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Genetic polymorphisms in the aryl hydrocarbon receptor signaling pathway as potential risk factors of menopausal hot flashes

Ayelet ZIV-GAL, MBA[a], **Lisa GALLICCHIO, Ph.D.**[b], **Susan R. MILLER, Sc.D.**[c], **Howard A. ZACUR, MD, Ph.D.**[c], and **Jodi A. FLAWS, Ph.D.**[a,d]

[a]Comparative Biosciences, University of Illinois, Urbana, IL, USA

[b]Mercy Medical Center, Baltimore, MD, USA

[c]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Objective—To determine if genetic polymorphisms in the aryl hydrocarbon receptor signaling pathway are associated with menopausal hot flashes via hormone levels.

Study design—Women (n=639) aged 45–54 years completed a study survey and provided blood for genetic and hormone analyses. The associations were analyzed using multivariable logistic regression and generalized linear models.

Results—Women carrying *CYP1B1* (rs1800440) GG genotype had 3-fold greater odds of experiencing hot flashes for ≥1 year compared to the AA genotype [adjusted odds ratio (aOR): 3.05 (1.12–8.25)]. Adding serum estradiol concentrations to the confounder-adjusted model resulted in a non-significant association [aOR: 2.59 (0.91–7.18)]. Carriers of both *CYP1B1* (rs1800440) G and *CYP1B1* (rs1058636) G alleles had higher odds of experiencing hot flashes for ≥1 year compared to women homozygous for the major alleles [aOR: 1.77 (1.06–2.96)], even after adjustment for serum estradiol.

Conclusion—*CYP1B1* is associated with menopausal hot flashes via pathways that may involve changes in serum estradiol concentration.

Keywords

AHR; *CYP1B1*; hot flashes; polymorphism; risk factor

Introduction

Menopausal hot flashes are one of the main reasons that millions of women seek medical care during their menopausal transition.[1,2] It is estimated that about 5 million women in the United States alone will experience hot flashes each year.[2] Most of these women will

[d]Corresponding Author (and address for reprints): Jodi A. Flaws, Ph.D.; Department of Comparative Biosciences, University of Illinois, 2001 S. Lincoln Ave. Urbana, Illinois, 61802. Phone: 217-333-7933; Fax: 217-244-1652; jflaws@illinois.edu.

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experience hot flashes for several months to 5 years, and some will even have hot flashes for up to 30 years.[2–4] Nevertheless, little is known about the etiology of hot flashes[2–4] although several risk factors have been identified. Such risk factors include African American race, high body mass index (BMI), cigarette smoking, and low serum estrogen concentrations[3–8] Additionally, a limited number of previous studies have shown that certain genetic polymorphisms in estrogen metabolizing enzymes (e.g. *CYP1B1*, rs1056836)[9,10] and estrogen receptors[11,12] are associated with an increased odds of hot flashes in midlife women. Thus, it is possible that additional genetic variations are associated with the risk of menopausal hot flashes.

The aryl hydrocarbon receptor (AHR)is a highly conserved transcription factor that is present in many cell types. Upon ligand binding and activation of the receptor, the cytoplasmic AHR translocates to the nucleus and heterodimerizes with the AHR nuclear translocator (ARNT).[13] This complex can then induce the transcription of specific genes that can further lead to estrogen biosynthesis (i.e. aromatase) or degradation (i.e. *CYP1B1*).[13–16] The AHR repressor (AHRR) can block the activation of the AHR signaling pathway.[13]

In the current study, we chose to focus on certain single nucleotide polymorphisms (SNPs) in the AHR signaling pathway as potential risk factors of menopausal hot flashes for several reasons. First, the AHR interacts with the estrogen biosynthesis pathway, and its activity can affect the concentrations of sex steroid hormones.[13–16] Given the known associations between genetic polymorphisms (e.g. *CYP1B1*) and serum estrogens concentrations,[9,17] as well as between low serum estrogens concentrations and hot flashes,[3,4,18,19] it is possible that women with hot flashes are more likely to carry genetic polymorphisms in the AHR signaling pathway genes than women without hot flashes. We specifically examined the potential associations between the *AHR*-rs2066853 (missense), *AHRR*-rs2292596 (missense), *ARNT*-rs2228099 (synonymous), *CYP1B1*-rs1800440 (missense), and *CYP1B1*-rs1056836 (missense) and selected hot flashes outcomes, although little is known regarding the functional significance of the selected polymorphisms. The exception is the *CYP1B1* (rs1800440) polymorphism, for which Hanna et al.[20] reported a higher catalytic activity for the variant allele compared to the non-variant allele in an in-vitro study. In a previous report, we found that a genetic polymorphism in the AHR signaling pathway (*CYP1B1* rs1056836) was associated with hot flashes.[9,10] Therefore, we hypothesized that common SNPs reported in the *AHR, ARNT, AHRR*, and *CYP1B1* genes, and combinations of these SNPs, are associated with the risk of hot flashes. In addition, we examined if the selected SNPs were associated with altered sex steroid hormone concentrations or ratios and sex hormone binding globulin (SHBG) to determine if these differences could account for any observed association between the selected SNPs and hot flashes.

Materials and Methods

Participants (n=639) were recruited for a cross-sectional study of risk factors for menopausal hot flashes in midlife women. Detailed methods of participant recruitment are described elsewhere.[8] Briefly, between 2000 and 2004, generally healthy women residing in Baltimore city and the surrounding counties were sent recruitment letters via mail. Potential participants had to meet the following eligibility criteria: aged 45 to 54 years old, not taking hormonal therapy, not pregnant, and no history of cancer.[8] Further, because the study was designed to examine the health of women undergoing the menopausal transition, women were eligible only if they reported having at least three menstrual periods in the last 12 months. Therefore, all women in the study were either pre- or peri-menopausal. Eligible women were scheduled to come to the clinic during the morning hours and were instructed to fast overnight prior to the visit. During the clinic visit, each participant had their weight and height measured, and a blood sample was drawn for genetic and hormone

concentrations analyses. The participants also completed the detailed study survey, which included questions regarding demographics; medical, family, and reproductive history; past exogenous hormone use, menopausal symptoms; and lifestyle habits (e.g. smoking, diet). All participants in this study gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards.

