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Occupational Health

AHR gene-dioxin interactions and birthweight in the Seveso Second Generation Health Study

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Abstract

Background: 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) is proposed to interfere with fetal growth via altered activity of the aryl hydrocarbon receptor (protein: AHR; gene: *AHR*) pathway which regulates diverse biological and developmental processes including xenobiotic metabolism. Genetic variation in *AHR* is an important driver of susceptibility to low birthweight in children exposed to prenatal smoking, but less is known about these genetic interactions with TCDD, AHR's most potent xenobiotic ligand.
Methods: The Seveso Women's Health Study (SWHS), initiated in 1996, is a cohort of 981 Italian women exposed to TCDD from an industrial explosion in July 1976. We measured TCDD concentrations in maternal serum collected close to the time of the accident. In 2008 and 2014, we followed up the SWHS cohort and collected data on birth outcomes of SWHS women with post-accident pregnancies. We genotyped 19 single nucleotide polymorphisms (SNPs) in *AHR* among the 574 SWHS mothers.
Results: Among 901 singleton births, neither SNPs nor TCDD exposure alone were significantly associated with birthweight. However, we found six individual SNPs in *AHR* which adversely modified the association between maternal TCDD and birthweight, implicating gene-environment interaction. We saw an even stronger susceptibility to TCDD due to interaction when we examined the joint contribution of these SNPs in a risk allele score. These SNPs were all located in noncoding regions of *AHR*, particularly in proximity to the promoter.
Conclusions: This is the first study to demonstrate that genetic variation across the maternal *AHR* gene may shape fetal susceptibilities to TCDD exposure.

Key words: Dioxins, aryl hydrocarbon receptor, Seveso, birthweight, gene-environment, polymorphisms

Key Messages

- Some genetic subpopulations of *AHR*, a gene coding for a key transcription factor involved in xenobiotic metabolism, may be more susceptible to *in utero* TCDD exposure than others.
- We observed six polymorphisms in regulatory regions of *AHR*, particularly in proximity to the gene's promoter, that adversely modified the association between maternal TCDD exposure and child birthweight.
- Analyses using a risk allele score suggested that the combination of these risk variants may jointly influence susceptibility to dioxin.
- *AHR* polymorphisms and maternal TCDD levels were not significantly associated with birthweight when examined independently, supporting the presence of gene-environment interaction.

Introduction

In 1976, an industrial accident near Seveso Italy resulted in one of the highest residential exposures to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in history.[1–5] TCDD, a common by-product of industrial and combustive processes, is a highly lipophilic, persistent organic pollutant, and a known carcinogen[6] and endocrine disruptor.[7] The half-life of TCDD is relatively long, in the order of 7–10 years in humans.[8] TCDD has been shown to cross the placenta[9,10] and *in utero* exposure to TCDD and dioxin-like chemicals has been linked in animal studies to altered immune function, glucose regulation, steroidogenesis and neurobehavioural and bone development.[11–19]

Animal studies have also suggested that TCDD may impact fetal growth,[15,20–23] possibly via altered activity of the aryl hydrocarbon receptor (protein: AHR; gene: *AHR*), a nuclear receptor and transcription factor active in many tissues including the placenta.[24–29] Upon binding TCDD in the cell cytoplasm, AHR moves to the nucleus, where it induces expression of several xenobiotic metabolizing enzymes such as cytochrome P450s, and exhibits cross-talk with pathways of hormone synthesis.[30] In addition to detoxification, the AHR pathway regulates a myriad of biological processes related to development, cell growth, apoptosis and immune function.[31]

Of the epidemiological studies that have examined biological markers of maternal dioxin exposures and birthweight, three found no association,[32–34] another three found adverse associations[35–37] and three had adverse associations that did not reach statistical significance.[38–40] In the Seveso Women's Health Study (SWHS), a follow-up study of women exposed to high levels of TCDD from an industrial explosion in 1976, we previously reported a non-significant inverse association between serum TCDD concentrations and birthweight of first post-explosion births [adjusted-β = −47.7 g, 95% confidence interval (CI): −107.3, 11.9 for a 10-fold increase in serum TCDD concentration].[38,41] The lack of consistency across the literature examining the effects of TCDD on birthweight may be due to the wide variation in sample size, difference in exposure levels and, perhaps, genetic variation represented in these study populations.

Inter-strain and interspecies differences in AHR ligand-binding affinities suggest that genetic variation in *AHR* may influence susceptibility to TCDD.[42] Human evidence supporting this hypothesis is drawn from two Japanese studies that found that a polymorphism in maternal *AHR* (rs2066853) conferred significant reductions in birthweight in pregnant women who smoked cigarettes (components of cigarette smoke, such as benzo(a)pyrene, also bind AHR).[43] A more recent study of Japanese infants ($n = 421$), which examined the relationship between this single nucleotide polymorphism (SNP) and prenatal dioxins toxic equivalency (TEQ), reported no relationship with birthweight;[44] consideration of additional *AHR* SNPs in larger cohorts with higher exposures to specifically TCDD, the most potent compound of the TEQ, is warranted.

