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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

Background: Exposure to disinfection by-products (DBPs) during pregnancy was associated with reduced foetal 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 *GSTM1* 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.

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

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been exposed at home during their whole pregnancy to an average water supply THM levels above 29.7 μg/L (90th percentile of the distribution of concentrations in participants water supply systems) were at higher risk of SGA (<10th 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 (Fig. 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 Fig. 1).

2.2. 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.

2.3. Definition of cases and controls

Cases of SGA were all term singleton newborns with birth weight less than the sex-specific 10th 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.

2.4. 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.

2.5. 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, THMs and HAAs 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 monochloroacetic, 0.9 μg/L for dichloroacetic, 0.4 μg/L for trichloroacetic, 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 for a typical day. (See Supplemental material Methods S1 for details).

2.6. 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 Fig. 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).