Age and race/ethnicity were self-reported. BMI was calculated based on height and weight measurements of the participant at the clinic visit and categorized either as normal BMI (≤24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese (≥30.0 kg/m^2). Smoking status at the time of enrollment (current/former/never) was determined using the questions “Have you ever smoked cigarettes?” and “Do you still smoke cigarettes?” A woman’s history of ever experiencing midlife hot flashes was determined using the question “Have you ever experienced hot flashes?” Women experiencing hot flashes were further queried regarding hot flash severity (mild, moderate, or severe), frequency (daily, weekly, or monthly), and duration (number of months/years). Hot flash outcomes examined in the analyses were: ever experienced hot flashes, moderate or severe hot flashes, daily hot flashes, and hot flashes experienced for one year of greater (long duration). For all hot flash outcomes, the comparison group was never experienced hot flashes.

SNPs genotyped to address the hypotheses were: *AHR*-rs2066853 (Arg554Lys; G>A), *AHRR*-rs2292596 (Pro185Ala; C>G), *ARNT*-rs2228099 (Val89Val; G>C), and *CYP1B1*-rs1800440 (Asn453Ser; A>G). Additionally, genotype data for the SNP *CYP1B1* rs1056836 (Leu432Val; C>G) that was reported in a previously published manuscript from this study were also used in the analyses.[9,10]

Genomic DNA was isolated from the donated blood samples using the GeneElute Blood Genomic DNA kit (Sigma, USA) and was amplified through polymerase chain reactions (PCR) based on published primers.[21,22] Genotyping of *AHR, ARNT*, and *AHRR* SNPs was done using the allele-specific PCR method. Each reaction tube of *AHR, ARNT*, and *AHRR* contained: DNA sample 3μL, HotStarTaq *Plus* Master mix (QIAGEN, USA) 10μL, 10X reaction buffer 2μL (QIAGEN, USA), forward and reverse primers (50mM) 0.1μL of each, allele-specific primer (50mM) 0.1μL (*AHR*) 0.02μL (*AHRR, ARNT*), and RNase free water 4.7μL (*AHR*) 4.78μL (*ARNT, AHRR*). Each *CYP1B1* PCR reaction tube contained: DNA sample 3μL, HotStarTaq *Plus* DNA polymerase 0.1μL (5U/μl; QIAGEN, USA), 10X reaction buffer 2μL (QIAGEN, USA), forward and reverse primers (50mM) 0.1μL of each, RNase free water 14.3μL (QIAGEN, USA), and dNTPs (10mM; Invitrogen, USA) 0.4μL. PCR protocols included: activation at 95°C for 5 minutes, denaturation at 94°C for 45 sec, annealing at 63°C (*AHR, AHRR*), 57°C (*ARNT*), or 60°C (*CYP1B1*) for 45 seconds, elongation at 72°C for 45 seconds [denaturation to elongation steps were repeated 30 cycles (*AHR, CYP1B1*) or 33 cycles (*AHRR, ARNT*)], final elongation at 72°C for 5 minutes, and lastly 10°C for 5 minutes. *CYP1B1* PCR products were digested with restriction enzyme (MwoI). All products were visualized by 2.5% (*AHR, ARNT, AHRR*) or 3.0% (*CYP1B1*) agarose gel electrophoresis. Participants were categorized according to the genotype for each SNP as homozygous for the major allele, heterozygous, or homozygous for the minor allele.

Serum concentrations of estradiol, estrone, testosterone, progesterone and SHBG were measured using enzyme-linked immunosorbent assays. All assays were performed as previously described.[8] Briefly, the assays were run using the manufacturers’ instructions. All assays were conducted in the same laboratory by a single investigator. All samples were run in duplicate and mean values for each participant were used in the analyses. The laboratory personnel were blind with respect to any information concerning study subjects.

In addition, positive controls containing known amounts of the tested hormone or SHBG were included in each batch. Further, some samples were run in multiple assays to ensure that the assay values did not dramatically shift over time.[8] The minimum detection limits for the estradiol, estrone, testosterone, androstenedione, progesterone, and SHBG assays were 7 pg/ml, 10 pg/ml, 0.04 ng/ml, 0.03 ng/ml, 0.1 ng/ml, and 0.1 nmol/L, respectively. No estradiol, estrone, testosterone, androstenedione, or SHBG measurements were below the limit of detection. For progesterone measurements that were below the limit of detection (n = 66), the value was set at the limit of detection (0.1 ng/ml). The average intra-assay coefficient of variation was 3.3±0.17% for estradiol, 4.8±0.25% for estrone, 2.2±0.56% for testosterone, 2.5±0.60% for androstenedione, 2.1±0.65% for progesterone, and 2.4±0.67% for SHBG. The average inter-assay co-efficient of variation for all assays was less than 5%.[7] To assess the amount of unbound serum estradiol or testosterone, ratios of serum estradiol to SHBG and serum testosterone to SHBG were calculated as described previously to generate free serum estradiol indices and free serum testosterone indices.[8]

The associations between the SNPs and both the categorical covariates and hot flash outcome variables were analyzed using chi-square tests. Both unadjusted and confounder-adjusted odds ratios (OR) and 95% confidence intervals (CI) for associations between the SNPs and the hot flash outcome variables were generated using logistic regression models. The following covariates were included as potential confounders in all of the logistic regression models, as they were associated with ever experiencing hot flashes in previous analyses from this study or in the published literature: race, BMI, smoking status, age. In addition, race-stratified analyses were conducted to examine differences in the analyzed associations between Caucasian and African American women in the sample; however, the OR estimates did not substantially differ and, therefore, we determined that race was not an effect modifier. Thus, instead of presenting the race-stratified results, we treated race as a confounder in the analyses along with BMI, age and smoking status. Lastly, generalized linear models were used to examine the associations between the SNPs and hormone concentrations, ratios, and SHBG. Analyses were performed using SAS Version 9.1 (Cary, NC). A p-value of less than 0.05 was considered to be statistically significant.

