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Associations of polygenic risk score and polybrominated biphenyl exposure on age at menarche in the Michigan polybrominated biphenyl registry

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Bachelor of General Studies
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2016

Faculty Thesis Advisor: Michele Marcus, PhD, MPH

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Rollins School of Public Health of Emory University
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Abstract

Associations of polygenic risk score and polybrominated biphenyl exposure on age at menarche in the Michigan polybrominated biphenyl registry

By Leah Moubadder

Numerous small nucleotides polymorphisms and endocrine disrupting chemicals have been associated with changes in the timing of menarche – a complex reproductive event – but, little is known about their interactions. We examined the direct associations of exposure to polybrominated biphenyl (PBB) exposure, an endocrine disruptor, and a previously established polygenic risk score (PRS) with age at menarche and the role of gene-environment interactions. Data from the PBB registry were analyzed (n=219). Age at menarche was obtained by self-reported through a health history questionnaire. We measured PBB levels from whole blood and peripheral blood samples were genotyped and imputed. Linear regression models were used to test the association between PRS and age at menarche, PBB and age at menarche, and investigate effect modification. PRS was positively associated with age at menarche among the total cohort but did not reach statistical significance. PBB was positively associated with age menarche, but only among those exposed to PBB in childhood, and was independent of PRS. Finally, we observed no evidence of gene-environment interaction between PBB exposure and PRS. These data lend support to the hypothesis that age at menarche is a polygenic trait and that timing of menarche is influenced by both environmental and genetic factors.

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Leah Moubadder

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CHAPTER 1: BACKGROUND

Age at menarche from a life course perspective and as a complex health event

Menarche is a notable health event that signifies the beginning of the reproductive cycle through the onset of oocyte functioning (13). There is a mounting body of research linking the timing of menarche with the risk of certain chronic conditions later in life (Table 1), thus highlighting the significance of this reproductive event beyond its primary function. However, how timing of menarche is predicted is not fully understood; which may be due, at least in part, to the combined genetic and environmental effects contributing to age at menarche. This notion is heavily supported by epidemiologic studies that have identified ethnic-group variations in declining age at menarche (14, 15) (although age at menarche has been declining in industrialized countries since the mid-1900s (16)) and the steady decrease in the correlation between mother-daughter age at menarche over time (15). However, there is a paucity of research examining the joint associations of environmental and genetic factors on timing of menarche.

Table 1. Health risks associated with early or late timing of menarche

Early	Late
Breast cancer (1)	Cardiovascular disease (2, 3)
Endometrial cancer (4)	Osteoporosis (5)
Gestational diabetes (6)	
Type II diabetes (7)	
Accelerated biological aging (8)	
Cardiovascular disease (2, 3)	
Multiple sclerosis (9)	
Asthma (10)	
Depression (11)	
Obesity (12)	

Endocrine disruptors and age at menarche

While improved public health and nutrition in the last century are to credit for a portion of the secular trends in timing of menarche, prior research also underscores the introduction of endocrine-disrupting compounds (ECDs) – a heterogenous group of chemicals that interfere with hormone function (17). Animal models have provided mechanistic evidence to suggest that menarche is sensitive to exposure to estrogenic or antiandrogenic compounds, especially during prenatal and peripubertal periods (16). Additionally, epidemiologic research has evaluated the effect of numerous EDCs on timing of menarche.

Table 2. Current epidemiologic evidence on the associations between endocrine-disrupting chemicals and age at menarche

Study	Sample size	Exposure(s)	Outcome metric	Effect size (95% CI)
Blank et al. (18)	327	Polybrominated biphenyl *in utero*	Early age at menarche	MR_a=3.4 (1.3-9.0)
Warner et al. (19)	282	Dioxin	Age at menarche	HR_b=0.05 (0.83-1.09)
Chen et al. (20)	271	Polybrominated diphenyl ethers	Age at menarche	β= -0.10 (-0.33- 0.13)
Buttke et al. (21)	440	Environmental phenols	Early age at menarche	HR_b=1.09 (1.01-1.19)
McGuinn et al. (22)	987	Bisphenol A	Early age at menarche	OR_c=0.57 (0.30-1.80)
Binder et al. (23)	200	2,5-dichlorophenol	Early age at menarche	HR_b=1.13 (1.01-1.27)
		Benzophenone-3		HR_b=1.17 (1.06-1.29)
Attfield et al.(24)	1257	Polybrominated diphenyl ethers	Early age at menarche	HR_b=0.75 (0.58-0.97)
		Polychlorinated biphenyl		HR_b=0.67 (0.5-0.89)
		Organochlorine pesticide		HR_b=0.66 (0.50-0.89)