In the present analysis, we investigate whether maternal *AHR* gene variation modifies the association between maternal exposure levels of TCDD and birthweight in the children born after the Seveso explosion to mothers who participated in SWHS, a follow-up study of women living in Seveso, Italy, at the time of the accident.

Methods

Study population and procedures

In 1996, 20 years after the explosion, the Seveso Women's Health Study was initiated. Eligible women were aged 40 years or younger on 10 July 1976, resided in the most contaminated areas and had blood samples collected soon after the explosion. A total of 981women (80% of those eligible) participated.[5] In 2008 and 2014, we followed up these participants. Details of the study procedure for the 2008 and 2014 studies are described elsewhere.[45,46]

At each follow-up visit, women were interviewed in a private room at the Hospital of Desio by a nurse interviewer who was blinded to participant TCDD levels. Information was obtained on medical and reproductive history, with detailed information on each pregnancy and on demographic and lifestyle factors. Between 1976 and 2016, a total of 574 SWHS mothers reported 943 live births occurring after the 1976 accident. We obtained genetic information on 567 mothers (98.8%), corresponding with 929 births. Seven women (who had 14 live births) either did not consent to biobanking their blood specimens or did not have adequate amounts of blood specimen for DNA isolation. We excluded an additional 27 multiple births and one singleton with missing birthweight, leaving 901 singletons from 562 mothers for the primary analyses (Table 1). The study was approved by the institutional review boards of the participating institutions and we obtained written informed consent from all mothers before participation.

Table 1. Descriptive statistics of mothers with genetic data in the SWHS, 1996–2014

Characteristic	*n*	(%)	1976 serum TCDD (ppt) median (IQR)	
Total women	562	(100.0)	61.3	(29.0, 163.0)
Total live births	901	(100.0)		
Age at explosion (years)				
0–10	164	(29.2)	157.5	(51.8, 320.5)
11–20	239	(42.5)	53.4	(25.4, 105.0)
21–30	139	(24.7)	41.3	(22.0, 80.9)
31–40	20	(3.6)	39.0	(27.7, 66.8)
Menarche status at explosion				
Premenarche	211	(37.5)	131.0	(50.5, 286.0)
Postmenarche	351	(62.5)	44.4	(22.5, 86.7)
Pre-explosion parity				
0	451	(80.3)	71.1	(31.7, 192.0)
1	71	(12.6)	36.6	(21.1, 70.4)
≥2	40	(7.1)	35.6	(24.5, 69.1)
Maternal education at last follow-up				
<Required	105	(18.7)	42.5	(23.5, 73.4)
Required/high school	432	(76.9)	64.7	(30.0, 188.0)
University	25	(4.5)	75.7	(30.2, 180.0)
Age at pregnancy (years)				
<25	152	(16.9)	46.4	(21.5, 103.5)
25–29	309	(34.3)	55.5	(28.6, 131.0)
30–34	270	(30.0)	67.2	(29.9, 187.0)
≥35	170	(18.9)	64.3	(35.0, 176.0)
Smoking during pregnancy				
No	813	(90.2)	61.2	(29.3, 164.0)
Yes	88	(9.8)	50.0	(20.7, 102.0)
Weight gain during pregnancy (kg)[a]				
<10	190	(21.1)	67.9	(38.2, 141.0)
10–14	421	(46.7)	54.3	(26.7, 136.0)
15–19	171	(19.0)	62.8	(22.0, 214.0)
≥20	96	(10.7)	70.9	(30.0, 204.0)
Low birthweight (<2500 g)				
No	844	(93.7)	60.4	(28.4, 157.0)
Yes	57	(6.3)	64.7	(31.7, 131.0)
Preterm (<37 weeks)				
No	839	(93.1)	60.4	(28.4, 156.0)
Yes	62	(6.9)	76.2	(33.2, 206.0)
Infant sex				
Male	473	(52.5)	55.0	(27.2, 130.0)
Female	428	(47.5)	67.0	(29.9, 179.5)

[a]Missing data on pregnancy weight gain for 23 live births.

Outcome assessment

Birthweights and gestational duration were based on maternal report. In a small sample ($n = 139$), we confirmed reported birthweights using hospital records. These data indicated that women slightly over-reported birthweight, by 22 g on average, but this was non-differential by TCDD exposure.[41]

TCDD analysis

TCDD was measured in archived maternal serum samples collected near the time of the explosion by high-resolution gas chromatography/high-resolution mass spectrometry methods at the Centers for Disease Control and Prevention (CDC).[47,48] Details of the serum sample selection and TCDD concentrations are presented elsewhere.[5,49] Before statistical analysis, maternal serum TCDD levels were adjusted for blood lipid concentrations by dividing TCDD on a whole-weight basis by total serum lipid content, estimated from measurements of triglycerides and total cholesterol.[50] Serum TCDD levels were reported in picograms per gram lipid or parts per trillion (ppt). The median lipid-adjusted limit of detection (LOD) for the full population was 18.8 ppt. Quantifiable results less than the method detection limits were reported when observed. Otherwise, samples below the LOD (9.4% in the full cohort) were assigned a value equal to one-half of the LOD.[51] By considering the 1976 TCDD levels, we examine the hypothesis that the mother's primary dose permanently altered her reproductive system or oocytes, possibly resulting in persistent epigenetic changes that could impact fetal growth.[52]