Results

Characteristics and genotype distributions of the study sample by hot flash status are presented in Table 1. Women with hot flashes were significantly older and more likely to be African American, to be overweight or obese, and to smoke cigarettes compared to women without hot flashes. The percentages of women with genetic polymorphisms were not significantly different between women with and without hot flashes. We used the Hardy-Weinberg equation to estimate if the observed genotype frequencies in our study sample differed from the predicted genetic variation at equilibrium of the general population. All SNPs excluding *AHR* and *AHRR* were in Hardy-Weinberg equilibrium (data not shown), indicating that there was a stable frequency distribution of these genotypes in our study compared to the predicted variation of the general population. In the unadjusted analyses, the *AHR* SNP was associated with ever experiencing hot flashes (Table 2). Specifically, women homozygous for the *AHR* minor allele (rs2066853 A) had increased odds of any hot flashes (OR: 2.67; 95% CI: 1.13–6.33) compared to women homozygous for themajor allele (rs2066853 G). However, after adjustment for age, race, BMI, and smoking status, this association was attenuated and no longer statistically significant (OR: 2.44; 95% CI: 0.99–6.01). Similarly, the combined genotypes of the *AHR* + *CYP1B1* (rs1800440) SNPs were significantly associated with ever experiencing hot flashes only in the unadjusted statistical model (OR: 1.93; 95% CI: 1.00–3.75). The combined genotypes of the *CYP1B1* SNPs (rs1800440 + rs1056836) were also associated with ever experiencing hot flashes, but only in the confounder adjusted model (OR: 1.51; 95%CI: 1.01–2.31). None of the other SNPs or

combinations of SNPs were significantly associated with ever experiencing hot flashes in the unadjusted or adjusted analyses.

The results of the analyses examining the selected SNP genotypes and the severity, frequency, and duration of hot flashes are shown in Table 3. Hot flash severity was significantly associated only with the combined genotypes of the selected SNPs in *CYP1B1.* Specifically, carriers of at least one minor allele for both *CYP1B1* SNPs (rs1800440 G + rs1056836 G) had greater odds of moderate or severe hot flashes compared to women who were homozygous for the major allele of both *CYP1B1* SNPs (OR: 1.68; 95% CI: 1.00–2.82). Long lasting hot flashes (hot flashes experienced for a year or more) were more likely to be experienced among women homozygous for minor allele of the *AHR* (rs2066853 A), *CYP1B1 (*rs1800440 G), or combined SNPs of the *CYP1B1* (rs1800440 G + rs1056836 G) compared to women who were homozygous for the major alleles; however, only the associations between the *CYP1B1* SNP (rs1800440) and combined *CYP1B1* SNPs (rs1800440 + rs1056836) remained statistically significant after adjustment for the other covariates. Hot flash frequency was not significantly associated with any of the SNPs or their combinations.

The associations between the selected SNPs and hormones as well as SHBG are shown in Table 4. Women homozygous for the major allele of *AHR* (rs2066853 G), *ARNT* (rs2228099 G), and *CYP1B1* (rs1800440 A) had significantly higher serum concentrations of SHBG, estrone and estradiol, respectively, compared to carriers of the minor alleles (homozygous or heterozygous) of those genes. Additionally, significant associations were observed for the combined genotypes of *AHR* (rs2066853) + *AHRR* (rs2292596) and SHBG and free testosterone index and between *AHR* (rs2066853) + *ARNT* (rs2228099) and serum estradiol, estrone, and testosterone concentration (Table 5). No other statistically significant associations were observed between the individual SNPs or their combinations and hormone concentrations, ratios or SHBG.

Lastly, to examine whether the statistically significant associations observed between the SNPs and hot flashes outcome variables in the adjusted analyses were due to observed differences in hormone concentrations, the hormone variable was added to the multivariable logistic regression model for the specific SNP and hot flashes outcome where a statistically significant association was found. Adding the serum estradiol concentration variable to the statistical model resulted in attenuation of the association between *CYP1B1* (rs1800440) and hot flashes experienced for more than a year (OR: 2.59; 95% CI: 0.91–7.18). No further analyses were conducted for the combined *CYP1B1* SNPs and either of the other hot flash outcomes as the combined *CYP1B1* genotypes were not significantly associated with any of the hormone concentrations.

Comment

Findings from this study suggest that SNPs in the *CYP1B1* gene(rs1800440, rs1056836) are associated with the risk of menopausal hot flashes, independent of known risk factors for hot flashes. Furthermore, our results indicate that genetic polymorphisms in the AHR signaling pathway (i.e. *AHR, ARNT, CYP1B1* and the combined genotypes *AHR+ARNT; AHR +AHRR*) are correlated with sex steroid hormone concentrations, SHBG concentration, and hormone ratios.

In our current study, women homozygous for the minor allele of the *AHR* SNP (rs2066853 A) had almost a three-fold greater odds of any hot flashes and hot flashes experienced for more than a year in our unadjusted models compared to women homozygous for the major allele (rs2066853 G). These associations, however, were attenuated when the statistical model was adjusted for BMI, race, age, and smoking status. This finding suggests that the

AHR SNP is not an independent risk factor for hot flashes and that BMI, race, age, and smoking status confound the observed associations with hot flashes. These findings are in accordance with studies conducted in our sample and others indicating that high BMI, African American race, and cigarette smoking are strongly associated with increased odds of hot flashes[5–8,23–25] In our study sample, women who were homozygous for the major *AHR* allele (rs2066853 G) included a significantly higher percentage of African American women (22.3% versus 16.0%), and women with higher BMI (BMI>30.0 kg/m^2; 60% versus 7.9%) compared to women homozygous for the minor allele.