Christensen et al.(25)	218	Perfluorooctanoate *in utero*	Early age at menarche	OR_c=1.25 (0.61-1.68)
		Perfluorooctane sulfonate *in utero*		OR_c=0.85 (0.53-1.36)

[a]Menarche ratio
[b]Hazard ratio
[c]Odds ratio

As summarized in Table 2, the effects of EDCs are relatively inconsistent – with chemical exposures associated with earlier and later ages at menarche. However, this is to be expected, given the heterogeneity of these compounds. Future research is necessary to build more research on individual compounds, replicate these findings in different populations, and in larger sample sizes.

Genetic factors and age at menarche

Reminiscent to environmental exposures, there is a growing body of research lending support to genetic components of timing of menarche. Familial and twin studies have provided particularly strong evidence; where the estimated heritability of age at menarche ranges from 50-70% (26-28). Moreover, with the relatively recent advancements in large-scale genomic research, genome-wide association studies (GWAS) have discovered numerous genetic variants which predict timing at menarche.

Most notably, a recent meta-analysis comprising of 40 studies utilized GWAS data from 23andMe, UK Biobank and the ReproGen consortium to develop a polygenic risk score (PRS) – a genetic score based on a combination of statistically independent small nucleotide polymorphisms (SNPs) that are associated with the outcome of interest – to predict age at

menarche. Investigators derived 389 independent signals, with per-allele effect sizes ranging from ~1 week to 5 months. Additionally, the PRS explained approximately 7.4% of the population variance in age at menarche, thus corresponding to ~25% of the estimated heritability (29).

CHAPTER II: MANUSCRIPT

Associations of polygenic risk score and polybrominated biphenyl exposure on age at menarche in the Michigan polybrominated biphenyl registry

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ABSTRACT

Numerous small nucleotides polymorphisms and endocrine disrupting chemicals have been associated with changes in the timing of menarche – a complex reproductive event – but, little is known about their interactions. We examined the direct associations of exposure to polybrominated biphenyl (PBB) exposure, an endocrine disruptor, and a previously established polygenic risk score (PRS) with age at menarche and the role of gene-environment interactions. Data from the PBB registry were analyzed (n=219). Age at menarche was obtained by self-reported through a health history questionnaire. We measured PBB levels from whole blood and peripheral blood samples were genotyped and imputed. Linear regression models were used to test the association between PRS and age at menarche, PBB and age at menarche, and investigate effect modification. PRS was positively associated with age at menarche among the total cohort but did not reach statistical significance. PBB was positively associated with age menarche, but only among those exposed to PBB in childhood, and was independent of PRS. Finally, we observed no evidence of gene-environment interaction between PBB exposure and PRS. These data lend support to the hypothesis that age at menarche is a polygenic trait and that timing of menarche is influenced by both environmental and genetic factors.

INTRODUCTION

Age at menarche, defined as the age at first occurrence of menstruation, is a complex reproductive milestone that serves as a strong indicator for disease susceptibility later in a

women's life. A convincing body of research has associated the timing of menarche – particularly earlier age at menarche – with adverse health outcomes. For instance, earlier age at menarche is associated with an increased risk of breast cancer (30), endometrial cancer (4), gestational diabetes (6), type II diabetes (7), and multiple sclerosis (9); whereas cardiovascular disease is associated with earlier and later ages at menarche (3).

Both genetic and environmental factors have been examined extensively – both of which have been determine to be associated with age at menarche – however, the steady population decrease in age at menarche continues to eludes investigators (31). Given the complex physiologic mechanisms preceding menarche, investigation into gene-environment interactions are needed (16, 32), yet there is a dearth of research exploring these interactions.

In previous research by our group, exposure to polybrominated biphenyl (PBB), an endocrine disrupting compound, was found to decrease age at menarche among those who were exposed to high levels *in utero*; and that while age at menarche is heritable, heritability may change based on PBB exposure (18, 27). Additionally, a recent meta-analysis comprising of 40 studies and ~ 370,000 females, developed a polygenic risk score (PRS) to explain approximately 7.4% of the population variance in age at menarche, and 25% of the heritability (29). Given the heritability of we utilized the recently established PRS and PBB exposure to explore gene-environment interactions on age at menarche.

