planned the study and revised critically the paper to be published, Y.G. co-planned the study, provided the biological samples which came from a cohort study and gave helpful comments on the initial draft, M.R. planned drinking water sampling, chlorination by-products (CBPs) analysis and gave helpful comments on previous version of the paper, A.B. planned and oversaw the analysis and revised the final version to be published.

Conflict of interest

None declared.

Interactions were found between mother and neonate carrying *CYP17A1* rs4919687 A and rs743572 G alleles and maternal exposure to total trihalomethanes or five regulated HAAs species. However, these interactions became non statistically significant after correction for multiple testing.

There is some evidence, albeit weak, of a potential effect modification of the association between CBPs and SGA by SNPs in *CYP17A1* gene. Further studies are needed to validate these observations.

Descriptive keywords

CYP1A2, *CYP2A6*, *CYP2D6*, *CYP17A1*; Single nucleotide polymorphism; Gene-environment interaction; Intra-uterine growth restriction; Small-for-gestational-age

1. Introduction

Drinking water disinfection is necessary for inactivating or killing pathogenic microorganisms. Disinfection by-products (DBPs) are chemicals resulting from the disinfection process. Chlorine is a cost-effective and widespread drinking water disinfectant. However, it reacts with organic matter and bromide in water and leads to formation of potentially toxic compounds known as chlorination by-products (CBPs) (Rook, 1974). Trihalomethanes (THMs) and haloacetic acids (HAAs) are the most prevalent CBPs and the most abundant among more than 600 DBPs identified (Richardson et al., 2007). Some toxicological and epidemiologic studies on CBPs have provided evidence of association with reduced fetal growth (Costet et al., 2012; Grellier et al., 2010; Levallois et al., 2012; Nieuwenhuijsen et al., 2009; Tardiff et al., 2006; Villanueva et al., 2015) whereas other studies did not show such association (Patelarou et al., 2011; Villanueva et al., 2011). In fact, there is little knowledge about the CBPs toxicological molecular process leading to fetal growth restriction. To date, few studies considered genetic susceptibility, which may shed light on potential mechanism of CBPs toxicity on vulnerable individuals.

Infante-Rivard (2004) found that newborns carrying one or two C alleles of the cytochrome P450 (CYPs) isoenzyme *CYP2E1* gene rs3813867 single nucleotide polymorphism (SNP) were at higher risk of being small-for-gestational-age (SGA) if their mothers had been exposed to total THMs above 90th percentile (corresponding to about 30 µg/L) during their whole pregnancy. Subsequently, Danileviciute et al. (2012) observed that women with the highest exposure to THMs and carrying a deletion of the Glutathione S-Transferase M1 gene (*del GSTM1*) were at higher risk of delivering low birth weight babies. However, no relationship was found with the SGA outcome. Deletion of the Glutathione S-Transferase Theta 1 gene (*del GSTT1*) in mothers was also studied, but no significant statistical interaction was found (Danileviciute et al., 2012). More recently, in a larger-scale study, Levallois et al. (2016) did not observe statistically significant replication of previously reported positive modification of the effect of THMs or HAAs uterine exposure by mothers or newborns *CYP2E1* rs3813867 C allele or *del GSTM1*. However, interactions with other *CYP2E1* polymorphisms were observed. Kogevinas et al. (2016) also found weak evidence of the potential modification of effect of exposure to THMs on SGA by maternal *CYP2E1*

rs743535 genotype and *del GSTT1*, but these interactions did not persist after correction for multiple testing.

Previous studies mainly targeted a few xenobiotic-metabolizing genes (*CYP2E1*, *GSTM1*, *GSTT1*). Additional investigations concerning other genetic factors are needed to better understand how physiopathogenesis of fetal growth restriction relates to DBPs exposure. Cytochromes, CYP1A2, CYP2A6 and CYP2D6 are phase 1 xenobiotic-metabolizing enzymes (Ingelman-Sundberg, 2002) which are involved in metabolic pathways of CBPs. Toxicological studies demonstrated that human isoenzymes CYP1A2 and CYP2A6 metabolize the bromodichloromethane (BDCM), a THM specie formed when water contains bromide (Allis and Zhao, 2002; Zhao and Allis, 2002). In an experimental study, Backer et al. (2008) examined THMs levels in blood samples collected at baseline and 10 and 30 min after the shower and reported that genetic variants in *CYP2D6* gene were significant predictors of both baseline and post showering blood THMs concentrations.

CYP17A1 gene encodes the cytochrome isoenzyme CYP17A1 whose functions are key branch points in sex steroid hormones biosynthesis in placenta, adrenal and gonads (Escobar et al., 2011; Sharp et al., 2004). *CYP17A1* polymorphisms might modulate the risk of SGA (Yamada et al., 2004) and two case-control studies provided evidence that interaction between *CYP17A1* polymorphisms and environmental chemicals might affect fetal growth. Delpisheh et al. (2008) suggested a positive interaction between maternal *CYP17A1* genotype A1A1 and prenatal alcohol consumption for SGA. Furthermore, Chand et al. (2014) found that maternal *CYP17A1* genotype A1A1 and high level in cord blood of endosulfan, an organochlorine pesticide, caused an estimated reduction in birth weight.

We evaluated the association between maternal and child SNPs in *CYP1A2*, *CYP2A6*, *CYP2D6*, *CYP17A1* genes and SGA, thereafter assessing the potential interaction between prenatal exposure to drinking water CBPs and SNPs in these CYPs genes on SGA in mother-child pairs. The overall aim of our study was to characterize potential mechanism of CBPs toxicity related to the risk of fetal growth impairment and to identify factors which may modify this risk. Presently, water regulations of CBPs are based on their carcinogenic properties. If CBPs effects on fetal growth were demonstrated in genetically susceptible subgroups, it would provide strong argument for revision of water regulation taking into account potential reproductive outcomes.

2. Materials and Methods

2.1. Study design and population

Mother-child pairs were originally from a population-based case-control study conducted prospectively in the Quebec City (Canada) metropolitan area between August 2006 and April 2008 (Levallois et al., 2012). This study investigated the association between maternal exposure to THMs or HAAs and the occurrence of SGA (Levallois et al., 2012). Eligible women (and their child) had to be 16 years or older, had not resided in more than two residences in the study area or away from their residence for more than a month during their entire pregnancy. Among 2647 women (and their child) participating to the original study, 2517 (95 %) accepted to be contacted again for a follow-up study (Levallois et al., 2016). A

total of 1717 mothers and 1620 children provided DNA samples either from blood (for participants to a concurrent cohort study (Giguere et al., 2015) or saliva (for participants recontacted for the follow-up study).