In this study, we also found that *CYP1B1* (rs1800440) was significantly associated with long lasting hot flashes in the confounder adjusted statistical model that included BMI, race, age, and smoking status. This association was attenuated when serum estradiol was added to the model, suggesting that serum estradiol concentration may mediate the association between *CYP1B1* (rs1800440) and long lasting hot flashes. Though there is limited information regarding the direct effects of this SNP on CYP1B1 activity,26 its potential effects in the context of various haplotypes, or its association with hot flashes,[27] it is possible that the SNP leads to an increase in CYP1B1 enzyme activity. This, in turn, may reduce serum estradiol concentration and thus increase the risk of hot flashes. Hanna et al.[20] reported a higher catalytic activity for the CYP1B1(rs1800440) variant than the non-variant form in an in-vitro study. Specifically, in our study, increased enzyme activity is possible in the light of the significant association between the SNP and lower serum estradiol concentration.

The association between *CYP1B1* (rs1800440) and hot flashes is intriguing because the observed OR in the confounder adjusted model was relatively high (OR: 3.05) compared to other published risk factors for hot flashes, such as cigarette smoking and race (OR: 1.55,[7] risk ratio: 2.08[23] respectively). Moreover, it is the second SNP in the *CYP1B1*[9,10] that has been reported to be significantly associated with hot flashes. In one of our previous studies, women heterozygous or homozygous for the *CYP1B1* minor allele (rs1056836 C) had a 27% higher risk (RR: 1.27, 95% CI 1.00–1.61) of long lasting hot flashes compared to women who were homozygous for the major allele (rs1056836 C).[9] In our combined analysis of the two SNPs in *CYP1B1* (rs1800440 + rs1056836), the increased odds of long lasting hot flashes was still observed, but with a lower OR than of *CYP1B1* (rs1800440) alone. Another finding that suggests a potential role for these SNPs in the way women experience hot flashes is that the combined *CYP1B1* genotypes were also associated with hot flash severity. Generally, we can only speculate that due to a relative proximity of the SNPs in the *CYP1B1* gene (codons 432 and 453), there may be some cross effects that attenuate the odds of long lasting hot flashes or that other factors that were not assessed in our current study attenuated the odds of long lasting hot flashes when we analyzed the combination of the two SNPs.

We also observed that the *AHRR* (rs229296) and *ARNT* (rs2228099) SNPs were not significantly associated with any of the hot flash outcomes. The data suggest that these SNPs do not play a central role in the etiology of hot flashes despite their major role in the AHR signaling pathway.

Overall, this study had several strengths as well as limitations. Some of the strengths are the relatively large sample size and the novel focus on selected individual SNPs and their combinations in the AHR signaling pathway. Additionally, this study included generally healthy women during their menopausal transition, a time in which hot flashes are likely to be experienced. Since hot flashes can be experienced differently between individuals, the study participants were asked specific questions regarding different aspects of hot flashes (i.e. any hot flashes, severity, duration, frequency). Nevertheless, the presented results must

be considered in the context of the study limitations. Although we focused on selected SNPs that are common in the population, we only examined a limited number of them, and in a defined set of study participants. Potentially, there may be other SNPs that affect the risk of hot flashes solely or when combined with other SNPs.[28] Moreover, the numbers of women who were homozygous for the minor allele of *AHR*, *AHRR* and *CYP1B1* were relatively small. Thus, we might have a type 2 error/lack of statistical power to detect possible effects of some of the minor alleles. It is also possible that our study design (cross-sectional) did not allow us to identify more statistically significant associations due to the high variability in the way and/or the timing women experience hot flashes.

In conclusion, our results suggest that some genes in the AHR signaling pathway may play a role in the etiology of hot flashes. Further studies should investigate the potential roles of individual SNPs, combinations of SNPs, and hormone concentrations to obtain a better understanding of their potential role as risk factors for menopausal hot flashes.

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Reference List

1. Kronenberg F. Menopausal hot flashes: Randomness or rhythmicity. Chaos. 1991; 1(3):271–278. [PubMed: 12779925]
2. Kronenberg F, Downey JA. Thermoregulatory physiology of menopausal hot flashes: a review. Can J Physiol Pharmacol. 1987; 65(6):1312–1324. [PubMed: 3304594]
3. Ziv-Gal A, Flaws JA. Factors that may influence the experience of hot flushes by healthy middle-aged women. J Womens Health (Larchmt). 2010; 19(10):1905–1914. [PubMed: 20831431]
4. Whiteman MK, Staropoli CA, Benedict JC, et al. Risk factors for hot flashes in midlife women. J Womens Health (Larchmt). 2003; 12(5):459–472. [PubMed: 12869293]
5. Gallicchio L, Visvanathan K, Miller SR, et al. Body mass, estrogen levels, and hot flashes in midlife women. Am J Obstet Gynecol. 2005; 193(4):1353–1360. [PubMed: 16202725]
6. Gallicchio L, Miller SR, Visvanathan K, et al. Cigarette smoking, estrogen levels, and hot flashes in midlife women. Maturitas. 2006; 53(2):133–143. [PubMed: 16368467]
7. Cochran CJ, Gallicchio L, Miller SR, et al. Cigarette smoking, androgen levels, and hot flushes in midlife women. Obstet Gynecol. 2008; 112(5):1037–1044. [PubMed: 18978103]
8. Schilling C, Gallicchio L, Miller SR, et al. Genetic polymorphisms, hormone levels, and hot flashes in midlife women. Maturitas. 2007; 57(2):120–131. [PubMed: 17187946]
9. Visvanathan K, Gallicchio L, Schilling C, et al. Cytochrome gene polymorphisms, serum estrogens, and hot flushes in midlife women. Obstet Gynecol. 2005; 106(6):1372–1381. [PubMed: 16319265]
10. Schilling C, Gallicchio L, Miller SR, et al. Relation of body mass and sex steroid hormone levels to hot flushes in a sample of mid-life women. Climacteric. 2007; 10(1):27–37. [PubMed: 17364602]
11. Crandall CJ, Crawford SL, Gold EB. Vasomotor symptom prevalence is associated with polymorphisms in sex steroid-metabolizing enzymes and receptors. Am J Med. 2006; 119(9 Suppl 1):S52–S60. [PubMed: 16949389]
12. Takeo C, Negishi E, Nakajima A, et al. Association of cytosine-adenine repeat polymorphism of the estrogen receptor-beta gene with menopausal symptoms. Gend Med. 2005; 2(2):96–105. [PubMed: 16115604]
13. Abel J, Haarmann-Stemmann T. An introduction to the molecular basics of aryl hydrocarbon receptor biology. Biol Chem. 2010; 391(11):1235–1248. [PubMed: 20868221]
14. Puga A, Ma C, Marlowe JL. The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. Biochem Pharmacol. 2009; 77(4):713–722. [PubMed: 18817753]