METHODS

Study Sample

Participants were selected from the Michigan PBB Registry. The registry was originally developed by the Michigan Department of Community Health (MDCH) in 1976 in response to an agricultural accident where Michigan residents were exposed to high levels of PBB. The PBB registry consists of individuals who were living on farms quarantined due to PBB contamination, consumers of farm products, workers of the chemical plant that manufactured PBB, and their family members. Original recruitment by the MDCH has been described elsewhere (33). Participants in the registry have been followed since the 1970s and have completed questionnaires regarding their health history and provided blood samples. In 2011, the registry was transferred to Emory University and original registry members' children and additional members of the community who were exposed to PBB have since been enrolled (http://pbbregistry.emory.edu).

For purposes of this study, female participants were selected if they: (1) reported their age at menarche via a health history questionnaire, (2) had a recent (2004-present) buffy coat or whole blood sample available for DNA extraction, and (3) had PBB levels and lipid measurements of their serum. A total of 220 satisfied these criteria and were selected. Informed consent was obtained from each individual prior to their participation. Study protocols were approved by the Institutional Review Board at Emory University.

Assessment of age at menarche

Participants completed detailed questionnaires which included a question about age at first menstrual period. If a participant completed multiple questionnaires and different ages were self-reported, the first reported age at menarche was used to reduce the likelihood of recall bias. One participant was excluded from the analysis for a reported age at menarche of 31, which was assumed to be a reporting error.

Measurement of PBB exposure

There are a total of 209 possible congeners of PBB, depending upon the total number and position of the bromine atoms around the biphenyl ring. PBB-153 (2,2',4,4',5,5' - hexabromobiphenyl) is the predominant PBB congener and makes up approximately 61% of FireMaster FF-1 - the commercial product responsible for contaminating the food supply of Michigan residents. Three additional congeners - PBB-77, PBB-101, PBB-180 – were previously measured in members of the registry using gas chromatography-tandem mass spectrometry. The limit of detection (LOD) was 2 pg/mL for PBB-153, 4.5 pg/mL for PBB-77, 3.9 pg/mL for PBB-101, and 5.6 pg/mL for PBB-180. The extraction recovery ranged from 83.2-99.2%. The accuracy ranged from 89-119%, and the precision ranged from 2.8-8.5%. Any values below the LOD in a sample were imputed as the LOD divided by the square root of 2 (34). The congeners were then summed to give a total PBB measurement per person. If available, maternal serum PBB levels were used as a proxy for individual-level PBB exposure to better estimate *in utero* exposure. This condition applied to 19 out of the 24 participants exposed prenatally. For this reason, analyses with PBB are stratified by

generation of exposure – those exposed *in utero* and those exposed during childhood – and by maternal serum PBB level or PBB level measured in adulthood.

Lipid measurement

Total lipid amounts were calculated based on methods described elsewhere. Briefly, a Triglyceride Quantification Assay Kit (Abnova Corporation) was used to measure the total triglyceride content in serum, and a Cholesterol Assay Kit (Caymen Chemical Company) was used to measure total cholesterol content in serum. Both were completed in accordance with manufacturer's instructions.

Genotyping and imputation

Peripheral blood samples were collected from participants as part of the ongoing PBB Registry activities between 2004 – 2015. Whole blood was centrifuged at 3000 rotations per minute to separate the plasma from the buffy coat. Buffy coats were aliquoted and stored at -80° C. DNA was then extracted from the buffy coat samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The manufacturers protocol was followed for genotyping the autosomal SNPs on the Illumina Multi-Ethnic Global BeadChip; where further details regarding genotyping procedures have been described elsewhere. For quality control, SNPs were excluded for a low call rate (<95%), a minor allele frequency (MAF) less than 0.05, or deviation from Hardy-Weinberg equilibrium with a p-value less than 0.001. A total of 636,593 SNPs passed quality control filters and were subsequently imputed, on the

1000 Genomes reference panel, using the Michigan Imputation Server. Two of the 389 SNPs from the established PRS were not imputed. Therefore, only data on 387 SNPs were available for this analysis. All genetic data were managed in PLINK v1.9 and VCFtools.