SNP selection and genotyping

We used the HapMap browser[53] in the Caucasian population of European descent (CEPH) and the 1000 Genomes Toscani in the Italia population (TSI), to choose SNPs from *AHR* which were expected to have minor allele frequencies greater than 5% in our Italian Caucasian sample. In cases where SNPs were in linkage disequilibrium, we sought an appropriate tagging SNP representative of this group of co-varying SNPs, to conserve study power and resources. These candidate SNPs were further pared down to those with known or suspected functional relevance as reported in the literature or as listed in the open access Regulome SNP database (Stanford University).[54] We particularly prioritized SNPs linked to xenobiotic exposure and fetal development, though we considered *AHR* SNPs associated with any health outcome. Our final genotyping assay comprised 18 SNPs across 50 kb of the *AHR* gene as well as one SNP in *AHR*'s upstream intergenic region. Location of the SNPs and their physical distribution are presented in Table 2 and Supplementary Figure 1, available as Supplementary data at *IJE* online.

Maternal DNA for genotyping was isolated from archived blood using a QIAamp Blood DNA Maxi kit (QIAGEN, Valencia, CA, USA) with some modification as previously described.[55] High-throughput genotyping of selected SNPs was performed using the multiplex platform

Table 2. Description of *AHR* SNPs genotyped in SWHS mothers, 1996-2014

SNP	Location (chr: bp)	Alleles	MAF (%)	Functional consequence
rs6968865	7: 17 247 645	T/A	41.2	Upstream, intergenic region
rs3757824	7: 17 296 411	A/G	18.5	intron, regulatory region variant
rs10249788	7: 17 298 523	C/T	12.4	intron, regulatory region variant
rs713150	7: 17 300 533	C/G	25.7	intron, regulatory region variant
rs17722841	7: 17 303 970	G/C	14.2	intron
rs2282885	7: 17 305 990	T/C	35.3	intron
rs3802083	7: 17 309 283	G/A	38.9	intron
rs17779352	7: 17 310 002	T/C	8.1	exon, synonymous variant
rs1476080	7: 17 318 249	C/A	37.2	intron, regulatory region variant
rs2237297	7: 17 319 970	C/T	9.3	intron
rs17137566	7: 17 320 897	T/C	15.7	intron
rs4236290	7: 17 323 944	T/C	12.0	intron
rs6960165	7: 17 328 461	A/G	24.1	intron
rs2158041	7: 17 328 796	G/A	23.8	intron
rs3802082	7: 17 330 557	T/A	15.3	intron
rs7811989	7: 17 331 739	G/A	27.3	intron
rs2066853	7: 17 339 486	G/A	9.8	exon, missense variant
rs2040623	7: 17 341 038	T/G	19.4	intron
rs2106728	7: 17 342 116	A/G	35.2	intron

iPLEX (Sequenom, San Diego, CA) at the Genomics Center at the University of Minnesota. The main steps involved multiplex polymerase chain reaction (PCR), single-base primer extension and finally mass spectrometry to determine the genotype. Quality assurance procedures for genotyping included assessment of randomly distributed blank samples and duplicates of participant samples. Call rates were above 98% for all 19 SNPs. Samples with lower success rates were resolved with additional genotyping. All genotype distributions were in accordance with Hardy-Weinberg equilibrium assumptions.

Statistical analyses

TCDD measures were analysed as a $\log_{10}$-transformed, continuous variable. We considered those covariates that were used in previous reports of birthweight in this cohort or identified a priori as confounders between *in utero* dioxin and birthweight in a directed acyclic graph (DAG) (Supplementary Figure 2, available as Supplementary data at *IJE* online). These covariates included maternal age at pregnancy (continuous, years), year of pregnancy, smoking during pregnancy (yes/no), parity (0, 1 or >2 pregnancies), maternal height (cm), pre-accident history of delivering a low birthweight infant, child sex and gestational age (maternal report in weeks). Model parameters were estimated with use of generalized estimating equations (GEE) with exchangeable correlation of the variance structure to account for siblings.[56] We first re-evaluated the association between TCDD and birthweight in this analytical sample for comparison with our previously reported result of an adverse but non-significant relationship.[38,41]

Before model fitting, linkage disequilibrium between SNPs was assessed with r^2 and observed SNP distributions and correlations were compared with those in the TSI of the 1000 Genomes. Genotype analyses considered two penetrance models with reasonable power given our sample sizes: (i) additive allelic penetrance (i.e. groups inheriting 0, 1 or 2 minor alleles at each SNP); and (ii) dominant penetrance (i.e. inheriting 0 vs at least 1 minor allele). In models assuming dominant penetrance of the minor 'variant' allele, the genotype at each SNP was analysed as an indicator variable. In separate analyses assuming additive allelic penetrance, the genotype was analysed categorically and if a dose-response pattern was observed with the increasing number of variant alleles (0, 1, 2), an ordinal variable was used. In both scenarios, the reference group was the genotype with 0 variant alleles.