15. Wormke M, Stoner M, Saville B, et al. The aryl hydrocarbon receptor mediates degradation of estrogen receptor alpha through activation of proteasomes. Mol Cell Biol. 2003; 23(6):1843–1855. [PubMed: 12612060]

16. Safe S, Wormke M. Inhibitory aryl hydrocarbon receptor-estrogen receptor alpha cross-talk and mechanisms of action. Chem Res Toxicol. 2003; 16(7):807–816. [PubMed: 12870882]

17. Napoli N, Rini GB, Serber D, et al. The Val432Leu polymorphism of the CYP1B1 gene is associated with differences in estrogen metabolism and bone density. Bone. 2009; 44(3):442–448. [PubMed: 18977467]

18. Erlik Y, Meldrum DR, Judd HL. Estrogen levels in postmenopausal women with hot flashes. Obstet Gynecol. 1982; 59(4):403–407. [PubMed: 7078891]

19. Overlie I, Moen MH, Holte A, et al. Androgens and estrogens in relation to hot flushes during the menopausal transition. Maturitas. 2002; 41(1):69–77. [PubMed: 11809345]

20. Hanna IH, Dawling S, Roodi N, et al. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res. 2000; 60(13):3440–3444. [PubMed: 10910054]

21. Merisalu A, Punab M, Altmae S, et al. The contribution of genetic variations of aryl hydrocarbon receptor pathway genes to male factor infertility. Fertil Steril. 2007; 88(4):854–859. [PubMed: 17559847]

22. Fukatsu T, Hirokawa Y, Araki T, et al. Genetic polymorphisms of hormone-related genes and prostate cancer risk in the Japanese population. Anticancer Res. 2004; 24(4):2431–2437. [PubMed: 15330195]

23. Miller SR, Gallicchio LM, Lewis LM, et al. Association between race and hot flashes in midlife women. Maturitas. 2006; 54(3):260–269. [PubMed: 16423474]

24. Thurston RC, Sowers MR, Sutton-Tyrrell K, et al. Abdominal adiposity and hot flashes among midlife women. Menopause. 2008; 15(3):429–434. [PubMed: 18204407]

25. Whiteman MK, Staropoli CA, Langenberg PW, et al. Smoking, body mass, and hot flashes in midlife women. Obstet Gynecol. 2003; 101(2):264–272. [PubMed: 12576249]

26. Rylander-Rudqvist T, Wedren S, Granath F, et al. Cytochrome P450 1B1 gene polymorphisms and postmenopausal breast cancer risk. Carcinogenesis. 2003; 24(9):1533–1539. [PubMed: 12844487]

27. Woods NF, Mitchell ES, Tao Y, et al. Polymorphisms in the estrogen synthesis and metabolism pathways and symptoms during the menopausal transition: observations from the Seattle Midlife Women's Health Study. Menopause. 2006; 13(6):902–910. [PubMed: 16977255]

28. Wong JM, Harper PA, Meyer UA, et al. Ethnic variability in the allelic distribution of human aryl hydrocarbon receptor codon 554 and assessment of variant receptor function in vitro. Pharmacogenetics. 2001; 11(1):85–94. [PubMed: 11207035]

Table 1

Sample characteristics (Baltimore metropolitan area, 2000–2004)

Variable	Women without hot flashes	Women with hot flashes	*p* value
	n (%)[a]	n (%)[a]	
Total sample size	267	372	
Age group (years)			
45 to 49	194 (72.7)	219 (58.9)	<0.01
50 to 54	73 (27.3)	153 (41.1)	
Race			
Caucasian	230 (86.1)	302 (81.2)	0.01
African American	28 (10.5)	66 (17.7)	
Other	8 (2.9)	3 (0.8)	
Body mass index (kg/m^2)			<0.01
<25.0	127 (47.6)	146 (39.2)	
25.0 to 29.9	81 (30.3)	94 (25.3)	
≥30.0	59 (22.1)	131 (35.2)	
Cigarette smoking			0.01
Current	16 (6.0)	42 (11.3)	
Former	94(35.2)	153 (41.1)	
Never	157 (58.8)	176 (47.3)	
***AHR* (rs2066853)**			0.06
+/+ (GG)	190 (71.4)	244 (65.8)	
+/− (GA)	69 (25.9)	103 (27.7)	
−/− (AA)	7 (2.7)	24 (6.5)	
***AHRR* (rs2292596)**			0.99
+/+ (CC)	111 (41.7)	157 (42.3)	
+/− (CG)	144 (54.1)	199 (53.6)	
−/− (GG)	11 (4.2)	15 (4.1)	
***ARNT* (rs2228099)**			0.82
+/+ (GG)	84 (31.6)	126 (34.0)	
+/− (GC)	136 (51.1)	182 (49.0)	
−/− (CC)	46 (17.3)	63 (17.0)	
***CYP1B1* (rs1800440)**			0.19
+/+ (AA)	186 (69.9)	251 (67.5)	
+/− (AG)	74 (27.8)	102 (27.4)	
−/− (GG)	6 (2.3)	19 (5.1)	
***CYP1B1* (rs1056836)**			0.30
+/+ (CC)	77 (28.9)	87 (23.5)	
+/− (CG)	122 (45.9)	180 (48.7)	
−/− (GG)	67 (25.2)	103 (27.8)	

