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Association of In Utero Exposure to Polybrominated Diphenyl Ethers With the Risk of Hypospadias

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IMPORTANCE Polybrominated diphenyl ethers (PBDEs) are added to many consumer products as flame retardants, and their endocrine-disrupting properties are a growing health concern in pregnancy.

OBJECTIVE To investigate whether in utero PBDE exposure as measured in maternal hair is associated with increased risk for hypospadias.

DESIGN, SETTING, AND PARTICIPANTS In this case-control study, the setting was the urology clinic of a tertiary pediatric hospital between January 3, 2011, and April 1, 2013. Participants were children diagnosed as having hypospadias and their mothers and a control group of children without hypospadias and their mothers. Dates of data analysis were September 3, 2017, to December 28, 2017.

EXPOSURES Gestational exposure to 8 PBDEs as measured in the 3-cm segment closest to the skull of maternal hair by gas chromatography–mass spectroscopy as a proxy for in utero exposure. The mothers resided in the same household for the duration of their pregnancy.

MAIN OUTCOMES AND MEASURES Difference in total maternal hair PBDE levels between the hypospadias and control groups.

RESULTS Total PBDE levels were significantly higher among mothers of infants with hypospadias (n = 152) (total PBDE level, 51.4 pg/mg; interquartile range, 35.8-78.5 pg/mg) than among controls (n = 64) (total PBDE level, 35.8 pg/mg; interquartile range, 18.1-69.9 pg/mg) (P = .02). Of the 152 women with sufficient hair samples for analysis in the case group, 89 completed a questionnaire and were included in a multivariable analysis, and of the 64 women with sufficient hair samples for analysis in the control group, 54 completed a questionnaire and were included in a multivariable analysis. Adjusting for potential confounders, hypospadias was associated with a relative 48.2% (95% CI, 23.2%-65.4%) higher maternal level of total PBDE levels in the multivariable analysis.

CONCLUSIONS AND RELEVANCE In this analysis, mothers of children with hypospadias were exposed during pregnancy to significantly higher levels of PBDEs. The results of this study suggest that level of exposure to PBDEs during gestation may have a role in the etiology of hypospadias.

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Supplemental content

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Hypospadias is a congenital birth defect affecting approximately 1 in every 300 male infants.[1] It is characterized by the incomplete fusion of the urethral folds.[1,2] Hypospadias exhibits 3 major phenotypic subtypes with increasing severity, including anterior, middle, and posterior. The usual treatment for restructuring the urethral opening is by surgical repair when the infant is 6 months old.[1] Evidence of rise in the prevalence of male reproductive health disorders in the last 50 years has led to the hypothesis that increasing levels of persistent environmental chemicals may be harmful to reproduction.[3-5]

Polybrominated diphenyl ethers (PBDEs) are environmental chemicals with powerful endocrine-disrupting properties. In developed countries, hypospadias has increased in incidence by approximately 50% in the last 40 years,[6] roughly coinciding with the introduction of PBDEs. These ubiquitous synthetic chemicals have been used since the 1970s to decrease the flammability of furniture, electronics, plastics, and textiles. Because PBDEs are physically, and not chemically, combined with the material being treated, they are able to leach into the home or work environment. Humans are exposed to these chemicals via inhalation or ingestion of household or workplace dust in which PBDEs accumulate.[7,8] Infants and toddlers are especially at risk for exposure to PBDEs in dust because of their time spent on the ground or floor.[9] One study[10] found a nonsignificant trend toward elevated PBDEs in midpregnancy serum samples of mothers whose sons had hypospadias.

Hair levels of PBDEs have been established as a suitable noninvasive biomarker for assessing long-term systemic PBDE exposure.[11] The objective of this study was to investigate whether in utero PBDE exposure as measured in maternal hair is associated with increased risk for hypospadias.

Key Points

Question Do the endocrine-disrupting polybrominated diphenyl ethers have a role in the etiology of hypospadias?

Findings In this case-control study of 152 mothers of infants with hypospadias and 64 mothers of infants without hypospadias who provided sufficient hair samples for analysis, 89 and 54 completed a questionnaire and were included in a multivariable analysis. Total maternal hair polybrominated diphenyl ether levels were significantly higher among mothers of infants with hypospadias than among controls in this case-control study.