To evaluate the main effects of each SNP, we fitted multivariate models of birthweight regressed on maternal genotype controlling for the above covariates and TCDD levels. We then considered crude and adjusted models of interaction between maternal genotype and TCDD on birthweight by constructing a cross-product term of genotype and TCDD (i.e. $SNP_{Maternal}$ x TCDD). TCDD was considered as a continuous variable ($\log_{10}$-transformed) and as a categorized variable to explore GxE interaction allowing for nonlinear associations between TCDD and birthweight. The lowest TCDD category with no variant alleles served as the reference group. We examined cumulative associations across SNPs through calculation of a genetic risk allele score by summing, for each individual, the number of risk alleles (1 point if $\geq$1 risk allele present) across all SNPs that were significant ($p_{int} < 0.1$) in the individual SNP models and, in a sensitivity analysis, by weighting the SNP components by the size of their coefficients in the individual models. Genotypes associated with lowered birthweight in interaction with TCDD were designated as the 'risk' genotype and summed across all SNPs included in the score. The score was then examined in models with and without interaction with maternal TCDD levels. The score, a common method that conserves study power and potentially gives insight into underlying biological mechanisms, was examined as a continuous variable.[57]

Multiple births were excluded from the initial models, but were added back in a sensitivity analysis. Additional sensitivity analyses considered models excluding preterm (<37 weeks' gestation)[58] births and models restricted to the mother's first birth after the explosion, using robust regression. All analyses were performed in Stata 13 (StataCorp LP, College Station, TX) with the exception of Hardy-Weinberg and linkage disequilibrium statistics and the Benjamini-Hochberg false-discovery rate (FDR) correction for multiple comparisons which were performed using the *SNP assoc* and *p.adjust* packages in R, version 3.4.3,[59] respectively.[60,61]

Results

Descriptive statistics of study sample

Maternal 1976 serum TCDD concentrations across demographic and pregnancy characteristics are presented in Table 1. Mothers in this sample averaged 14.7 (±7.7) years of age at the time of the accident. The average maternal age at pregnancy across all 901 births was 29.6 years (±5.3) and 20% of women had a history of pregnancy preceding the accident. The majority of mothers (81.4%) had educational attainment beyond the compulsory education in Italy. Women who were younger in 1976 tended to have higher levels of serum TCDD, higher levels of educational attainment at follow-up and were slightly older at time of pregnancy than SWHS women who were adults at the time of the accident, a pattern described in previous studies of

the SWHS.[4,49] The median TCDD level in maternal serum was 61.3 ppt [interquartile range (IQR): 29.0 163.0] in 1976. The mothers in our analytical sample ($n = 562$) did not differ in sociodemographic characteristics or medical history from the sample of all mothers with eligible children in the Seveso Second Generation Health Study ($n = 574$).

Among the 901 singleton births, the average birthweight was 3264 (+/−526) g. The number of low birthweight (<2500 g)[62] and preterm infants (<37 weeks)[58] was 57 (6.3%) and 62 (6.9%), respectively. Without considering genotype, a 10-fold increase in 1976 serum TCDD was associated with a non-significant reduction in birthweight ($\beta = -33.65$, 95% CI: -85.11, 17.80; $P = 0.20$), adjusting for covariates. These findings are similar to what has been observed in previous studies in this cohort, although marginally different due to the slightly different sample who had DNA available for analysis.[38,41,46]

Minor allele frequencies for the 19 SNPs, shown in Table 2, ranged from 8.1% (rs17779352) to 41.2% (rs6968865). The allelic frequencies in the SWHS population were similar to those observed in the 1000 Genomes TSI.[63] Linkage disequilibrium was relatively low among the *AHR* SNPs, with 5% of pairwise LD comparisons exceeding an $r^2 > 0.8$ (see Supplementary Figure 1, available as Supplementary data at *IJE* online, for LD Plot).

Associations between SNPs and birthweight

We observed no association between any of the 19 SNPs in *AHR* on birthweight (Supplementary Table 1, available as Supplementary data at *IJE* online). The only SNP with a suggestive association was rs2066853. Under an additive model of penetrance, each additional risk allele (A) was associated with a -62 g reduction in birthweight relative to the wild-type GG mothers ($P_{trend} = 0.085$). However, this trend was based on seven mothers with the AA genotype, and therefore a dominant model (adjusted β = -67.42, 95% CI: -145.13, 10.30; $P = 0.089$) was used in subsequent sensitivity analyses to conserve power (Figure 1). The only other SNP (rs2237297) that was notably associated with birthweight (adjusted β = -58.04, 95% CI: −137.49, 21.42; $P = 0.152$) is in high LD with rs2066853 ($r^2 = 0.93$) in this population.