[a]Due to missing information, some columns do not add up to the total n listed, and percentages do not add up to 100

Table 2

The associations between the selected SNPs and ever experiencing hot flashes

Genotype	Women without hot flashes	Women with hot flashes	Unadjusted Model		*b* Adjusted model	
	n[a]	n[a]	OR	95% CI	OR	95% CI
***AHR* (rs2066853)**						
+/+ (GG)	190	244	1.00	Referent	1.00	Referent
+/− (GA)	69	103	1.16	0.81,1.67	1.08	0.72, 1.60
−/− (AA)	7	24	**2.67**	**1.13, 6.33**	2.44	0.99, 6.01
***AHRR* (rs2292596)**						
+/+ (CC)	111	157	1.00	Referent	1.00	Referent
+/− (CG)	144	199	0.98	0.71, 1.35	1.03	0.73, 1.46
−/− (GG)	11	15	0.96	0.43, 2.18	0.84	0.36, 1.98
***ARNT* (rs2228099)**						
+/+ (GG)	84	126	1.00	Referent	1.00	Referent
+/− (GC)	136	182	0.89	0.63, 1.27	0.86	0.59, 1.24
−/− (CC)	46	63	0.91	0.57, 1.46	0.83	0.51 1.35
***CYP1B1* (rs1800440)**						
+/+ (AA)	186	251	1.00	Referent	1.00	Referent
+/− (AG)	74	102	1.02	0.72, 1.46	1.07	0.74, 1.55
−/− (GG)	6	19	2.35	0.92, 5.99	2.49	0.95, 6.48
AHR and AHRR						
+/+ and +/+	78	90	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	145	221	1.32	0.91, 1.91	1.29	0.88, 1.88
+/−,−/− and +/−, −/−	43	60	1.21	0.74, 1.98	1.12	0.67, 1.86
AHR* and *ARNT						
+/+ and +/+	63	85	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	148	200	1.00	0.68, 1.48	0.92	0.61, 1.38
+/−,−/− and +/−, −/−	55	86	1.16	0.73, 1.85	0.99	0.60, 1.65
AHR* and *CYP1B1						
+/+ and +/+	123	159	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	128	177	1.07	0.77, 1.48	1.00	0.72, 1.41

Genotype	Women without hot flashes	Women with hot flashes	Unadjusted Model		*b* Adjusted model	
	n[a]	n[a]	OR	95% CI	OR	95% CI
+/−,−/− and +/−, −/−	14	35	**1.93**	**1.00, 3.75**	1.87	0.95, 3.70
***CYP1B1* and *CYP1B1* (rs1056836)**						
+/+ and +/+	75	84	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	112	169	1.35	0.91, 2.00	**1.51**	**1.01, 2.31**
+/−,−/− and +/−, −/−	78	117	1.34	0.88, 2.05	1.54	0.97, 2.42

Reference group for each examined SNP was women homozygous for the major allele SNP= single nucleotide polymorphism

[a]Due to missing information, some columns do not add up to the total n listed, and percentages do not add up to 100

[b]Confounder adjusted model; Adjusted for race, body mass index, age, and smoking status

Table 3

The associations between the selected SNPs and type of hot flashes[a]