Polygenic risk scores

The PRS were created based on the results from a large meta-analysis which comprised of 40 studies from the ReproGen consortium (N = 179,117), 23andMe (N = 76,831), and UK Biobank (N = 73, 397) studies (29). From the meta-analysis, SNPs were selected only if they had results from at least two of the three studies, a combined MAF greater than 0.1%, passed a two-tailed threshold of significance ($P < 5 \times 10^{-8}$) , and were in low linkage disequilibrium to each other ($r^2 < 0.5$). Variants who satisfied these criteria but were within 1 Mb of one another were considered to be located on the same locus. This resulted in 389 statistically independent signals.

Statistical Analysis

Analyses for PRS on age at menarche were conducted on 219 women who self-reported their age at menarche via a health history questionnaire, consented to the collection of DNA and had PBB levels measured. Descriptive statistics on key variables is provided in Table 1.

We also investigated the interaction between PBB exposure and the PRS as a measure of combined effects of established SNPs on age at menarche in a subset of 144 women who

were exposed to PBB prior to reaching menarche. The calculation of the PRS has been described elsewhere. Briefly, the established PRS used in this analysis was calculated for each study participant using the formula:

$$PRS = \beta_1 x_1 + \beta_2 x_2 + \cdots \beta_k x_k$$

where β_k was the per-allele effect size (indicated by β-coefficient) provided by the meta-analysis for age at menarche association with the minor allele for SNP k, x_k was the number of alleles for that same SNP (0, 1 or 2), and n = 389 was the total number of SNPs. Scores were computed using PRSice and were adjusted for the first ten principal components as a measure of ancestry.

Univariate and multivariable linear regression models were used to assess the associations of PRS on age at menarche, PBB on age at menarche, and the interaction between PRS and PBB. We tested for the interaction between PBB and PRS by adding an interaction term (PBB × PRS) to the model. Analyses with PBB include additional models adjusting for lipids as a potential confounder. To account for skewness, total PBB values were natural log-transformed for all analyses. PRS, lipids and age at menarche were approximately normally distributed, as demonstrated in Figure 1. Statistical analyses were performed using the R statistical package version 3.5.1. An alpha value of 0.05 was used to determine statistical significance.

RESULTS

Description of study participants

A total of 219 women met the inclusion criteria for PRS, and a subset of 144 women for the interaction analyses. The participant demographics, stratified by generation of PBB exposure among those exposed to PBB before their age at menarche, are presented in Table 1. The average age of blood draw was 51 years old (range: 31-85 years) and the average age of exposure, among those exposed to PBB in childhood, was approximately 8 years old (range: 2-16 years). Participants were highly exposed to PBB (range: 0.1-184 ng/ml) as compared to the 95th percentile for a representative sample of the United States. When using the PBB levels measured in adulthood, those who were exposed *in utero* had lower exposure, on average, than those exposed during childhood (*in utero* = 0.03 ppb; childhood average = 0.33 ppb). Among the women who reported their race and ethnicity, all were White and non-Hispanic.

Main effects: PRS and PBB exposure on age at menarche

After quality control measures, 163 SNP's from the established PRS were used to calculate PRS in our sample. As shown in Table 2, in the total cohort, increasing PRS increased age at menarche (β = 167.59, standard error [SE] = 159.95), explained less than 1% of the variation in our model, and was not associated with age at menarche. PBB was positively associated with age at menarche, but the magnitude of the association varied by generation (Table 3). There was a significant, but modest, association of PBB and age at menarche, after

adjustment for lipids, among those exposed in childhood (β = 0.63, SE = 0.27). PBB was modestly associated with an increase in age at menarche among those exposed *in utero* and when PBB exposure was assessed via maternal serum measurements (β = 0.58, SE = 0.34), but the association diminished when using measurements of the participant's PBB levels in adulthood (β = 0.07, SE = 0.27).

As demonstrated in Table 4 and Figure 1, PRS is significantly associated with age at menarche, at median PBB and lipid levels – and thus independent of PBB and lipids – in both generations of exposure (*in utero*: β = 789.12, SE = 232.58; in childhood: β = 555.60, SE = 176.20). Further, PBB continues to be positively associated with age at menarche among those exposed to PBB in childhood, when PRS is held constant.