The population of the present study is a sub-sample of participants successfully genotyped in the follow-up study (Levallois et al., 2016). Details on participants are given on the flow diagram presented in Fig. 1 and described elsewhere (Levallois et al., 2016).

To reduce a potential stratification bias, non-Caucasian participants (about 3%), identified from a self-administered questionnaire, were removed from the initial sample. We also excluded mother-child pairs with missing value on child genotype (32 cases and 66 controls), mother body mass index BMI (5 cases and 15 controls), passive smoking during pregnancy (5 controls) and CBPs exposure (19 cases and 67 controls). Finally, preterm neonates born before 32 weeks' gestation were excluded. A total of 1432 mother-child pairs were then retained for analysis (see Fig. 1).

2.2. Ethical considerations

The ethical review board of CHU de Québec approved the initial case-control study, the gene-environment component of the study and the use of the cohort biobank samples (SC12-01-159). All participants provided written informed consent for biological samples collection and genetic analysis.

2.3. Cases and controls definition

Cases (n=287) were defined as SGA singleton born at 32 weeks or more with birth weight less than the sex-specific 10th percentile of weight for gestational age according to the most recent Canadian standards (Kramer et al., 2001).

Controls (n=1145) were also singleton born at 32 weeks or more with birth weight at or above the 10th percentile of the same sex-specific standard for gestational age. Cases and controls status were determined in the initial study (Levallois et al., 2012) using information recorded on birth certificates with the approval of the Commission d'accès à l'information du Québec (The Quebec Office for access to information). Controls were randomly selected among singletons born the same calendar week in the same geographical study area.

2.4. Interview of participants

Information of participants on potential risk factors for SGA, water consumption (number of glasses/day, frequency of showers and baths) as well as water treatment home-devices and water handling (boiling or letting stay in the fridge) were collected in the initial study by telephone interview two months after birth. See Levallois et al. (2012) for further details.

2.5. CBPs exposure assessment

The detailed methods on drinking water sampling, chlorination by-products (CBPs) analysis and exposure assessment are given in Levallois et al. (2012) study. In brief, CBPs exposure was based on the analysis of each trihalomethane (THM) and haloacetic acid (HAA) concentration in the water distribution system serving participants' residence and on the

estimation of these CBPs absorbed doses. Tap water was sampled monthly from April 2006 to April 2008 at 46 sites distributed across the 9 surface-water systems and at one site for each of the 7 groundwater systems. Surface-water systems sampling sites were selected based on system characteristics influencing the spatial variations of CBPs in order to have at least one sample in each subsystem with homogenous concentrations of CBPs. Four THMs (chloroform, bromodichloromethane, chlorodibromomethane, and bromoform) and five regulated HAAs (monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic, dibromoacetic acids) were analysed. The third trimester of pregnancy was the focus period of exposure assessment because it is usually considered as a critical temporal window for detecting CBPs effects on fetal growth (Wright et al., 2003). A spatio-temporal model was used to estimate each CBP concentration on tap water at participants' residence during the third trimester (Levallois et al., 2012).

Doses of CBPs (expressed in $\mu\text{g}/\text{day}$) absorbed by each mother were estimated for THMs multiroute exposure (ingestion, inhalation and dermal exposure) whereas HAAs uptake was only considered for ingestion, because of their low volatility and low lipophilic properties. Ingested dose of each THM and HAA was calculated during a typical 24-hour day by multiplying the daily ingested volume of water with the estimated concentration of each CBP in tap water during the third trimester (after adjustment for home water treatment devices and other handling). Multiroute exposure to each THM was expressed as total (ingestion + inhalation + dermal) absorbed dose. Intakes from inhalation and dermal absorption during one typical day of third trimester were calculated for each mother using a physiologically based toxicokinetic model (PBPK) and added to the estimated ingested dose.

In this study, maternal exposure to CBPs was evaluated by total THMs (TTHMs) and the sum of the five regulated HAAs (HAA5) internal doses. The main specific species of THMs (chloroform) and HAA5 (dichloroacetic and trichloroacetic acids) as well as the sum of the three brominated trihalomethanes (bromodichloromethane, chlorodibromomethane, and bromoform) internal dose was also considered as indexes of exposure.

2.6. Genetic data collection and laboratory methods

Blood samples of mothers who participated to the concurrent cohort study (Giguere et al., 2015) were drawn at the first prenatal visit between 10 and 18 weeks, while cord blood of their neonates was sampled after delivery. Saliva was sampled using the ORAGENE-DNA kits (OG-500 and OG-575; GENOTK, Kanata, On, Canada) mailed to potential participants. DNA isolation from blood samples was performed from 200 μl of whole blood using a QIAamp DNA Blood kit (Qiagen), according to the protocol recommended by the manufacturer. DNA isolation from saliva was performed according to the GENOTEK protocol with minor adaptations. DNA isolated from blood and saliva sample were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Blood and saliva samples collection and DNA extraction were described in details in the previous gene-environment study (Levallois et al., 2016).

SNPs selection was realised to best capture the genetic diversity of the loci of interest with a systematic approach using the Tagger program (Broad Institute, Cambridge, MA). To optimize cost-efficiency, tag SNPs were selected by using linkage disequilibrium (LD) pattern with r^2 threshold 0.8 and minor allele frequency of at least 5% (based on the

Caucasian samples from Great Britain (GBR), Tuscany (TSI) and Utah of Western, and Northern European origin (NEU) of the 1000 Genomes project (IGSR)).

CYP17A1 rs743572 G allele were found to reduce the risk of SGA (Yamada et al., 2004). In addition to *CYP17A1* rs743572 G allele selected according to the literature review, additional SNPs within *CYP17A1*, *CYP1A2*, *CYP2A6* and *CYP2D6* genes were selected for genotyping as described above. Using the SNP Annotation and Proxy Search (SNAP) tool (<http://www.broadinstitute.org/mpg/snap/ldsearch.php>), rs743572 tags no other *CYP17A1* SNPs with a $r^2 \geq 0.8$ in the 1000 Genomes samples of European ancestry.