Genotype	n	Moderate or severe hot flashes				n	Hot flashes for 1 year				n	Daily hot flashes			
		Unadjusted model		[b] Adjusted model			Unadjusted model		[b] Adjusted model			Unadjusted model		[b] Adjusted model	
		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI
***AHR* (rs2066853)**															
+/+ (GG)	152	1.00	Referent	1.00	Referent	159	1.00	Referent	1.00	Referent	52	1.00	Referent	1.00	Referent
+/− (GA)	69	1.25	0.84, 1.86	1.14	0.73, 1.76	65	1.13	0.76, 1.68	0.95	0.61, 1.49	28	1.48	0.87, 2.53	1.46	0.81, 2.64
−/− (AA)	14	2.50	0.98, 6.35	2.21	0.82, 5.96	17	**2.90**	**1.17, 7.17**	2.37	0.90, 6.21	5	2.61	0.80, 8.56	2.66	0.70, 0.11
***AHRR* (rs2292596)**															
+/+ (CC)	90	1.00	Referent	1.00	Referent	105	1.00	Referent	1.00	Referent	35	1.00	Referent	1.00	Referent
+/− (CG)	132	1.13	0.79, 1.63	1.22	0.83, 1.79	128	0.94	0.66, 1.34	0.97	0.66, 1.42	48	1.06	0.64, 1.75	1.12	0.66, 1.90
−/− (GG)	13	1.46	0.62, 3.41	1.30	0.53, 3.18	8	0.77	0.30, 1.99	0.60	0.22, 1.66	2	0.58	0.12, 2.73	0.59	0.12, 3.02
***ARNT* (rs2228099)**															
+/+ (GG)	84	1.00	Referent	1.00	Referent	83	1.00	Referent	1.00	Referent	28	1.00	Referent	1.00	Referent
+/− (GC)	109	0.80	0.54, 1.19	0.77	0.51, 1.16	118	0.88	0.59, 1.30	0.87	0.58, 1.31	42	0.93	0.53, 1.61	0.82	0.45, 1.47
−/− (CC)	42	0.91	0.55, 1.53	0.81	0.47, 1.39	40	0.88	0.52, 1.48	0.83	0.48, 1.43	15	0.98	0.48, 2.02	0.79	0.36, 1.70
***CYP1B1* (rs1800440)**															
+/+ (AA)	159	1.00	Referent	1.00	Referent	163	1.00	Referent	1.00	Referent	59	1.00	Referent	1.00	Referent
+/− (AG)	64	1.01	0.68, 1.50	1.04	0.68, 1.58	64	0.99	0.67, 1.47	1.10	0.72, 1.67	24	1.02	0.59, 1.76	1.14	0.63, 2.05
−/− (GG)	13	2.53	0.94, 6.82	2.62	0.94, 7.31	15	**2.85**	**1.08, 7.52**	**3.05**	**1.12, 8.25**	3	1.58	0.38, 6.50	1.60	0.36, 7.20
AHR* and *AHRR															
+/+ and +/+	53	1.00	Referent	1.00	Referent	65	1.00	Referent	1.00	Referent	21	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	136	1.38	0.91, 2.10	1.38	0.89, 2.13	134	1.11	0.74, 1.66	0.98	0.64, 1.49	45	1.15	0.64, 2.07	1.24	0.66, 2.31
+/−,−/− and +/−, −/−	46	1.57	0.92, 2.71	1.44	0.81, 2.55	42	1.17	0.69, 2.01	0.99	0.55, 1.74	19	1.64	0.80, 3.38	1.61	0.74, 3.50
AHR* and *ARNT															
+/+ and +/+	58	1.00	Referent	1.00	Referent	55	1.00	Referent	1.00	Referent	16	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	120	0.88	0.57, 1.35	0.79	0.51, 1.24	132	1.02	0.66, 1.57	0.93	0.59, 1.46	48	1.28	0.68, 1.52	1.11	0.56, 2.20
+/−,−/− and +/−, −/−	57	1.13	0.67, 1.88	0.94	0.53, 1.64	54	1.13	0.67, 1.89	0.90	0.51, 1.60	21	1.50	0.71, 3.16	1.24	0.54, 2.83
AHR* and *CYP1B1															
+/+ and +/+	98	1.00	Referent	1.00	Referent	102	1.00	Referent	1.00	Referent	33	1.00	Referent	1.00	Referent

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Genotype	n	Moderate or severe hot flashes				n	Hot flashes for 1 year				n	Daily hot flashes			
		Unadjusted model		[b] Adjusted model			Unadjusted model		[b] Adjusted model			Unadjusted model		[b] Adjusted model	
		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI
+/+ and +/− or −/−	115	1.13	0.78, 1.63	1.06	0.72, 1.55	118	1.11	0.77, 1.60	1.01	0.69, 1.48	45	1.31	0.79, 2.19	1.28	0.74, 2.22
+/−,−/− and +/−, −/−	22	1.97	0.96, 4.06	1.72	0.82, 3.64	21	1.81	0.88, 3.74	1.71	0.81, 3.62	7	1.86	0.70, 4.99	2.11	0.75, 5.95
***CYP1B1* and *CYP1B1* (rs1056836)**															
+/+ and +/+	48	1.00	Referent	1.00	Referent	53	1.00	Referent	1.00	Referent	18	1.00	Referent	1.00	Referent
+/+ and +/− or −/−	113	**1.58**	**1.01, 2.46**	**1.81**	**1.13, 2.89**	110	1.39	0.90, 2.16	**1.64**	**1.02, 2.62**	41	1.53	0.82, 2.85	1.84	0.93, 3.65
+/−,−/− and +/−, −/−	74	1.48	0.92, 2.40	**1.68**	**1.00, 2.82**	79	1.43	0.90, 2.30	**1.77**	**1.06, 2.96**	26	1.39	0.70, 2.74	1.74	0.82, 3.69

Reference group for each examined SNP was women homozygous for the major allele; SNP= single nucleotide polymorphism

[a]The comparison group for all hot flash outcomes was women who reported that they never experienced hot flashes

[b]Confounder adjusted model; Adjusted for race, body mass index, age, and smoking status

Table 4

The association between SNPs and hormone serum concentrations, ratios, and SHBG concentration