Gene-environment interactions

In crude models, the variant alleles of four of the 19 SNPs (rs6968865, rs3757824, rs10249788, rs2040623) exhibited interaction with 1976 maternal TCDD levels on birthweight (Supplementary Table 2, available as Supplementary data at *IJE* online). When these models were adjusted for covariates,

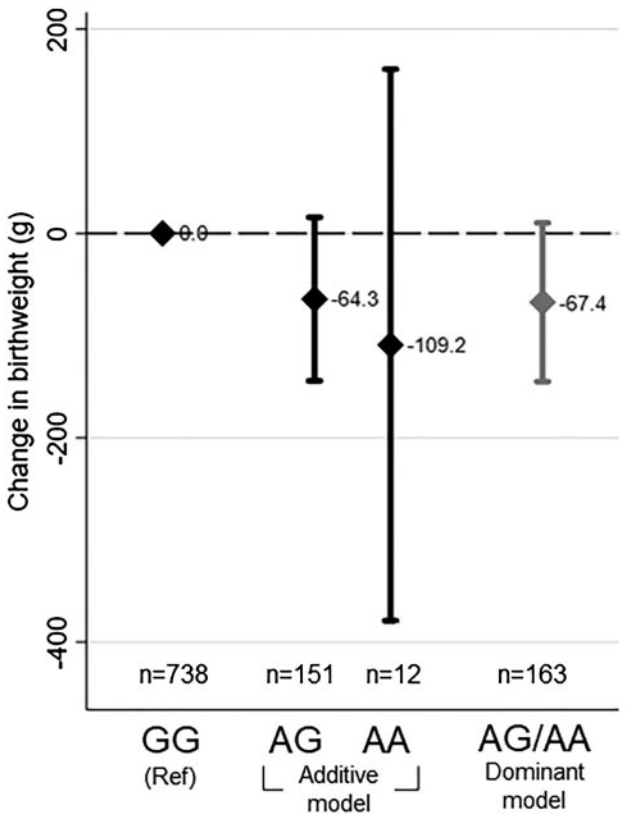

Figure 1. Main effects of maternal *AHR* SNP rs2066853 on birthweight under dominant and additive genetic models, SWHS, 1996-2014. Models adjusted for gestational age (weeks), history of low birthweight, maternal age at pregnancy, parity, maternal height, maternal smoking, birth year, child sex.

effect sizes were attenuated, but these four SNPs and two others (rs2282885, rs2106728) suggested gene-environment interaction (Table 3). Further, these six interactive SNPs were clustered around the promoter and first and last introns of *AHR*. We observed five SNPs (rs6968865, rs3757824, rs10249788, rs3802082, rs2040623) for which presence of the variant allele in mothers was associated with strong adverse associations between TCDD and birthweight relative to the reference group of homozygous wild-type mothers. For example, a 10-fold increase in TCDD was associated with -149.09 g (95%CI: -252.34, -45.83; $P = 0.015$) and −1.76 g (95%CI: -59.89, 56.37; $P = 0.953$) reductions in birthweight among children of mothers with the variant (CC/CT) and wild-type (TT) rs10249788 genotypes, respectively. This interactive association was particularly notable at the low and high ends of TCDD exposure where, continuing with the example of rs10249788, the variant allele was protective at low TCDD levels but crossed to adverse at higher TCDD exposures (Figure 2). We observed this pattern of GxE interaction, whereby the risk allele conferred fetal hypersusceptibility to TCDD, for three other SNPs (rs6968865, rs3757824, rs2040623). For two SNPs, rs2282885 and rs2106728, the variant allele exhibited a

Table 3. GEE regression models of 1976 log10TCDD exposure on child birthweight stratified by genotype at each locus using dominant model of inheritance, SWHS, 1996–2014