SNPs were genotyped by Sequenom Technology at the “plateforme de génotypage” of CHU de Québec-Université Laval. Details on DNA quantification and SNPs genotyping methods are given in the previous study (Levallois et al., 2016). Briefly, for DNA quantification, double stranded DNA concentration was assessed using the QuantiFluor dsDNA system (Promega Corporation, Madison, USA). SNPs were genotyped from 30 ng of genomic DNA using the iPLEX chemistry on a MALDI-TOF mass spectrometer (Sequenom). Cluster plots for all SNPs were manually evaluated to assess data quality. SNPs clusters performing poorly were excluded. Overall, 9 SNPs were genotyped.

2.7. Statistical analysis

R software (version 3.2.2) was used for statistical analysis. To compare characteristics between mothers of SGA cases and mothers of controls, a Pearson Chi-2 test was used for qualitative variables and a Cochran-Armitage test was used for ordinal variables such as maternal age, annual household income and prepregnancy body mass index (BMI). Deviation from Hardy Weinberg equilibrium was tested among controls and a significance level of p-value < 0.006 (0.05/9 genotyped SNPs) was used to reject the null hypothesis that the genotype frequencies were in equilibrium. The independent sample t-test was used for comparison of exposure to CBPs between cases and controls. Multiple unconditional logistic regression was used to calculate odds ratio (ORs) and their 95% confidence interval (95% CI) with controlling for the calendar month of birth as in Levallois et al. (2016).

Multiroute absorbed doses of THMs, chloroform (CHCl_3), brominated trihalomethanes (THMBr) and ingested doses of HAA5, dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were defined as low or high based on the 80th percentile of their exposure distribution on controls subjects (<80th percentile vs. 80th percentile). This threshold corresponds about to current standards of chlorination by-products in drinking water of 80 µg/L and 60 µg/L respectively for total THMs and HAAs (Gouvernement du Québec, 2016; United States Environmental Protection Agency, 2010). THMs and HAAs associations with SGA were determined by comparing high exposure (80th percentile) to low exposure (<80th percentile, the reference category). We determined the Spearman's correlation (r^2) between internal doses of chlorination by-products species.

In order to assess the association between SNPs in candidate genes and SGA, we calculated ORs for children with one or two copies of the allele relative to children with no copy and ORs for a child whose mother had one or two copies of the allele relative to the child whose mother had no copy, except for homozygotes genotype with frequency < 5%, which were

coded under a dominant model (allele carrier status: yes/no). To control confounding coming from the parent-offspring correlation, genotype variables for both mother and child were included in the same regression model.

To assess interaction on the multiplicative scale, SNP genotypes were coded as allele counts (0, 1, 2) in order to maximise statistical power. Genetic variables for both mother and child 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). Also for assessing interaction, additional models were constructed for the different routes of exposure to trihalomethanes (ingestion vs. inhalation plus dermal absorption). Known risk factors which associated in univariable analysis with SGA (with $p < 0.25$) were considered in the multiple logistic regression model: preeclampsia, maternal chronic disease, prepregnancy BMI, parity, prematurity, maternal age, maternal education, annual income, and maternal passive and active smoking during pregnancy, as well as coffee and alcohol consumption. Bonferroni adjustment for multiple testing was performed for the analysis by setting the significance level for multiplicative interaction to $0.05/27 = 0.002$ (27 for 9 SNPs and 3 CPBs because HAA5, TTHMs and THMBR were not highly correlated between themselves, see Table S-I (in supplementary data)).

Studying interaction on an additive scale is of interest from a public health perspective and for assessing the biological basis of interaction (Rothman et al., 2008). A measure of additive interaction, the relative excess risk due to interaction (RERI), was estimated for statistical interactions with p -value < 0.05 on the multiplicative scale. The function “epi.interaction” from the package epiR was used to estimate RERIs in a case-control study and to calculate their 95% CI using the delta method as described by Hosmer and Lemeshow (1992). Dominant genetic models were tested and genetic variables for both mother and child were included in the same model. In the absence of additive interaction, the $RERI = 0$.

3. Results

3.1. Characteristics of participants

Mothers of cases and controls were mostly 25 to 34 years of age (Table I). Mothers of cases tended to be poorer, nulliparous and had lower prepregnancy BMI. Occurrence of preeclampsia during the pregnancy was slightly more frequent among mothers of cases than mothers of controls. Likewise, passive and active smoking, as well as coffee and alcohol consumption, during pregnancy were more frequent among mothers of cases than mothers of controls.

3.2. Participants exposure to CBPs and CBPs association to SGA

Mean internal doses of CBPs were slightly higher in mothers of cases than mothers of controls particularly for HAAs internal doses, but t-test did not report any significant

difference between means of the two groups (Table II). The Spearman correlation between chloroform and total trihalomethanes (TTHMs) internal doses was high (0.99) as well as the correlation between HAA5 and DCAA (0.99) and TCAA (0.98) internal doses (Supplementary data, Table S-I). Association between maternal third trimester exposure to THMs and HAAs (high exposure 80th percentile vs. low exposure <80th percentile) and SGA were non statistically significant (Supplementary data, Table S-II).

3.3. Genotyping and SNPs association to SGA

All SNPs successfully genotyped within *CYP17A1*, *CYP1A2*, *CYP2A6* and *CYP2D6* genes had allele frequencies >5% and no significant departures from the Hardy Weinberg equilibrium were observed for all genes genotypes among controls (Table III). Moreover, all genotyped SNPs in each gene were not in linkage disequilibrium between each other with $r^2 < 0.2$.

Genotyped SNPs in maternal and newborns *CYP1A2*, *CYP2A6*, *CYP2D6* and *CYP17A1* genes were not significantly associated with SGA (Table IV) after correction for multiple testing (Bonferroni correction, p-value < 0.006 (0.05/9 SNPs)). ORs of heterozygous or homozygous were non-significantly different from the null value of 1 (all 95% CI included the null value 1).

3.4. Statistical interactions

Complete results of the interactions on the multiplicative scale, ORs (95% CI) and p-values are presented in supplementary data (Tables S-III to S-VI).

We found positive multiplicative interactions ($p < 0.05$) between TTHMs (OR, 95% CI: 2.3 (1.3; 4.1)/OR, 95% CI: 2.0 (1.1; 3.6)) and HAA5 (OR, 95% CI: 2.1 (1.2; 3.6)/OR, 95% CI: 2.1 (1.2; 3.6)) internal doses and child *CYP17A1* rs4919687 A and rs743572 G alleles whereas negative interactions ($p < 0.05$) between TTHMs (OR, 95% CI: 0.5 (0.2; 0.9)/OR, 95% CI: 0.5 (0.3; 0.9)) internal dose and mothers *CYP17A1* rs4919687 A and rs743572 G alleles were found, see Tables V, VI-a and Tables S-III, S-IV (in Supplementary data,).