Interaction of PRS and PBB exposure on age at menarche

Analyses of the interaction between PRS and PBB exposure are presented in Table 4. We found no evidence of statistical interaction at $p < 0.05$. However, after adjustment for total lipids and when stratified by generation of exposure, the combined effects of the PRS genotype and PBB exposure is associated with a decreased age at menarche among exposed in childhood (β = -37.17, SE = 315.60) – but the estimates were imprecise and did not reach statistical significance. Moreover, in the interaction models, PRS significantly predicts age at menarche in total cohort (β = 635.70, SE = 172.10) and after stratification by generation of exposure (*in utero*: β = 803.60, SE = 231.17; in childhood: β = 539.00, SE = 16.37). At $p <$ 0.05, no significant interaction is observed.

DISCUSSION

In this study of women enrolled in the PBB registry, we showed that a previously established genetic risk score and PBB exposure may predict age at menarche, with differences between those exposed to an endocrine-disrupting chemical by stages of development. Among these participants, there was evidence that PBB increases a female's age at menarche, but only when exposed in childhood; and that genetic susceptibility increases age at menarche, independent of PBB exposure. We found no evidence of gene-environment interaction

An overwhelming body of research supports a genetic component in the timing of menarche. Due to the relatively small effects of individual SNPs, and the wealth of large-scale genomic studies on age at menarche, a PRS of 389 independent signals was recently developed – the largest number of independent genetic signals to predict age at menarche to date. Among our total cohort, PRS explained only 0.05% of the variation in our model. When restricted to the women exposed to PBB during childhood, but before menarche, the PRS explained more of the variation in our model (approximately 7%), which is consistent with the meta-analysis. However, it is important to note that in our study the PRS was comprised of only 163 of the 389 SNPs, and our sample size was limited.

Considerable research has linked PBB to reproductive health outcomes, including timing of menarche. A prior study completed by the PBB registry found that females exposed to high levels of PBB *in utero* had an earlier age at menarche compared to those exposed to lower

levels of PBB *in utero* (18). While we did not observe a similar result among those exposed *in utero*, our sample size for this comparison was very limited. Additionally, in the previous study, *in utero* PBB levels for all participants were estimated using maternal serum PBB measurements and extrapolated time to pregnancy using a model of PBB elimination. While our study utilized maternal PBB levels to estimate *in utero* exposure, these data were only available for 19 of the 24 women exposed *in utero*. Further, we did not employ a maternal elimination rate model in our analyses.

Strengths and limitations

To our knowledge, no previous studies have examined the interaction between a genetic risk score and endocrine disrupting chemical exposure on timing of menarche – a significant reproductive event. Due to the well-defined time frame of PBB exposure, a strength of this study is the ability to assess chemical exposure by life stages that have been shown to be meaningful in assessing the effect of the environment on age at menarche. Further strengths include the use of the combined effect of SNP's to evaluate genetic risk and the biological measurements of both PBB and PRS.

Given the uncommon nature by which this population was exposed to PBB – a high level of exposure over a relatively short period of time – a limitation of this study is the potential lack of generalizability of findings to different exposures or populations. PBB belongs to a large group of endocrine disrupting chemicals; a great deal of which are still in commercial use today. A more likely mechanism of exposure to similar chemicals is modest and over a

longer period of time. Additionally, with some exceptions, the majority of participants had their PBB levels measured well after reaching their age at menarche; therefore, we were unable to definitively assess PBB exposure prior to the participant's first menstruation. Although there was no interaction in this study, this provides support for the independent effects of genetics and environmental exposures on age at menarche.

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Table 1. Cohort Demographics

	Total Cohort (*N* = 219)	**Exposed to PBB *in utero* (*N* = 24)**	**Exposed to PBB in childhood (*N* = 120)**
Current Age (Years)[a]	51.58 ± 10.10	38.18 ± 2.58	47.94 ± 3.86
Age Exposed (Years)[a]	11.86 ± 10.13	-	7.71 ± 3.73
Total PBB (ppb)[b]	0.39 (7.27)	0.03 (10.08)	0.33 (4.95)
Total Maternal PBB (ppb)[b,e]	-	3.01 (7.32)	-
Lipids (ng/g lipid)[a, d]	731.20 ± 208.25	624.30 ± 142.63	756.2 ± 222.98
Race			
White/Non-Hispanic[c]	209 (95%)	23 (96%)	116 (97%)
Missing[c]	10 (5%)	1 (4%)	4 (3%)