		0 minor alleles			1 or 2 minor alleles				
SNP	Genetic model	β_{adj} (95%CI)		P	β_{adj} (95%CI)		P	P_{int}	FDR
rs6968865	AA + TA vs TT	23.23	(−66.93, 113.40)	0.614	−62.34	(−124.58, -0.10)	0.050*	0.034	0.17
rs3757824	GG + AG vs AA	18.44	(−45.40, 82.28)	0.571	−126.75	(−210.00, -43.49)	0.003**	0.021	0.14
rs10249788	TT + CT vs CC	−1.76	(−59.89, 56.37)	0.953	−149.09	(−252.34, -45.83)	0.005**	0.015	0.14
rs713150	GG + CG vs CC	−46.10	(−119.31, 27.11)	0.217	−2.95	(−78.30, 72.40)	0.939	0.741	0.80
rs17722841	AA + GA vs GG	−50.31	(−109.15, 8.53)	0.094	−7.97	(−111.77, 95.83)	0.880	0.704	0.80
rs2282885	CC + TC vs TT	−86.66	(−164.08, -9.24)	0.028*	10.86	(−57.15, 78.87)	0.754	0.067	0.22
rs3802083	AA + GA vs GG	−36.32	(−131.41, 58.78)	0.454	−35.05	(−95.96, 25.86)	0.259	0.654	0.80
rs17779352	CC + TC vs TT	−37.35	(−90.59, 15.90)	0.169	15.29	(−150.22, 180.81)	0.856	0.618	0.80
rs1476080	AA + CA vs CC	−14.00	(−103.84, 75.83)	0.760	−46.64	(−109.23, 15.96)	0.144	0.360	0.62
rs2237297	TT + CT vs CC	−23.65	(−80.81, 33.51)	0.417	−79.12	(−191.05, 32.81)	0.166	0.369	0.62
rs17137566	CC + TC vs TT	−14.76	(−76.99, 47.46)	0.642	−84.39	(−172.78, 4.00)	0.061	0.409	0.62
rs4236290	CC + TC vs TT	−11.52	(−70.17, 47.13)	0.700	−103.61	(−210.38, 3.16)	0.057	0.161	0.45
rs6960165	GG + AG vs AA	−48.01	(−118.69, 22.67)	0.183	−9.59	(−83.44, 64.25)	0.799	0.772	0.80
rs2158041	AA + GA vs GG	−47.28	(−117.89, 23.32)	0.189	−16.02	(−90.12, 58.08)	0.672	0.861	0.85
rs3802082	AA + TA vs TT	4.04	(−59.70, 67.79)	0.901	−88.91	(−174.30, -3.53)	0.041*	0.221	0.53
rs7811989	AA + GA vs GG	−20.32	(−94.72, 54.09)	0.593	−46.31	(−117.47, 24.85)	0.202	0.370	0.62
rs2066853	AA + GA vs GG	−26.83	(−83.60, 29.94)	0.354	−59.57	(−175.78, 56.65)	0.315	0.416	0.62
rs2040623	GG + TG vs TT	36.74	(−29.19, 102.68)	0.275	−116.13	(−197.27, -34.99)	0.005**	0.021	0.14
rs2106728	GG + AG vs AA	−80.23	(−158.02, -2.45)	0.043*	6.88	(−60.90, 74.66)	0.842	0.061	0.22

Models adjusted for gestational age (weeks), history of low birthweight, maternal age at pregnancy, parity, maternal height, maternal smoking, birth year, child sex. $P_{interaction}$ from model with an interaction term between log10TCDD and genotype.

*P-value significant at alpha <0.05.

**P-value significant at alpha <0.01.

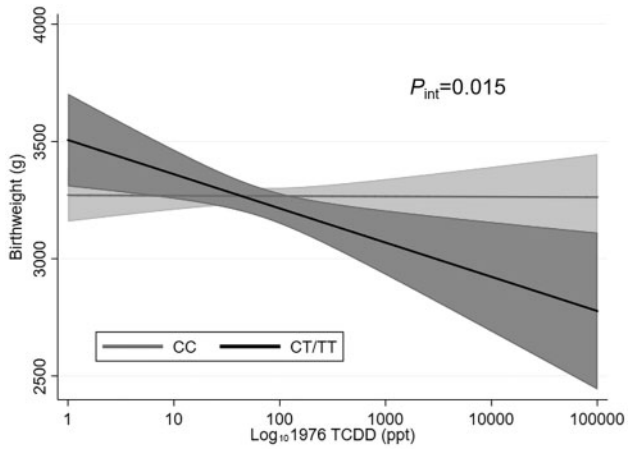

Figure 2. Interactive effects of maternal rs10249788*Maternal log10TCDD on birthweight under a dominant genetic model, SWHS, 1996–2014. Models adjusted for gestational age (weeks), history of low birthweight, maternal age at pregnancy, parity, maternal height, maternal smoking, birth year, child sex.

protective influence on the association between TCDD and birthweight.

We developed a genetic risk allele score to explore the joint association of multiple *AHR* SNPs in interaction with TCDD on birthweight. We considered the six SNPs that were interactive with TCDD. Two of these SNPs (rs2040623 and rs2106728), located at the end of the *AHR* gene, were excluded from the score as they were in high LD with two other SNPs already in the score, rs2282885 and rs3757824 (r^2 >0.84), respectively, and did not contribute appreciably to the association or variance of the models. Thus, the final risk allele score was based on the four SNPs located in the intergenic and promoter regions only (rs6968865, rs3757824, rs10249788, rs2282885). Considered independently of dioxin interaction, the risk allele score had no association with birthweight (adjusted β = -3.84, 95% CI: -27.20, 19.53; P = 0.75). However, the score adversely modified the association of TCDD and birthweight (P_{int} = 0.001) (Figure 3). For example, a 10-fold increase in TCDD was not associated with birthweight among 199 children whose mothers had the lowest risk allele score (adjusted β = −2.41, 95% CI: -119.65, 114.83; P = 0.97). In contrast, TCDD was associated with significant decreases in birthweight in children of mothers with scores greater than 1, with the largest reductions observed when mothers carried all four risk genotypes (adjusted β = -192.93, 95% CI: −314.85, −71.01; P = 0.002).