A negative multiplicative interaction ($p = 0.02$; OR, 95% CI: 0.5 (0.3; 0.9)) was also found between mother *CYP1A2* rs2470890 C allele and internal dose of HAA5, Tables VI-a and (Supplementary data, Table S-IV). No interactions were found between mother or child *CYP1A2* rs2470890 C allele and DCAA, TCAA and THMs (Supplementary data, Table S-IV) and between mother or child genotyped SNPs in *CYP2A6* and *CYP2D6* and either THMs or HAAs (Supplementary data, Tables S-III and S-IV).

However, none of these multiplicative interactions including those between each route of exposure to THMs and SNPs in all CYPs genes (Supplementary data, Tables S-V and S-VI) remained statistically significant after Bonferroni correction for multiple testing. Moreover, interactions on the additive scale (RERI) were not statistically significant for all reported multiplicative interactions at the nominal $p < 0.05$ significance level (Tables V, VI-a and VI-b).

4. Discussion

The study of genetic variants in *CYP1A2*, *CYP2A6*, *CYP2D6* and *CYP17A1* genes might shed light on the causal nature of association between SPCs and SGA.

We found a negative interaction ($p < 0.05$) between ingested dose of HAA5 and mother *CYP1A2* rs2470890 C allele, but no interactions were found with genotyped SNPs in *CYP2A6* and *CYP2D6* genes. Positive interactions ($p < 0.05$) between TTHMs and HAA5 internal doses and child *CYP17A1* rs4919687 A and rs743572 G alleles were also found whereas negative interactions between TTHMs doses and mothers *CYP17A1* rs4919687 A and rs743572 G alleles were reported at the same nominal p-value threshold. However, none of these interactions remained statistically significant after correction for multiple testing.

4.1. Assessment of multiplicative interaction between CBPs and genetic variants in *CYP1A2*, *CYP2A6* and *CYP2D6* genes

CYPs 1–3 families are responsible of oxidative reactions in xenobiotic-metabolizing pathway (Zhou et al., 2009) involve in detoxification processes. Some of these CYPs, particularly CYP1A, 1B and 2E subfamilies play a role in the bioactivation of the vast majority of xenobiotic and may transform innocuous chemicals into reactive metabolites (Ioannides and Lewis, 2004). Cytochrome CYP2E1 is the primary enzyme involved in the metabolism of low doses of chloroform (Meek et al., 2002) and it generates free radicals and electrophilic phosgene which cause oxidative stress (Gemma et al., 2003; Nieuwenhuijsen et al., 2009). Oxidative stress may result from CYPs metabolism that releases excessive reactive oxygen species or activate chemicals (Gonzalez, 2005). There is some evidence that maternal oxidative stress during pregnancy may lead to adverse foetal development such as abnormal fetal growth (Biri et al., 2007; Mert et al., 2012).

Polymorphisms in xenobiotic-metabolizing CYPs and those involve in bioactivation may contribute to different susceptibility to toxicants and risk of disease (Daly, 2015; Zhou et al., 2009). In addition to CYP2E1, the CYPs that metabolically activate toxicants are limited to a few forms including CYP1A2 and CYP2A6 isoenzymes (Rendic and Guengerich, 2012). In this study, SNPs in maternal and neonates *CYP1A2*, *CYP2A6*, *CYP2D6* genes were not associated with SGA (Table IV). This is similar to results of a recent European mother-child cohort study which did not find association between others SNPs in these genes (not in linkage disequilibrium with our studied SNPs) and risk of SGA (Kogevinas et al., 2016). These findings suggest that the effect of genetic variants in *CYP1A2*, *CYP2A6* and *CYP2D6* genes on SGA is either absent or very small.

Enzymes coded by *CYP1A2*, *CYP2A6* and *CYP2D6* genes also play a role in CBPs biotransformation. CYP1A2 and CYP2A6 enzymes metabolized low dose of BDCM with lower catalytic efficiency (Allis et al., 2002; Allis and Zhao, 2002; Zhao and Allis, 2002). Backer et al. (2008) showed that the genotype homozygous for the allele *CYP2D6**4 (rs3892097 T) was associated with increased chloroform (and thus presumably decreased metabolism) in blood 10 min after showering when compared with the wild type. We reported a negative multiplicative interaction between HAA5 and mother *CYP1A2* rs2470890 C allele, but we did not find interaction with HAA5 main species and THMs

family compounds. However, the negative interaction was no longer significant when multiple testing (Bonferroni correction) was taken into consideration. Further, we did not find interactions between THMs or HAAs and genotyped SNPs within *CYP2A6* and *CYP2D6* genes. Of note, in the 1000 Genomes samples of European ancestry, our genotyped SNPs in *CYP2D6* gene were not in linkage disequilibrium with the *CYP2D6* rs3892097 T allele presented in Backer et al. (2008). However, these results are in line with those of the European mother-child cohort study, which did not find interaction between TTHMs and others SNPs within *CYP1A2*, *CYP2A6* and *CYP2D6* genes in relation to SGA (Kogevinas et al., 2016).

4.2. Assessment of multiplicative interaction between CBPs and genetic variants in *CYP17A1* gene

Cytochrome *CYP17A1* mediates both steroid 17 α -hydroxylase and 17,20-lyase activity and function at key steps in the genesis of human sex steroid hormones (Sharp et al., 2004). Genetic variants in *CYP17A1* are likely to affect fetal growth. The *CYP17A1* rs743572 is an A/G/T SNP locates in the 5' untranslated region 34 base pairs upstream of translation initiation site (dbSNP: <http://www.ncbi.nlm.nih.gov/snp/>). In a cohort study, Yamada et al. (2004) reported higher risk of SGA in women homozygous for the allele *CYP17A1* A1 (rs743572 A) than in carriers of the allele *CYP17A1* A2 (rs743572 G) with an OR=3.41 (95% CI=1.18–9.84). Indeed, *CYP17A1* A2 (rs743572 G) allele was hypothesized to increase the production of precursor androgens and their subsequent conversion to estrogens resulting into the creation of an additional Sp1 binding site with enhanced *CYP17A1* promoter activity and increased rate of transcription (Feigelson et al., 1998). Estrogens stimulate placental function and cell proliferation and are thus important determinants of fetal growth (Bukovsky et al., 2003). In our study, association between mother or child *CYP17A1* rs743572 G allele and SGA was not statistically significant (Table IV). It is worth pointing that we considered both maternal and child genotype in the same logistic regression model to control confounding coming from the parent-offspring correlation, while Yamada et al. (2004) analysed only maternal genotype. Thus the association found with maternal genotype may be confounded by the effect of the newborn genotype (Shi et al., 2008). The genotype homozygous for *CYP17A1* rs4919687 A allele could be a genetic risk marker of coronary artery disease (Dai et al., 2015). Its relationship with SGA is unclear, but maternal cardiovascular morbidities such as preeclampsia was associated with SGA (Zezza et al., 2014). In our study, mothers and newborns carrying rs4919687 A allele and two others SNPs within *CYP17A1* gene were not associated with SGA neonates (Table IV).