[a]Mean and standard deviation

[b]Geometric mean and geometric standard error

[c]Frequency and proportion

[d]Missing information for 3 participants

[e]Applicable to 19 out of 24 participants

Table 2. Regression coefficients for the association of PRS and age at menarche

	Total Cohort				
Variable in the model	**Total N**	**β**	**Std. error**	**Adjusted r^2**	***P***
PRS	219	167.59	159.95	0.005	0.30

Table 3. Regression coefficients for the association of PBB and age at menarche, stratified by generation of exposure

Variables in the model	Exposed to PBB *in utero*: Maternal PBB levels[a]					Exposed to PBB *in utero*: PBB levels measured in adulthood[b]					Exposed to PBB in childhood				
	Total N	β	Std. error	Adjusted r^2	*P*	Total N	β	Std. error	Adjusted r^2	*P*	Total N	β	Std. error	Adjusted r^2	*P*
PBB	19	0.58	0.34	<0.00	0.1	24	0.001	0.27	<0.00	0.98	120	0.40	0.23	0.02	0.09
PBB	-	-	- -	-	-	24	0.07	0.27	<0.00	0.80	117	0.63	0.27	0.03	0.02
Lipids[c]		-	- -		-		-0.002	0.002		0.28		0.0001	0.0007	0.21	0.89

[a]PBB exposure was estimated by maternal serum PBB levels

[b]PBB exposure was estimated by individual levels measured in adulthood

[c]Lipid measurements unavailable for maternal PBB levels

Table 4. Regression coefficients for the interaction of PRS and PBB on age at menarche, stratified by age of exposure

	Exposed to PBB *in utero*: Individual PBB levels					Exposed to PBB in childhood				
Variables in the model	**Total N**	**β**	**Std. error**	**Adjusted r^2**	***P***	**Total N**	**β**	**Std. error**	**Adjusted r^2**	***P***
PRS	24	789.12	232.58	0.31	0.003	117	555.60	176.20	0.10	0.002
PBB		-0.03	0.22		0.89		0.06	0.26		0.03
Lipids		-0.002	0.002		0.25		0.0002	0.0007		0.74
PRS	24	809.19	235.04	0.30	0.003	120	571.75	212.96	0.08	0.008
PBB		0.43	0.49		0.38		0.45	0.83		0.59
PRS × PBB		199.63	191.28		0.31		24.33	288.51		0.93
PRS	24	803.60	231.17	0.32	0.003	117	539.00	16.37	0.09	0.02
PBB		0.44	0.48		0.36		0.49	1.57		0.58
Lipids		-0.002	0.002		0.21		0.0002	0.0007		0.75
PRS × PBB		207.03	188.18		0.29		-37.17	315.60		0.91