Meaning Exposure to polybrominated diphenyl ethers during pregnancy may have a role in the etiology of hypospadias.

Methods

Inclusion and Exclusion Criteria

Eligible cases were biological mothers 18 years or older who had infant sons diagnosed as having isolated hypospadias by pediatric urologists at The Hospital for Sick Children, in Toronto, Ontario, Canada (HSC). To be included, the mothers had to have resided in the same household for the duration of their pregnancy and up to the time of hair collection. The age range for inclusion was based on evaluation by the pediatric urologists for infants with hypospadias. Consultation for surgical repair typically occurred between the ages of 3 and 18 months. Infants with hypospadias of either the anterior, middle, or posterior subtypes were included. Children were excluded if they were diagnosed as having a condition in which hypospadias was a component of a syndrome, including any additional genitourinary malformation. All study protocols were approved by the Research Ethics Board at HSC. Written informed consent was obtained from all participants.

Recruitment of Participants

Mothers of infants with hypospadias were recruited from the urology clinic at HSC between January 3, 2011, and April 1, 2013. Hair samples were collected from the mother and child, and a questionnaire was administered. For individuals who could not complete the questionnaire during their clinic visit, a prestamped envelope was provided to allow mailing after completion. Dates of data analysis were September 3, 2017, to December 28, 2017.

Participants in the control group qualified if they were biological mothers 18 years or older who had infant sons with no hypospadias between the ages of 3 and 18 months. They were recruited via referral from the Motherisk program at HSC. This program collected intake forms of callers who sought advice about medication exposure in pregnancy and screened for women who gave birth to an infant within the inclusion criteria age range (3-18 months). These women were contacted, informed about the study, and screened for eligibility. Similar to the mothers in the case group, the mothers in the control group had resided in the same home environment during pregnancy and up to the time of hair collection. For those who were eligible and interested in participation, an initial verbal informed consent was obtained, followed by mailing of a study package containing essential contact information, the study questionnaire, and instructions how to cut hair samples. Participants were informed that this study was voluntary and that they could withdraw at any time. Confidentiality was protected through the assignment of an encrypted number code, and all study data were stored on an encrypted USB in a secure, locked location.

Data Collection

The questionnaire given to mothers of both cases and controls had 9 sections. These consisted of questions about maternal and child health, lifestyle, and environmental information (**Table 1**).

Hair Collection

A sample of maternal hair was cut from the posterior vertex as close to the scalp as possible using sterilized stainless steel scissors. Because infant hair growth is variable, hair was cut from an area of the scalp that had the greatest amount. Hair samples were stored in paper envelopes covered in aluminum foil to prevent UV degradation at room temperature until analysis.

Hair Analysis for PBDEs

Eight congeners of PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209) were analyzed by a previously published gas chromatography-mass

Table 1. Maternal and Infant Characteristics of the Hypospadias and Control Groups

Variable	Hypospadias	Control	*P* Value
Maternal Characteristics	**(n = 89)**	**(n = 54)**	
Age, median (IQR), y	32.0 (29.0-36.0)	33.0 (30.0-34.0)	.45
Race/ethnicity, No. (%)			
White	69 (77.5)	42 (77.8)	.90
Other	20 (22.5)	12 (22.2)	
Income, No. (%)			
≤$39 999	7 (7.9)	3 (5.6)	.72
$40 000-$79 999	24 (27.0)	13 (24.1)	
≥$80 000	51 (57.3)	33 (61.1)	
No response	7 (7.9)	5 (9.3)	NA
Education, No. (%)			
Secondary	9 (10.1)	6 (11.1)	.83
University/college	62 (69.7)	36 (66.7)	
Postgraduate	18 (20.2)	12 (22.2)	
BMI			
Median (IQR)	23.3 (20.4-29.1)	23.9 (21.5-28.2)	.91
No response, No. (%)	11 (12.4)	6 (11.1)	NA
Smoking, No. (%)			
Yes	7 (7.9)	4 (7.4)	.84
During pregnancy	4 (4.5)	1 (1.9)	.72
Drinking during pregnancy, No. (%)	0	4 (7.4)	.42
Gestational diabetes, No. (%)			
Yes	7 (7.9)	3 (5.6)	.63
No response	2 (2.2)	0	NA
Infant Characteristics	**(n = 40)**	**(n = 54)**	
Age, median (IQR), mo	8.0 (5.0-12.0)	10.5 (7.5-12.0)	.25
Gestation length, No. (%)			
Full term, ≥37 wk	33 (82.5)	51 (94.4)	.43
Premature, <37 wk	6 (15.0)	3 (5.6)	
No response	1 (2.5)	0	NA
Birth weight, No. (%)			
<2500 g	9 (22.5)	5 (9.3)	.05
≥2500 g	25 (62.5)	48 (88.9)	
No response	6 (15.0)	1 (1.9)	NA
Breastfed, No. (%)	35 (87.5)	53 (98.1)	.21
Duration of breastfeeding, No./total No. (%)			
<3 mo	5/35 (14.3)	0/54	<.05
3-6 mo	14/35 (40.0)	3/54 (5.6)	
>6 mo	16/35 (45.7)	51/54 (94.4)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; NA, not applicable.