The steroidogenesis pathway is a target for the action of various endocrine-disrupting chemicals (Sanderson 2006; Patel et al. 2015). Genetic variation in *CYP17A1* and its interaction with potential endocrine disrupters during pregnancy may affect fetal development (Chand et al., 2014; Delpisheh et al., 2008). CBPs have been suggested to be endocrine disrupters (Nieuwenhuijsen et al., 2009) and elevated THMs exposure may lead to decreased serum total testosterone (Potter et al., 1996; Zeng et al., 2013). This suggests that THMs might produce androgenic deficiency and possibly cause interferences in steroidogenesis pathway.

To our knowledge, this is the first study to explore gene-environment interaction between prenatal exposure to CBPs and mother-child SNPs in *CYP17A1* gene on the risk of SGA. We found positive interactions between TTHMs and HAA5 internal doses and child *CYP17A1* rs4919687 A and rs743572 G alleles and negative interactions ($p < 0.05$) between TTHMs internal dose and mothers *CYP17A1* rs4919687 A and rs743572 G alleles (Table V, Table VI-a). The same multiplicative interactions were found with the main species of THMs and HAA5 families' compounds (Tables V and VI-b), but none of these interactions remained after correcting for multiple testing.

Fetal *CYP17A1* is implicated in male sexual differentiation and participates to the development and function of testis with a significantly high level of transcript in this tissue (Houmard et al., 2009). However, the implication of fetal *CYP17A1* gene in intra-uterine growth is unclear. During pregnancy, maternal androgen concentrations and production are dependent on fetal and placenta co-operation and the foetus is influenced by maternal hormones through placental barrier (Kuijper et al., 2013). It has been shown that the *CYP17A1* rs743572 G allele has bidirectional effects on human reproduction. Although this allele has been suggested to enhance fetal growth (Yamada et al., 2004), it could also increase susceptibility to recurrent pregnancy loss (Sata et al., 2003), by causing hormonal estrogen-progesterone imbalance in mother reproductive organs (Yamada et al., 2005).

4.3. Assessment of multiplicative interaction between each route of exposure to THMs and genetic variants in CYP1A2, CYP2A6, CYP2D6 and CYP17A1 genes

In addition to ingestion route, THMs are volatile and lipophilic compounds and therefore highly susceptible to being absorbed through inhalation and dermal route during showering, bathing and swimming (Erdinger et al., 2004; Haddad et al., 2006; Xu and Weisel, 2005). Exposure route affects the rates of metabolism and therefore the compound's potential toxicity. The ingested dose of volatile organic compounds is metabolized in the liver before entering the bloodstream whereas the dose from inhalation or dermal route is dispersed throughout the body without passing by the hepatic route, which leads to a much greater systemic CBPs dose (Ashley et al., 2005; Backer et al., 2000; Leavens et al., 2007; Weisel and Jo, 1996). Nevertheless, there were no significant interactions between SNPs in candidate genes and each route of exposure to THMs (ingestion vs. inhalation plus dermal absorption) after Bonferroni correction (Supplementary data, Tables S-V and S-VI).

4.4. Assessment of additive interaction between CBPs and genetic variants in CYP1A2, CYP2A6, CYP2D6 and CYP17A1 genes

Most frequently, epidemiology studies report departure from multiplicativity when characterizing interactions (Knol et al., 2009), but it is also important to consider measures of interaction on the additive scale which is most relevant for public health perspectives as well as biological or causal bases of interaction (de Jager et al., 2011; Rothman et al., 2008). Estimates of RERI are useful in detecting synergism between two factors within the sufficient cause framework (Rothman et al., 2008; VanderWeele and Robins, 2007). However, in our study, interactions on additive scale were not statistically significant (Tables V, VI-a and VI-b).

4.5. Strengths and limitations of the study

An important strength of our study is that it used a case-mother/control-mother design. In fact, statistical analysis took into account both maternal and the correlated fetal genotypes in the same regression model to reduce confounding bias (Shi et al., 2008). Moreover, we conducted a population-based study, which minimized selection bias (Rothman et al., 2008), and although our sample size was reduced to nearly 60% of the original sample, characteristics of participants of the present study were similar to those of the original sample (Levallois et al., 2012). The studied population was also limited to Caucasian mothers of French-Canadian ancestry for the large majority to reduce the stratification bias. The best available science was applied to optimize CBPs exposure assessment. All routes of exposure were considered for THMs which are volatile and lipophilic and internal dose was estimated using PBPK model taking into account the increase of mother body weight and body surface during pregnancy (Levallois et al., 2012).

Despite an advantageous number of study subjects compared to previous gene-environment studies, a major limitation of this gene-environment interaction study remains its limited statistical power. Furthermore, SGA neonates (<10th percentile) do not represent an homogenous group because SGA children might be either constitutionally small or authentically growth restricted. Neonates with a birth weight less than 10th percentile may be constitutionally small, whereas those with a birth weight greater than the 10th percentile may be authentically growth restricted (ie, pathologically small) (Sharma et al., 2016; Vayssiere et al., 2015). Therefore, even if SGA is correlated with intra-uterine growth restriction (IUGR) and may be used as a proxy for growth restriction both outcomes are not interchangeable (Bamberg & Kalache, 2004; Vayssiere et al, 2015), which could lead to some misclassification of growth restriction status. This could partially explain the loss of statistical significance of our results. In addition, the limited number of successfully genotyped SNPs within *CYP1A2*, *CYP2A6* and *CYP2D6* restricts analysis of these genes. Future study design (focalised on *CYP17A1*) should include a larger sample size, a better capacity to identify pathological SGA neonates directly, and innovate statistical analysis that would take into account the parent-offspring correlation more efficiently. If such well-designed larger-scale study fails to find any interaction, then there will be greater confidence in the absence of such interaction.