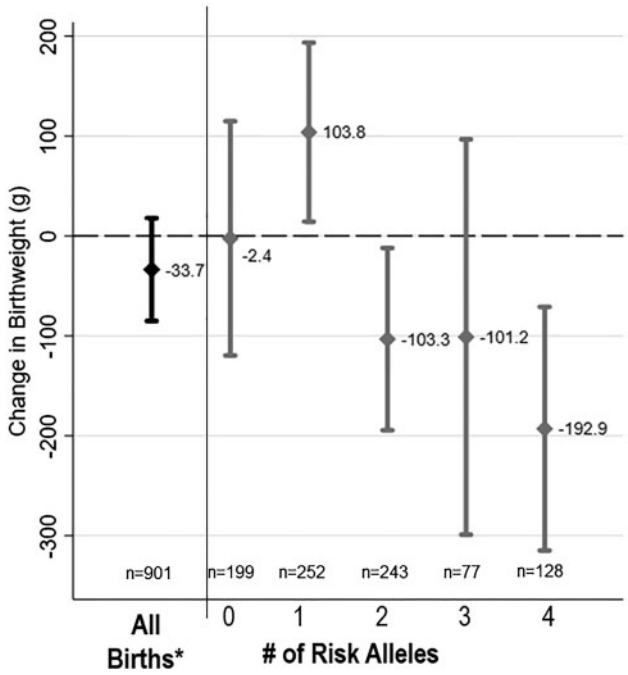

Figure 3. Association of maternal TCDD levels on birthweight, stratified by maternal risk allele score. Score based on four SNPs in *AHR*, SWHS, 1996–2014. Models adjusted for gestational age (weeks), history of low birthweight, maternal age at pregnancy, parity, maternal height, maternal smoking, birth year, child sex. *Model without consideration of risk allele score.

In sensitivity analyses, excluding preterm births did not appreciably change the findings of interaction between the *AHR* gene and TCDD exposure (Supplementary Table 3, available as Supplementary data at *IJE* online) nor did including multiple births (Supplementary Table 4, available as Supplementary data at *IJE* online). When we restricted to the first post-explosion birth, the GxE coefficients were also comparable but with diminished statistical precision due to the smaller sample sizes (Supplementary Table 5, available as Supplementary data at *IJE* online).When we accounted for multiple testing with FDR adjustment, the significance of the interactions for individual SNPs was attenuated (Table 3); nevertheless, the GxE interaction by the risk allele score, which mitigates issues of multiple-testing, remained statistically significant.

Discussion

In our previous research,[38,41] we found that birthweight was suggestively related to maternal TCDD blood concentrations and hypothesized that there may be a susceptible subgroup that is at higher risk. In this study, we examined whether variation in maternal *AHR* genotypes, coding for a transcription factor with diverse functions including dioxin metabolism, could explain heterogeneity in the effects of *in utero* dioxin exposure on birthweight. We found interactions between maternal serum TCDD levels and six SNPs in *AHR*'s regulatory regions, particularly in proximity to the gene's promoter. We observed an even stronger adverse interaction when we examined the joint contribution of these SNPs via a risk allele score. These novel results demonstrate for the first time an interactive association between *AHR* genetics and TCDD exposure on birthweight, supporting the existence of susceptible genetic subgroups, and add to previous evidence that variation in maternal *AHR* in interaction with smoking may influence fetal growth.[43,64,65] The only extant report of maternal *AHR* genetics (specifically, a single SNP, rs2066853) on fetal susceptibility to dioxins suggested no interaction;[44] our study, the first report in a population of European ancestry, builds upon this work by examining additional SNPs across the *AHR* gene and interaction across a wider distribution of TCDD in a population with background exposures to other dioxin-like compounds. Average reductions in birthweight (60–140 g) associated with TCDD in our study were in some cases as large as those reported among maternal smokers within high-risk genotypes in previous studies.[43,64,65] Susceptibility to TCDD culminated in an average 192-g reduction in birthweight among children of mothers carrying all four of the highest risk genotypes.

This finding is noteworthy, given that the independent associations of neither TCDD nor maternal *AHR* genotypes with birthweight were significant (though as we previously noted, the relationship between maternal TCDD and birthweight has trended in an adverse direction in earlier analyses). Specifically, we did not observe associations between 19 *AHR* SNPs and birthweight, though the variant allele at rs2066853 was associated with a large but statistically non-significant weight reduction. This lack of a relationship is consistent with what is known from genome-wide association studies (GWAS) of birthweight; AHR activity is integral to fetal development, but no variants have been linked to altered fetal growth in GWAS.[66,67] Nevertheless, previous GWAS studies of birthweight have only considered child's genetics and not maternal genetics, as examined here. This affirms the methodological caution advanced by Humblet *et al.*[68] that only testing for interaction among marginally significant SNPs may screen out and miss potentially important interactive loci in epidemiological studies.