[a]PBB exposure was estimated by individual levels measured in adulthood

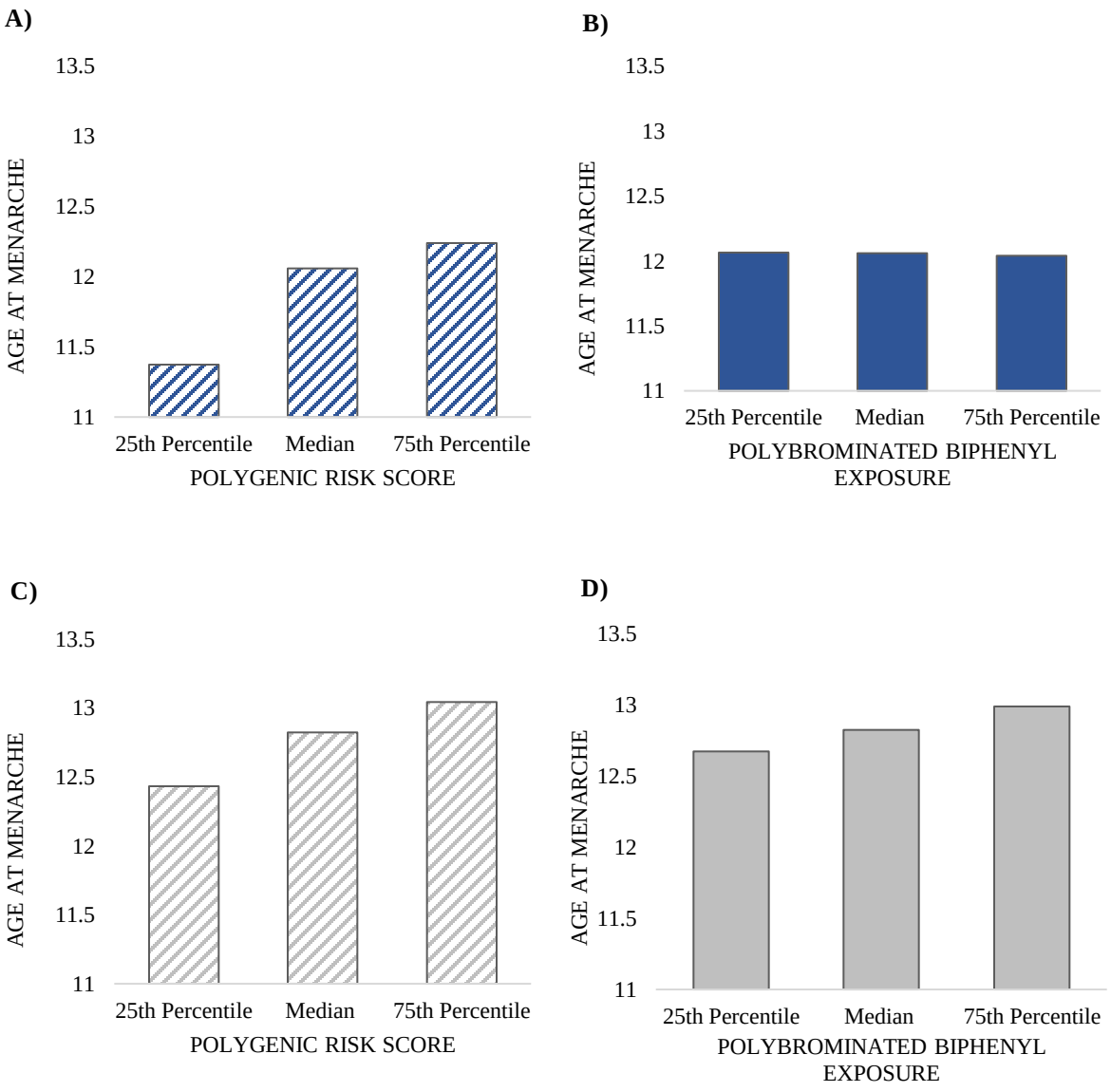

Figure 1. Predicted age at menarche by **A)** increasing polygenic risk score (PRS) at the median polybrominated biphenyl (PBB) and lipid levels among those exposed to PBB *in utero* (n=19); **B)** increasing PBB exposure at the median PRS and lipid levels, among those exposed to PBB *in utero* (n=19); **C)** increasing PRS at the median PBB and lipids level, among those exposed to PBB in childhood (n=117); and **D)** increasing PBB at the median PRS and lipid level, among those exposed to PBB in childhood (n=117). PBB exposure was estimated using individual levels for all participants.

CHAPTER III

SUMMARY

We evaluated the combined effects of 163 SNPs, as a PRS, on self-reported age at menarche in 219 women. Interaction of PRS and an endocrine disruptor, PBB, were examined in a subset of 144 women who were exposed to PBB prior to reaching their age at menarche. We found that PRS increased age at menarche but did not reach statistical significance. PBB was positively associated with age menarche, but only among those exposed to PBB in childhood. PRS was significantly associated with an increase in age at menarche when PBB was held constant; thus, we observed no evidence of gene-environment interaction between PBB exposure and PRS.

PUBLIC HEALTH IMPLICATIONS

Thesis findings lend support to the hypothesis that both genetic and environmental factors influence timing of menarche. Given the relatively small effects typically exhibited by individual SNPs, and the numerous SNPs that have been previously associated with age at menarche, these data suggest that menarche is a polygenic trait. It offers intriguing results on the implications of chemical exposures in different life stages. These findings can be used to inform future research in examining the effects of genetic and environmental exposures on age at menarche and encourage researchers to investigate interactions where possible.

Lastly, these data can be used to assist in influencing environmental health policies. Particularly, to encourage more prudent investigations on chemicals prior to their release and stricter regulations regarding their commercial use.

FUTURE DIRECTIONS

In regard to the genetic component of this research, future studies should examine the efficacy of the established PRS in racially similar and larger populations. Additionally, GWAS on age at menarche should be conducted in more diverse populations to develop a genetic risk score applicable to non-Whites. To validate the finding of PBB on age menarche, investigations should be broadened to other endocrine-disrupting chemicals. Studies of this nature in larger populations would also be powered to identify refined windows of susceptibility for environmental exposures on age at menarche