5. Conclusion

This study aimed to identify potential mechanisms underlining CBPs association and risk of SGA. After correction for multiple testing, we did not find significant interactions between genetic variants in *CYP1A2*, *CYP2A6*, *CYP2D6* and *CYP17A1* genes and exposure to THMs or HAAs on the occurrence of SGA. Further genetic and epidemiological research is required to draw definitive conclusions on the biological and causal role of CBPs exposure on the risk of pathological SGA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BDCM	bromodichloromethane
CBPs	chlorination by-products
CYPs	cytochrome P450
DBPs	disinfection by-products
DCAA	dichloroacetic acid
HAAs	haloacetic acids
HAA5	five regulated haloacetic acids
IUGR	Intra-uterine growth restriction
LD	linkage disequilibrium
TCAA	trichloroacetic acid
THMs	trihalomethanes
TTHMs	total trihalomethanes
SGA	small-for-gestational-age
SNP	single nucleotide polymorphism

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Highlights

- Chlorination by-products (CBPs) is a possible etiologic factor of small-for-gestational-age (SGA).
- Genetic susceptibility may modulate chlorination by-products (CBPs) effects on fetal growth.
- Genetic variants in *CYP17A1* gene could modify the risk of SGA related to CBPs.

Fig. 1.

Flow Chart starting from the participants to the previous gene-environment study (Levallois et al., 2016) with sample available for DNA extraction to participants to this study (1432 mother-child pairs).

Table I

Baseline characteristics of cases (n=287) and controls (n=1145) mothers

	Cases No. (%)	Controls No. (%)	p-value
Maternal age (years)			
< 25	24 (8)	93 (8)	
25–29	110 (38)	501 (44)	
30–34	120 (42)	412 (36)	p-trend=0.6
35	33 (11)	139 (12)	
Marital status			
Married	65 (23)	260 (23)	
Not married	222 (77)	885 (77)	1
Highest education level (years)			
12	55 (19)	232 (20)	
> 12	178 (81)	967 (84)	0.1
Annual household income (\$ Canadian)			
< 35,000	49 (17)	106 (9)	
35,000–69,999	107 (37)	496 (43)	p-trend=0.03 *
70,000	131 (46)	543 (47)	
Parity			
Nulliparous	196 (68)	557 (49)	
Parous	91 (32)	588 (51)	<0.001 *
Prematurity (<37 weeks)			
Preterms	22 (9)	68 (6)	
Terms	265 (92)	1077 (94)	0.3
Prepregnancy body mass index (Kg/m²)^a			
<19.8	82 (29)	176 (15)	
19.8–25.9	156 (54)	706 (62)	
26.0–29.9	26 (8)	143 (12)	p-trend=0.0007 *
>29.9	23 (8)	120 (10)	
History of chronic diseases			
Yes	25 (8)	77 (7)	
No	262 (91)	1068 (92)	0.2
Preeclampsia			
Yes	25 (9)	52 (5)	
No	262 (91)	1093 (95)	0.006 *
Coffee consumption^b			
Yes	151 (53)	526 (46)	
No	136 (47)	619 (54)	0.04 *
Maternal smoking^b			
Yes	51 (18)	126 (11)	
No	236 (82)	1019 (89)	0.003 *

	Cases No. (%)	Controls No. (%)	p-value
Maternal passive smoking at home			
Yes	34 (12)	76 (7)	0.003 [*]
No	253 (88)	1069 (93)	
Alcohol consumption ^b			
Yes	129 (45)	426 (37)	0.02 [*]
No	158 (55)	719 (63)	

^aPrepregnancy body mass index (BMI) was stratified according to the original population-based case-control study (Levallois et al., 2012) and a similar study conducted by Hoffman et al. (2008).

^bDuring pregnancy.

* p-value < 0.05 was considered to indicate statistical significance.

Table II

THMs and HAAs internal doses of cases and controls during the third trimester of pregnancy

CBPs internal doses (µg/day)	Cases	Controls	p-value
	mean (SD)	mean (SD)	
Trihalomethanes (THMs) multi-exposure doses			
Chloroform (CHCl ₃)	135.4 (145.7)	133.6 (151.0)	0.86
Brominated THMs (THMBr)	18.2 (16.8)	18.5 (16.6)	0.83
Total THMs (TTHMs)	153.6 (148.5)	152.1 (152.5)	0.88
Haloacetic acids (HAAs) ingested doses			
Dichloroacetic acid (DCAA)	14.3 (20.3)	12.1 (19.3)	0.09
Trichloroacetic acid (TCAA)	16.1 (26.3)	13.2 (25.7)	0.10
Total HAAs (5 species)	33.7 (49.9)	27.9 (46.9)	0.08

SD: standard deviation.

Table III

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

Chrom	Gene	rs number	Position ^a	Molecular ^b consequence	Clinical ^b significance	Allele	MA ^c	MAF ^d	MAFC ^e	HWE ^f Controls
10	CYP17A1									
		rs4919687	104595248	Intron	-	G:A	A	0.30	0.28	0.09
		rs3824755	104595849	Intron	-	G:C	C	0.11	0.07	0.04
		rs743572	104597152	utr variant 5 prime	^g	A:G	G	0.40	0.36	0.19
		rs284849	104591182	Intron	-	G:T	T	0.16	0.22	1.00
15	CYP11A2									
		rs2470890	75047426	synonymous	-	T:C	C	0.40	0.36	0.26
		rs12591078	75050854	-	-	C:T	T	0.31	-	-
		rs11072501	75051008	-	-	A:G	G	0.07	-	-
19	CYP2A6									
		rs34265315	41347494	-	-	A:G	G	0.056	0.063	0.80
		rs8192726	41354496	Intron	-	G:T	T	0.052	0.061	0.42
		rs4079369	41352762	Intron	-	G:C	C	0.13	-	-
		rs7250713	41355195	Intron	-	C:G	G	0.31	-	-
		rs1137115	41356281	synonymous	-	C:T	T	0.26	-	-
		rs11182204	41357795	2KB upstream	-	T:A	A	0.23	-	-
		3								
22	CYP2D6									
		rs28371725	42523805	Missense	-	A:G	A	0.11	0.08	1.00
		rs1081004	42525625	Intron	-	A:G	A	0.06	0.07	0.21
		rs76925852	42526763	Missense	-	C:T	T	0.05	-	-

^aPosition in genome assembly GRCh37.p13.

