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Association between exposure to organochlorine compounds and maternal thyroid status: Role of the *iodothyronine deiodinase 1* gene

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ABSTRACT

Introduction: Exposure to organochlorine compounds (OCs) may interfere with thyroid hormone (TH) homeostasis. The disruption of the deiodinase (DIO) enzymes has been proposed as a mechanism of action.
Aim: To evaluate the association between exposure to OCs and TH status in pregnant women, as well as to explore the role of genetic variations in the *DIO1* and *DIO2* genes.
Methods: The study population ($n = 1128$) was composed of pregnant women who participated in the INMA Project (Spain, 2003–2006). Hexachlorobenzene (HCB), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (4,4´-DDE), b-hexachlorocyclohexane (b-HCH), polychlorobiphenyl (PCB) congeners 138, 153 and 180, thyroid stimulating hormone (TSH), total triiodothyronine (TT3) and free thyroxine (FT4) were measured in serum samples taken during the first trimester of pregnancy (mean [standard deviation (SD)]: 13.5 [2] weeks of gestation). Polymorphisms in *DIO1* (rs2235544) and *DIO2* (rs12885300) were genotyped in maternal DNA. Sociodemographic and dietary characteristics were obtained by questionnaire.
Results: A 2-fold increase in HCB was associated with lower TT3 (% change = −1.48; 95%CI: −2.36, −0.60). Women in the third tertile for b-HCH had lower TT3 (% change = −3.19; 95%CI: −5.64, −0.67). The interactions between *DIO1* rs2235544 and PCB153 and b-HCH were statistically significant. The inverse association between PCB153 and TT3 was the strongest among women with AA genotype. Women with CC genotype presented the strongest inverse association between b-HCH and FT4.
Conclusion: Exposure to HCB and b-HCH was associated to a disruption in maternal TT3. The *DIO1* rs2235544 SNP modified the association between exposure to some of the OCs (specifically b-HCH and PCB153) and maternal thyroid hormone levels. These results strengthen the hypothesis that DIO enzymes play a role in explaining the disruption of thyroid hormones in relation to exposure to OCs.

1. Introduction

Organochlorine compounds (OCs), such as hexachlorobenzene (HCB), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (4,4′-DDE), b-hexachlorocyclohexane (b-HCH), and polychlorobiphenyls (PCBs), are synthetic chemicals with high resistance to degradation, which not only facilitates their bioaccumulation in living organisms, but also their biomagnification in the food chain. Their lipophilic nature gives them an affinity for adipose tissue, but they can also accumulate in

Abbreviations: 4,4′-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; b-HCH, b-hexachlorocyclohexane; DIO, deiodinase; FT4, free thyroxine; HCB, Hexachlorobenzene; LOD, limit of detection; OCs, organochlorine compounds; PCBs, polychlorobiphenyls; TBG, thyroxine-binding globulin; TH, thyroid hormones; TSH, thyroid stimulating hormone; TTR, transthyretin; TT3, total triiodothyronine.

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organs such as the liver, brain or pancreas (World Health Organization (WHO), 2003). The use and production of some OCs, including some pesticides and PCBs, were banned or regulated following the Stockholm Convention on Persistent Organic Pollutants (SCOPS, 2010), which has been ratified by over 180 countries. However, they are still found in food (Llop et al., 2010; Kartalović et al., 2016), environmental samples (Torre et al., 2016) and human tissues (Vizcaino et al., 2010).

Experimental studies have shown that some OCs can disrupt circulating thyroid hormones during development (Ulbrich and Stahlmann, 2004) and these hormones are essential for normal development in utero and in infancy (Dussault and Ruel, 1987). Thus, deficiency in maternal thyroid hormones may lead to neurodevelopmental disorders (Julvez et al., 2013), especially during the first half of pregnancy, when the fetus is totally dependent upon maternal thyroid production (Morreale de et al., 2004). Due to the importance of the possible environmentally mediated alterations in thyroid function in pregnant women and infants, several birth cohort studies have examined the effects of exposure to HCB, b-HCH, 4,4′-DDE, and PCBs on thyroid function during pregnancy or childhood; however, the results do not support a clear causal-effect pattern. Whereas associations were reported for PCBs and thyroid hormones in some studies in pregnant women or children (Koopman-Esseboom et al., 1994; Takser et al., 2005; Chevrier et al., 2008; Alvarez-Pedrerol et al., 2009; Berg et al., 2016), others found little or no evidence of any association (Steuerwald et al., 2000; Longnecker et al., 2000; Lopez-Espinosa et al., 2009, 2010; de Cock et al., 2014; Lignell et al., 2016). Similarly, some studies have described an association between prenatal exposure to some organochlorine pesticides, such as HCB, b-HCH or 4,4′-DDE, and thyroid hormone status (Lopez-Espinosa et al., 2009, 2010; Maervoet et al., 2007; Takser et al., 2005; Alvarez-Pedrerol et al., 2008; Li et al., 2014; Kim et al., 2015; Berg et al., 2016) but others have not (Steuerwald et al., 2000; Ribas-Fitó et al., 2003; de Cock et al., 2014).