spectroscopy method.[11,12] Briefly, 30 to 40 mg of maternal or child hair was used for analysis. For the maternal hair, the 3-cm proximal segment was used because this is representative of the last 3 months before enrollment into the study.[13] Child hair samples were not segmented, and the whole length was used. The limit of detection (LOD) ranged from 1 to 4 pg/mg, and the limit of quantification (LOQ) ranged from 3 to 12 pg/mg. All values below the LOD were assigned a value of 0. Trace values were assigned the LOQ value for each respective congener.

The proportion of values below the LOD was lower than 20.0%. The percentage recoveries ranged from 100% to 120.0%, with the exception of BDE-47 (135.2%), and the percentage coefficients of variation ranged from 13% to 19%, with the exception of BDE-209 (33.4%).

Statistical Analysis

The primary outcome of the study was the difference in total maternal PBDE levels between the hypospadias and control groups. Based on the available sample of 152 hypospadias cases, 40 controls would be needed to detect a difference in the mean total PBDE levels of 15 pg/mL between groups with 80% power and a type I error rate of 5%, assuming a baseline mean (SD) level of 35 (17) pg/mL. Maternal and infant characteristics were compared between cases and controls using Mann-Whitney test for continuous variables and Fisher exact test for categorical variables. Among all participants, the distribution of total PBDE levels was compared between cases and controls using Mann-Whitney statistics. Bonferroni correction was used to correct for multiple comparisons such that the level of statistical significance was set at 2-sided $P = .05$. Among the subset of

Table 2. Comparison of Potential Environmental Mechanisms That May Affect Hair Total PBDE Levels

Variable	No. (%) Hypospadias (n = 89)	Control (n = 54)	*P* Value	Median Total PBDE Level, pg/mg (n = 154)
Diet Consumption				
Meat				
Daily	67 (75.3)	32 (59.3)	.11	63.4
Weekly	18 (20.2)	18 (33.3)		44.7
Rarely/never	4 (4.5)	4 (7.4)		NA
Dairy				
Daily	69 (77.5)	40 (74.1)	.84	54.6
Weekly	15 (16.9)	10 (18.5)		52.5
Rarely/never	4 (4.5)	4 (7.4)		NA
Fish				
Daily	7 (7.9)	1 (1.9)	.82	NA
Weekly	62 (69.7)	38 (70.4)		54.7
Rarely/never	20 (22.5)	14 (25.9)		46.5
No response	0	1 (1.9)	NA	NA
Hair Treatments				
Coloring agents	44 (49.4)	22 (40.7)	.03	38.8
Permanent wave solutions	0	0	NA	NA
Straighteners/relaxers	4 (4.5)	1 (1.9)	.32	69.6
Highlighting agents	18 (20.2)	10 (18.5)	.78	54.8
Sprays/gels	49 (55.1)	38 (70.4)	.34	50.8
No response	0	1 (1.9)	NA	NA
Site of Residence				
City	38 (42.7)	22 (40.7)	.94	53.3
Urban	29 (32.6)	21 (38.9)		54.7
Rural	18 (20.2)	11 (20.4)		53.9
No response	4 (4.5)	0	NA	NA