^bTerminology and data from the National Center for Biotechnology Information Variation Viewer, retrieved on Jun 10th, 2016 (www.ncbi.nlm.nih.gov).

^cMA: minor allele.

^dMAF: minor allele frequency of 1000 Genomes EUR sub-populations (IGSR).

^eMAFC: minor allele frequency computed in the sample of mothers of controls. A missing value indicates that genotyping of the SNP failed.

^fHWE: Hardy Weinberg equilibrium was tested using Pearson Chi-2 test.

^gCYP17A1 rs743572 G allele had been found to reduce the risk of SGA (Yamada et al., 2004).

Table IV

SNP Genotypes association with small-for-gestational-age neonates

SNPs	Cases No. (%)	Controls No. (%)	Crude OR CI (95%)	Adjusted OR ^a CI (95%)	p-value ^b
Mothers <i>CYP17A1</i>					
rs4919687					
Wild type	137 (49)	595 (53)	1	1	0.03
Heterozygote	123 (44)	425 (38)	1.5 (0.9; 2.6)	1.4 (0.8; 2.5)	
Homozygote	19 (7)	98 (9)	1.2 (0.7; 2.1)	1.1 (0.6; 2.0)	
Newborns <i>CYP17A1</i>					
rs4919687					
Wild type	150 (54)	542 (48)	1	1	0.14
Heterozygote	99 (35)	489 (44)	0.6 (0.4; 1.0)	0.6 (0.4; 1.0)	
Homozygote	30 (11)	87 (8)	0.8 (0.5; 1.3)	0.9 (0.6; 1.5)	
Mothers <i>CYP17A1</i>					
rs3824755 ^c					
Wild type	245 (87)	969 (86)	1	1	0.54
Heterozygote	34 (12)	154 (14)	0.9 (0.6; 1.3)	0.9 (0.5; 1.3)	
Homozygote	1 (0.36)	1 (0.09)			
Newborns <i>CYP17A1</i>					
rs3824755 ^c					
Wild type	246 (88)	981 (87)	1	1	0.90
Heterozygote	34 (12)	137 (12)	0.9 (0.6; 1.4)	1.0 (0.6; 1.6)	
Homozygote	0 (0)	6 (0.53)			
Mothers <i>CYP17A1</i>					
rs743572					
Wild type	115 (41)	481 (43)	1	1	0.36
Heterozygote	130 (47)	485 (44)	1.1 (0.8; 1.5)	1.2 (0.9; 1.6)	
Homozygote	33 (12)	146 (13)	0.9 (0.6; 1.4)	1.0 (0.6; 1.7)	
Newborns <i>CYP17A1</i>					
rs743572					
Wild type	121 (43)	437 (39)	1	1	0.55
Heterozygote	122 (43)	540 (48)	0.8 (0.6; 1.1)	0.8 (0.6; 1.1)	
Homozygote	35 (12)	135 (12)	0.9 (0.6; 1.4)	0.9 (0.5; 1.4)	
Mothers <i>CYP17A1</i>					
rs284849 ^c					
Wild type	173 (61)	679 (61)	1	1	0.91
Heterozygote	100 (35)	382 (34)	1.0 (0.8; 1.3)	1.0 (0.7; 1.3)	
Homozygote	10 (4)	53 (5)			
Newborns <i>CYP17A1</i>					
rs284849 ^c					
Wild type	175 (62)	691 (62)	1	1	

SNPs	Cases No. (%)	Controls No. (%)	Crude OR CI (95%)	Adjusted OR ^a CI (95%)	p-value ^b
Heterozygote	99 (35)	384 (34)	1.0 (0.8; 1.3)	1.0 (0.7; 1.4)	0.95
Homozygote	9 (3)	39 (4)			
Mothers CYP1A2					
rs2470890					
Wild type	121 (43)	476 (42)	1	1	
Heterozygote	129 (45)	520 (47)	0.9 (0.6; 1.4)	0.9 (0.6; 1.5)	0.44
Homozygote	33 (11)	121 (11)	0.9 (0.6; 1.4)	1.0 (0.6; 1.7)	
Newborns CYP1A2					
rs2470890					
Wild type	112 (40)	489 (44)	1	1	
Heterozygote	139 (49)	503 (45)	1.1 (0.7; 1.7)	1.0 (0.7; 1.7)	0.82
Homozygote	32 (11)	125 (11)	0.9 (0.6; 1.4)	0.9 (0.5; 1.4)	
Mothers CYP2A6					
rs34265315^c					
Wild type	248 (88)	969 (88)	1	1	
Heterozygote	33 (12)	132 (12)	1.0 (0.6; 1.4)	1.1 (0.7; 1.7)	0.79
Homozygote	1 (0.35)	5 (0.45)			
Newborns CYP2A6					
rs34265315^c					
Wild type	250 (89)	965 (87)	1	1	
Heterozygote	31 (11)	139 (13)	0.9 (0.6; 1.3)	0.8 (0.5; 1.3)	0.47
Homozygote	1 (0.35)	2 (0.18)			
Mothers CYP2A6					
rs8192726^c					
Wild type	249 (88)	990 (89)	1	1	
Heterozygote	34 (12)	120 (11)	1.1 (0.7; 1.7)	1.2 (0.7; 1.9)	0.42
Homozygote	1 (0.35)	5 (0.45)			
Newborns CYP2A6					
rs8192726^c					
Wild type	251 (88)	979 (88)	1	1	
Heterozygote	32 (11)	134 (12)	0.9 (0.6; 1.4)	0.8 (0.5; 1.3)	0.47
Homozygote	1 (0.35)	2 (0.18)			
Mothers CYP2D6					
rs28371725^c					
Wild type	238 (85)	935 (84)	1	1	
Heterozygote	39 (14)	170 (15)	1.0 (0.7; 1.4)	1.0 (0.7; 1.5)	1.00
Homozygote	4 (1)	7 (1)			
Newborns CYP2D6					
rs28371725^c					
Wild type	241 (86)	943 (85)	1	1	
Heterozygote	39 (14)	157 (14)	0.9 (0.6; 1.3)	0.9 (0.6; 1.4)	0.75

SNPs	Cases No. (%)	Controls No. (%)	Crude OR CI (95%)	Adjusted OR ^a CI (95%)	p-value ^b
Homozygote	1 (0.36)	12 (1)			
Mothers <i>CYP2D6</i>					
rs1081004^c					
Wild type	242 (87)	952 (85)	1	1	
Heterozygote	37 (13)	154 (14)	0.9 (0.6; 1.3)	0.9 (0.6; 1.4)	0.62
Homozygote	0 (0)	10 (1)			
Newborns <i>CYP2D6</i>					
rs1081004^c					
Wild type	243 (87)	964 (86)	1	1	
Heterozygote	36 (13)	140 (12)	0.9 (0.6; 1.4)	1.0 (0.6; 1.5)	0.90
Homozygote	0 (0)	12 (1)			

^a Genotype variables for both mother and child were included in the same regression model.