The SNPs in the *AHR* risk allele score in our study may be important for development and toxicant metabolism. Previous experimental and epidemiological research indicate that the three SNPs in proximity to *AHR*'s promoter region may influence expression of the *AHR* gene,[69,70] suggesting a possible biological mechanism for the interaction we observed. For example rs6968865,

upstream of *AHR*, is a documented expression quantitative trait locus (eQTL) associated with expression of *AHR* in pancreas tissue (Genotype-Tissue Expression GTex Project, Broad Institute). The minor allele (T) of rs10249788, a SNP in *AHR*'s promoter, has been associated with higher expression of *AHR* than the C allele in human blood,[70] and downstream genes such as interleukin factors may be more upregulated in the presence of the TT genotype, independently of dioxin exposure.[71] A possible biological explanation for this expression difference is offered by a study in human endometrial cells, which observed that nuclear factor 1-C (NF1C), a suppressor of *AHR* expression, preferentially bound to promoters with the C-allele compared with the T-allele at rs10249788.[72] To the best of our knowledge, no studies on *AHR* regulation by rs3757824, another significant promoter SNP in our study that is located about 2 kb away from rs10249788, have been published, but the heterozygous genotype of this locus has been linked to higher risk of cryptorchidism in Italian boys,[73] suggesting a role in fetal development.

The most widely studied *AHR* variant in humans is rs2066853, an Arg554Lys missense mutation in exon 10, the codon site for *AHR*'s transactivation domain (TAD). Insight into the possible biological mechanism of rs2066853 is offered by a study that observed significantly higher mRNA levels of *AHR* in human lymphocytes with the wild-type genotype compared with the homozygote variant, Lys554Lys (AA).[74] However, functional studies of the polymorphism linking variation at rs2066853 to altered *CYP1A1* or *AHR* expression are mostly inconclusive.[75,76] Though this SNP had the largest main effect on birthweight in our study, the association was of borderline significance and we did not observe interaction of this SNP with dioxin exposure. This finding is consistent with a recent study in a Japanese cohort, which also did not report evidence of interaction between this SNP and dioxins/TEQ on birthweight.[44] However, in this same Japanese cohort, rs2066853 was associated with circulating levels of dioxins in pregnant mothers, suggesting that rs2066853 may still be related to efficiency of dioxin metabolism.[77]

To date, there is limited evidence on the functional relevance of rs2106728, found in the gene's last exon, on *AHR* activity but one study reported a large but insignificant association of this SNP with endometriosis in Japanese women.[78] This SNP and others could be worthy of further investigation in the SWHS, where dioxin exposure was observed to be non-significantly associated with a doubling of endometriosis risk.[79]

There are several possible pathways by which maternal xenobiotic-metabolizing genes such as *AHR* can alter the intrauterine environment and affect birthweight; for example, their activity contributes to xenobiotic levels in maternal-fetal circulation, placental function, endocrine regulation, and/or indirectly through the fetus's detoxification capacity inherited from the mother's alleles.[80] The relative contributions of these pathways are difficult to disentangle, but our study corroborates previous reports that maternal variation in other detoxifying proteins, particularly in the promoter region, may influence fetal susceptibility to toxicants.[81,82] For example, we previously found that maternal and child promoter variation in PON1, another multifunctional, detoxifying gene, has been linked to subsequent expression and detoxifying activity of organophosphate pesticides[83] and possibly modifies the relationship between prenatal organophosphate exposure and neurodevelopment.[84,85] However, whether *AHR* exhibits analogous genetic-epigenetic regulation at the site of the promoter and the contribution of the child's genetics is not yet known.

This study has several notable strengths, including the long follow-up of the SWHS through reproductive years, and one of the largest sample sizes among analyses examining GxE with *AHR*. The wide exposure distribution of TCDD in our study population also allowed for higher resolution of significant gene-dioxin interactions, particularly at the low and high ends of exposure where the interaction was most prominent. In addition, a candidate gene approach focusing on the *AHR* gene, where the biological plausibility is strong, conserved study power by limiting the number of multiple tests. Last, the genetic homogeneity in the SWHS limits confounding by population stratification.

Our study has several limitations. First, this is the earliest report examining this particular GxE association in a Caucasian population, and its generalizability to other ancestral populations is uncertain and warrants replication in additional cohorts. Second, though its potency for dioxin metabolism has been widely studied, AHR is just one player in a complex network of genes and proteins that mediate dioxin toxicity. Further, we only considered 19 main *AHR* SNPs among approximately 125 SNPs with >1% MAF across human populations, and we prioritized those evidencing functional significance for fetal development or detoxification. Third, in the interest of conserving study power, we used a dominant model of inheritance for all the risk alleles and an additive count of risk alleles across SNPs in composing the risk allele score on birthweight, assumptions which may not accurately reflect underlying biology. Future studies will investigate gene-dioxin interactions with respect to additional outcomes in the Seveso Women's Health Study and in the Seveso Second

Generation and the roles of other genes in the AHR pathway as well as the child's genotype.

Conclusions

This is one of the first studies to evaluate gene-dioxin interactions in a large, homogeneous cohort with a broad range of exposure levels. We used the prospective study design of the SWHS, high-quality TCDD measurements collected close to the time of the exposure, and an assessment of multiple polymorphisms across the *AHR* gene. We found six SNPs in maternal *AHR* that significantly potentiated the adverse relationship between maternal TCDD concentrations and child birthweight. *AHR* genetics may explain variation in human sensitivity to dioxin exposure, particularly among the offspring of TCDD-exposed mothers. Replication in additional cohorts or confirmation in mechanistic studies is warranted.

Supplementary Data

Supplementary data are available at *IJE* online.

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