Abbreviations: NA, not applicable; PBDE, polybrominated diphenyl ether.

participants with complete questionnaire data, adjusted differences in PBDE levels were estimated from a multivariable linear regression model that included advanced maternal age at delivery, obesity, use of assisted reproductive technology, maternal hypertension, thyroid disease, and birth weight.[14] Hair concentrations of PBDEs were log transformed such that the exponentiated coefficient for cases vs controls represented the ratio of average concentration between the cases and controls. Nonparametric (rank) correlation was used to study the correlation between maternal and neonatal total PBDE levels.

In an exploratory analysis, we investigated whether the hypospadias and control groups differed in proportions of mothers exposed to potential environmental factors that may affect hair total PBDEs using χ^2 test. All statistical analyses were performed using a software program (SPSS, version 20; SPSS Inc).

Results

A total of 235 mothers attending the urology clinic at HSC gave consent for themselves and their sons with hypospadias to participate in this study. Of these, 152 mothers who met the inclusion criteria and their 91 infants had sufficient amounts of hair to be analyzed. The incidence rates of the 3 hypospadias phenotypes were 99 anterior (65.1%), 32 middle (21.1%), and 21 posterior (13.8%). There were no statistical differences in the median maternal hair total PBDE levels detected among those who had infants diagnosed as having anterior, middle, or posterior hypospadias (eTable in the Supplement).

In the control group, approximately 300 women provided initial verbal consent when contacted over the telephone for follow-up. Of them, 64 mothers who met the inclusion criteria and their 40 infants were able to provide sufficient hair for analysis. Of the 152 women with sufficient hair samples for analysis in the case group, 89 completed a questionnaire and were included in a multivariable analysis, and of the 64 women with sufficient hair samples for analysis in the control group, 54 completed a questionnaire and were included in a multivariable analysis. Total PBDE levels of mothers who completed the questionnaire were not statistically different from those of mothers who did not complete the questionnaire for any single congener or their total levels (eTable in the Supplement). Those meeting the inclusion criteria but not providing hair samples did not differ from those providing hair samples on any study variable for both the hypospadias and control groups (eTable in the Supplement).

Bivariate analyses generally did not reveal differences between the case and control groups in potential confounders established a priori (Table 1). An exception was a significantly lower birth weight in the hypospadias group, and this was one of the confounders that was adjusted for in the multivariable analysis.

In univariate analysis, total maternal hair PBDE levels in cases were significantly higher than those in controls. Total PBDE levels were significantly higher among mothers of infants with hypospadias (n = 152) (total PBDE level, 51.4 pg/mg; interquartile range, 35.8-78.5 pg/mg) than among controls (n = 64) (total PBDE level, 35.8 pg/mg; interquartile range, 18.1-69.9 pg/mg) (P = .02). In the multivariable analysis adjusting for potential confounders, hypospadias was associated with a relative 48.2% (95% CI, 23.3%-65.4%) higher maternal level of total PBDE levels.

Infants with hypospadias had significantly higher total PBDE hair levels compared with their mothers: the medians were 78.2 pg/mg (range, 12.0-2090.6 pg/mg) and 51.4 pg/mg (range, 0-396.1 pg/mg), respectively (P < .05). In the entire study population, total PBDE levels in maternal hair did not correlate with infant hair levels (r^2 = 0.04, P > .90).

There were several differences between the groups in factors that may potentially affect PBDE levels. These included a shorter duration of breastfeeding, higher consumption of meat, and more common use of hair coloring agents among cases (**Table 2**).

Discussion

In our study, levels of exposure to the endocrine-disrupting PBDEs were significantly higher among mothers of children with hypospadias than among controls as indicated by hair measurements. The PBDE levels in hair are reflective of body burden and hence of in utero exposure.[11] These findings are

consistent with a case-control study[10] that found nonsignificant differences in the mean BDE-28, BDE-47, BDE-99, and BDE-100 in midpregnancy serum samples of mothers whose sons had hypospadias compared with controls.