^b Likelihood ratio test was used for SNPs where the three genotypes were coded separately and a Wald test (1 df) was used for SNPs coded as dominant for the minor allele. P-value < 0.006 (0.05/9 SNPs) was considered to indicate significance after Bonferroni correction.

^c SNP genotypes were coded as dominant for the minor allele (allele carrier status: yes/no) when genotype homozygous for the minor allele had frequency < 5%.

Table V

Association between exposure to THMs multi-exposure internal dose (high exposure 80th percentile in comparison to low exposure <80th percentile) and SGA for newborn and mother carriers of *CYP17A1* SNP genotypes

SNPs	Cases/Controls n	TTHMs		CHCl ₃			
		Adjusted OR ^a (95%CI)	p.interaction ^b	RER ^{ac} (95%CI)	Adjusted OR ^a (95%CI)	p.interaction ^b	RER ^{ac} (95% CI)
Newborns							
<i>CYP17A1</i> /rs4919687							
Wild type	150/542	1.2 (0.7; 1.9)			1.1 (0.6; 1.7)		
1 or 2 var. al	129/576	2.7 (1.5; 4.8) ^d	0.006	0.3 (−0.4; 1.0)	2.5 (1.4; 4.4) ^d	0.005	0.4 (−0.2; 1.1)
Newborns							
<i>CYP17A1</i> /rs743572							
Wild type	121/437	1.2 (0.7; 2.0)			1.1 (0.6; 1.8)		
1 or 2 var. al	157/675	2.4 (1.3; 4.3) ^d	0.02	0.3 (−0.4; 1.0)	2.3 (1.3; 4.0) ^d	0.01	0.4 (−0.2; 1.1)
Mothers							
<i>CYP17A1</i> /rs4919687							
Wild type	137/595	1.2 (0.7; 1.9)			1.1 (0.6; 1.7)		
1 or 2 var. al	142/523	0.6 (0.3; 1.1) ^d	0.02	−0.4 (−1.4; 0.5)	0.5 (0.3; 1.1) ^d	0.046	−0.2 (−1.2; 0.8)
Mothers							
<i>CYP17A1</i> /rs743572							
Wild type	115/481	1.2 (0.6; 2.0)			1.1 (0.6; 1.8)		
1 or 2 var. al	163/631	0.6 (0.3; 1.2) ^d	0.03	−0.4 (−1.3; 0.6)	0.6 (0.3; 1.2) ^d	0.046	−0.3 (−1.2; 0.7)

^aORs are adjusted for the following variables: calendar month of birth, maternal age, maternal education, annual household income, prepregnancy BMI, parity, prematurity, history of chronic disease, preeclampsia, active and passive smoking during pregnancy and coffee and alcohol consumption during pregnancy.

^bMultiplicative interaction p-value.

^cSNP genotypes were coded as allele carrier status (yes/no).

^dORs for carrier of one copy of allele.

Association between exposure to HAA5 internal dose (high exposure 80th percentile in comparison to low exposure <80th percentile) and SGA for newborn and mother carriers of *CYP1A2* and *CYP17A1* SNP genotypes

Table VI-a

SNPs	Cases/Controls n	HAA5		p. interaction ^b	RER ^{ac} (95% CI)
		Adjusted OR ^a (95%CI)			
Newborns					
CYP17A1/rs4919687					
Wild type	150/542	1.1 (0.7; 1.8)			
1 or 2 var. al	129/576	2.4 (1.4; 4.3) ^d	0.01		0.4 (−0.4; 1.2)
Newborns					
CYP17A1/rs743572					
Wild type	121/437	1.1 (0.7; 1.9)			
1 or 2 var. al	157/675	2.4 (1.4; 4.3) ^d	0.008		0.5 (−0.3; 1.3)
Mothers					
CYP1A2/rs2470890					
Wild type	121/476	2.5 (1.5; 4.1)			
1 or 2 var. al	162/641	1.3 (0.7; 2.4) ^d	0.02		−1.3 (−2.6; 0.07)

^a ORs are adjusted for the following variables: calendar month of birth, maternal age, maternal education, annual household income, prepregnancy BMI, parity, prematurity, history of chronic disease, preeclampsia, active and passive smoking during pregnancy and coffee and alcohol consumption during pregnancy.

^b Multiplicative interaction p-value.

^c SNP genotypes were coded as allele carrier status (yes/no).

^d ORs for carrier of one copy of allele.

Table VI-b

Association between exposure to dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) internal doses (high exposure 80th percentile in comparison to low exposure <80th percentile) and SGA for newborn and mother carriers of *CYP17A1* SNP genotypes

SNPs	Cases/Controls n	DCAA		TCAA			
		Adjusted OR ^a (95%CI)	p.interaction ^b	RER ^{ac} (95%CI)	Adjusted OR ^a (95%CI)	p.interaction ^b	RER ^{ac} (95%CI)
Newborns							
CYP17A1/rs491987							
Wild type	150/542	1.1 (0.7; 1.7)			1.2 (0.8; 1.9)		
1 or 2 var. al	129/576	2.5 (1.4; 4.4) ^d	0.008	0.4 (−0.5; 1.2)	2.4 (1.3; 4.2) ^d	0.04	0.3 (−0.5; 1.2)
Newborns							
CYP17A1/rs743572							
Wild type	121/437	1.0 (0.6; 1.7)			1.2 (0.7; 2.0)		
1 or 2 var. al	157/675	2.5 (1.4; 4.3) ^d	0.003	0.4 (−0.4; 1.2)	2.4 (1.4; 4.1) ^d	0.03	0.4 (−0.5; 1.2)

^a ORs are adjusted for the following variables: calendar month of birth, maternal age, maternal education, annual household income, prepregnancy BMI, parity, prematurity, history of chronic disease, preeclampsia, active and passive smoking during pregnancy and coffee and alcohol consumption during pregnancy.

^b Multiplicative interaction p-value.

^c SNP genotypes were coded as allele carrier status (yes/no).

^d ORs for carrier of one copy of allele.