The reasons for the heterogeneity observed in the epidemiological studies could be related to differences in design, such as the thyroid hormone studied (circulating or total triiodothyronine [T3] and thyroxine [T4]), the analytical method used (different immunoassays or equilibrium dialysis), the matrix where the thyroid hormones were determined (plasma or serum) or the period of pregnancy when thyroid hormones were analysed (different trimesters or at birth). Other influencing factors could be related to OC exposure, such as differences in the compounds analysed (e.g. PCBs represent a group of multiple individual compounds with different toxicity) and the wide range of concentrations to which the populations are exposed. In addition, the association between OC exposure and thyroid hormones could be confounded by the iodine status. Iodine is essential for thyroid synthesis (Glinoer, 2004) and fish is one important source of both iodine (Yang et al., 2014) and some OCs, especially PCBs (Llop et al., 2010).

Another possible cause contributing to this heterogeneity could be the genetic background. Changes in thyroid hormone levels and ratios have been suggested to be associated with genetic variation in the deiodinase (DIO) enzyme-encoding genes (de Jong et al., 2007; Panicker et al., 2008; Peltsverger et al., 2012). Thus, the C allele in rs2235544 *DIO type 1 (DIO1)* was associated with an increase in the free T3/T4 ratio and free T3 level (Panicker et al., 2008), the G allele in rs12885300 *DIO type 2 (DIO2)* with a decrease in free T4 level (Peltsverger et al., 2012) and the T allele in rs11206244 *DIO1* with an increased free T4 and lower T3 levels (de Jong et al., 2007). These DIO enzymes play a vital role in maintaining thyroid hormone homeostasis both at a serum and local tissue level. DIO1 and DIO2 are predominantly activating enzymes: both convert T4 to T3 by outer ring deiodination. DIO1 is found in liver, kidney, thyroid and pituitary in humans, and DIO2 can be present in skeletal muscle, central nervous system, pituitary, thyroid, heart and brown adipose tissue (Bianco et al., 2002). There is some, albeit weak, evidence of changes in deiodinase enzyme activity being associated to exposure to some OCs, such as PCB77, HCB and dioxins in experimental studies (Alvarez et al., 2005; Beck et al., 2006; Viluksela et al., 2004). In addition, in a Dutch cohort of 100 mother-infant pairs, a positive correlation was observed between some PCBs and the cord serum T3/rT3 ratio, an indicator of DIO *type 3* (*DIO3*) activity (Soechitram et al., 2017).

The INMA – INfancia y Medio Ambiente (Environment and Childhood) – project is a multicenter cohort study which aims to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development (http://www.proyectoinma.org/). The relationship between OC exposure and maternal thyroid hormones has already been studied in some of the INMA cohorts. Thus, maternal serum concentrations of 4,4′-DDE were associated with increased maternal thyroid stimulating hormone (TSH) and reduced free T4 (FT4) levels in a subsample from the Valencia region (Lopez-Espinosa et al., 2009). In Sabadell, levels of HCB and PCB congeners 180, 153 and 138 were related to lower maternal TT3 levels and higher FT4 levels (Alvarez-Pedrerol et al., 2009). The aim of this study is to evaluate the association between maternal OC concentrations and TSH, TT3 and FT4 in a pooled analysis for the INMA Valencia and Sabadell regions and to explore the role of genetic variations in *DIO1* and *DIO2* genes in this association.

2. Methods

2.1. The study population

The study subjects were pregnant women participating in the INMA birth cohort study. Details of the protocol and recruitment of women were reported previously (Guxens et al., 2012). Briefly, women were recruited in the first trimester of pregnancy and followed up until delivery in two regions of Spain: Valencia and Sabadell. Inclusion criteria were: being 16 or older, singleton pregnancy, planning to deliver at the study hospitals, not having followed an assisted reproduction programme, and without any problems of communication. Enrolment periods and number of women recruited were: November 2003 to June 2005 ($n = 855$) in Valencia, and July 2004 to July 2006 ($n = 657$) in Sabadell. Among them, only women with available data on maternal OC concentrations, genetics and thyroid hormones, and without thyroid pathology ($n = 1128$) were included in this study.

We obtained information about biomarkers, outcomes and covariates from 76% of the original cohort. We found statistically significant differences between the study population ($n = 1128$) and the excluded population ($n = 356$) according to the educational level and social class. Women who did not take part in this study were less educated and a higher proportion of them belonged to the lowest social class compared to the study population (data not shown).

The women participating in the study signed an informed consent form and the Ethics Committees of the centres involved in the study approved the research protocol.

2.2. OC analysis

Concentrations of HCB, b-HCH, 4,4′-DDE and three PCB congeners (IUPAC numbers: 138, 153, and 180) were determined in serum samples taken at the first trimester of pregnancy (mean [standard deviation (SD)]: 13.5 [2] weeks of gestation) as previously described (Goni et al., 2007; Vizcaino et al., 2010). Briefly, the OC concentrations were determined by gas chromatography with electron capture detection and confirmation by gas chromatography coupled to mass spectrometry in negative ion chemical ionization mode. Samples from Valencia were analysed at the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, in Barcelona (Spain) and samples from Sabadell were analysed in the Public Health Laboratory of the Basque Country. Both laboratories were in compliance with the Arctic Monitoring and Assessment Program for persistent organic pollutants in human serum (Centre de Toxicologie, Institut National de Santé Publique du Québec). Limits of detection (LODs) were