The hypospadias cases and controls were recruited among mothers seeking advice and management in the same single tertiary pediatric hospital. Therefore, not surprisingly, their characteristics were similar.

In general, we detected higher total PBDE levels in the hair of infants relative to their mothers. This is in agreement with previous studies[15-18] reporting infants' PBDE hair levels to be up to 3 to 4 times higher than their mothers' because they spend more time on the ground and floor and their hand-to-mouth behavior puts them at greater risk of exposure to PBDEs in dust.

The age range of the children was dictated by the age at urological consultation between 3 and 18 months. Because the mothers had resided at the same address during pregnancy and up to the follow-up, their hair levels of PBDEs were stable over this period. The median age of the children in the 2 groups was similar. We observed a nonsignificantly higher PBDE level in women who consumed meat on a daily basis. The PBDEs are lipophilic and bioaccumulative and are more commonly detected in several foods, including oils, fats, fish, meats, and dairy products.[19] Consumption of contaminated food products can contribute to total body burden of PBDEs. North Americans consume more meat than fish in their daily diet.[20,21] A large-scale study[20] that collected data from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) found that the total intake of meat (poultry and red meats) was significantly associated with PBDE body burden of Americans as measured in serum. Our data on hair PBDE levels corroborate these findings, lending credibility to our results. Consistent with previous studies,[22,23] women who used hair coloring agents at least once during the year tested had a significantly lower median total PBDE level compared with those who did not use such agents. The process of hair coloring involves the use of oxidation with hydrogen peroxide in a basic solvent, which damages the structural integrity of the matrix, thus weakening the ability of hair to retain chemicals.[24] This means that the true difference in total PBDE levels in the hypospadias group was probably even higher than that detected in our study.

Hypospadias distribution by phenotypic subtype was similar in our study to that previously reported[25]: approximately 70% of cases were considered mild (anterior), while the remaining 30% were considered moderate (middle) or severe (posterior). This contributes to the generalizability of our results. Normal urethral closure, which occurs during the 8th to 14th weeks of gestation, involves a continuous process of ventral fusion in the proximal to distal direction.[26,27] This process requires fetal synthesis of testosterone, conversion to dihydrotestosterone (DHT), DHT binding to the androgen receptor, and appropriate androgen receptor signaling. Because hormones essentially regulate reproductive development, any disturbances along the hypothalamic-pituitary-gonadal axis pathway resulting in androgen deficiencies or any mutations in genes involved in development may lead to genitourinary birth defects. The PBDEs have been shown to affect the estrogen receptor signaling pathway, and one of the proposed disruption mechanisms is direct binding of hydroxylated PBDEs to estrogen receptors.[28]

Limitations

Potential limitations of this study should be acknowledged. Advanced maternal age at delivery, maternal type 1 and type 2 diabetes, use of assisted reproductive technology, and maternal progesterone use have been suggested as possible risk factors for hypospadias.[14] However, these potential confounders have been accounted for in our analysis. The use of maternal hair as a proxy for maternal body burden of PBDEs has to be addressed. In a previous study,[11] excellent correlation was shown between hair PBDE levels and visceral concentrations in rats. In rat samples, significant correlations were observed for BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-209 in rat hair, serum, liver, and fat across doses, with r values ranging from 0.803 to 0.988. A recent study[29] using maternal hair BPDE levels has shown a similar association, with higher levels of PBDEs among mothers who gave birth to infants with cryptorchidism. This further suggests the role of PBDEs as endocrine disrupters in the embryotoxic effects of male genitourinary organs. To our knowledge, this is the first study to have used maternal hair as a proxy for PBDE exposure during pregnancy comparing infants with hypospadias with infants without hypospadias.

While the hair of cases was cut by the research team, the control hair was cut by the parents per instructions. The possibility that parents may have cut the hair differently than the research staff has to be addressed. Because we recruited only women residing in the same household throughout pregnancy and follow-up, it is unlikely that small deviations in cutting techniques would change the findings because hair levels of PBDEs are stable under these circumstances.

Conclusions

The results of our study suggest that exposure level to these endocrine disruptors during gestation may have a role in the etiology of hypospadias. More research is needed to identify the safety threshold of PBDEs relative to this congenital malformation and the effects of specific PBDE congeners.

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