Genotype	Estradiol (pg/mL)		Estrone (pg/mL)		Testosterone (ng/mL)		Progesterone (ng/mL)		SHBG (nmol/L)		Free estradiol index		Free testosterone index	
	Geometric mean 95% LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value
***AHR* (rs2066853)**														
+/+ (GG)	101.28 93.79, 109.37	0.21	136.10 127.27, 145.54	0.06	0.46 0.43, 0.49	0.41	0.83 0.73, 0.95	0.38	**59.62** **56.34, 63.08**	**0.05**	0.62 0.57, 0.68	0.77	2.66 2.44, 2.90	0.07
+/− (GA)	89.83 79.50, 101.49		129.25 116.19, 143.78		0.49 0.44, 0.53		0.76 0.61, 0.94		**54.66** **49.97, 59.79**		0.60 0.52, 0.70		3.09 2.69, 3.55	
−/− (AA)	88.63 66.48, 118.16		100.32 78.06, 128.94		0.49 0.29, 0.61		0.59 0.35, 0.98		**47.22** **38.22, 58.34**		0.69 0.49, 0.97		3.59 2.59, 4.97	
***AHRR* (rs2292596)**														
+/+ (CC)	95.41 86.50, 105.23	0.83	133.02 122.10, 144.92	0.80	0.45 0.42, 0.49	0.49	0.80 0.67, 0.94	0.86	59.22 55.10, 63.65	0.23	0.59 0.53, 0.66	0.29	2.65 2.37, 2.96	0.19
+/− (CG)	99.23 90.99, 108.21		130.82 121.28, 141.11		0.48 0.45, 0.51		0.81 0.69, 0.94		55.74 52.30, 59.41		0.65 0.59, 0.72		2.98 2.70, 3.29	
−/− (GG)	94.76 69.18, 129.81		143.49 108.98, 188.93		0.45 0.36, 0.57		0.69 0.39, 1.20		66.15 52.49, 83.37		0.53 0.36, 0.76		2.37 1.66, 3.39	
***ARNT* (rs2228099)**														
+/+ (GG)	105.59 94.55, 117.93	0.20	**143.30** **130.13, 157.79**	**0.04**	0.50 0.46, 0.54	0.14	0.85 0.70, 1.04	0.44	60.75 56.00, 65.90	0.24	0.64 0.56, 0.73	0.70	2.84 2.51, 3.22	0.73
+/− (GC)	92.80 84.83, 101.52		**131.28** **121.39, 141.97**		0.45 0.42, 0.48		0.81 0.69, 0.95		56.63 53.00, 60.50		0.60 0.54, 0.67		2.74 2.474, 3.04	
−/− (CC)	96.11 82.44, 112.03		**115.72** **101.24, 132.28**		0.47 0.42, 0.52		0.68 0.52, 0.90		54.51 48.68, 61.03		0.65 0.54, 0.78		2.97 2.49, 3.53	
***CYP1B1* (rs1800440)**														
+/+ (AA)	**97.00** **89.85, 104.71**	**0.04**	132.17 123.61, 141.31	0.19	0.46 0.44, 0.49	0.79	0.79 0.69, 0.91	0.98	57.90 54.71, 61.26	0.92	0.62 0.56, 0.67	0.08	2.77 2.54, 3.03	0.81
+/− (AG)	**103.28** **91.55–116.51**		136.10 122.48, 151.26		0.48 0.44, 0.52		0.80 0.65, 0.99		56.64 51.81, 61.92		0.67 0.58, 0.77		2.91 2.54, 3.34	
−/− (GG)	**66.41** **48.23, 91.44**		103.27 78.06, 136.61		0.44 0.35, 0.58		0.75 0.43, 1.33		57.00 49.74, 72.19		0.43 0.29, 0.62		2.68 1.86, 3.86	

95% LL,UL= 95% confidence limits; lower limit, upper limit

SHBG= sex hormone binding globulin

SNP= single nucleotide polymorphism

Table 5

The association between combined SNPs, hormone serum concentrations, ratios and SHBG concentration

Genotype	Estradiol (pg/mL)		Estrone (pg/mL)		Testosterone (ng/mL)		Progesterone (ng/mL)		SHBG (nmol/L)		Free estradiol index		Free testosterone index	
	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value	Geometric mean 95%LL, UL	*p* value
AHR* and *AHRR														
+/+ and +/+	97.75 86.39, 110.62	0.35	133.39 119.74, 148.59	0.15	0.43 0.40, 0.48	0.17	0.81 0.65, 1.01	0.50	**63.41** **57.91, 69.44**	**0.05**	0.57 0.49, 0.65	0.26	**2.37** **2.06, 2.72**	**0.02**
+/+ and +/− or −/−	100.15 92.11, 108.90		136.34 126.72, 146.68		0.48 0.45, 0.51		0.83 0.71, 0.96		**56.06** **52.72, 59.62**		0.66 0.59, 0.72		**2.95** **2.69, 3.25**	
+/−,−/− and +/−, −/−	87.80 74.97, 102.81		116.98 101.92, 134.28		0.49 0.43, 0.55		0.69 0.52, 0.91		**54.09** **48.17, 60.74**		0.60 0.50, 0.72		**3.12** **2.61, 3.73**	
AHR* and *ARNT														
+/+ and +/+	**110.08** **96.53, 125.52**	**0.05**	**149.34** **133.14, 167.50**	**0.04**	**0.51** **0.46, 0.56**	**0.04**	0.88 0.69, 1.11	0.43	**63.15** **57.33, 69.56**	**0.04**	0.64 0.55, 0.75	0.90	2.78 2.39, 3.23	0.12
+/+ and +/− or −/−	**96.76** **88.82, 105.41**		**129.74** **120.39, 139.83**		**0.44** **0.42, 0.47**		0.81 0.69, 0.94		**57.42** **53.91, 61.16**		0.62 0.56, 0.68		2.67 2.42, 2.94	
+/−,−/− and +/−, −/−	**87.14** **76.17, 99.69**		**122.00** **108.46, 137.22**		**0.49** **0.44, 0.54**		0.70 0.55, 0.89		**52.62** **47.66, 58.10**		0.61 0.52, 0.71		3.23 2.77, 3.77	
AHR* and *CYP1B1														
+/+ and +/+	100.75 91.57, 110.85	0.49	135.00 124.19, 146.74	0.31	0.45 0.42, 0.49	0.49	0.85 0.71, 1.00	0.62	60.42 56.33, 64.82	0.17	0.61 0.55, 0.69	0.89	2.59 2.33, 2.89	0.13
+/+ and +/− or −/−	96.07 87.64, 105.32		132.56 122.35, 143.64		0.48 0.44, 0.51		0.75 0.64, 0.89		55.67 52.04, 59.56		0.63 0.57, 0.71		2.96 2.67, 3.29	
+/−,−/− and +/−, −/−	87.47 69.56, 110.01		114.08 93.39, 139.36		0.49 0.42, 0.58		0.79 0.53, 1.19		53.28 45.02, 63.06		0.60 0.46, 0.79		3.20 2.47, 4.16	
***CYP1B1* and *CYP1B1* (rs1056836)**														
+/+ and +/+	99.47 87.57, 112.99	0.81	139.35 124.69, 155.73	0.39	0.49 0.45, 0.54	0.24	0.88 0.70, 1.10	0.51	56.42 51.40, 61.93	0.73	0.65 0.56, 0.75	0.58	3.03 2.62, 3.49	0.27
+/+ and +/− or −/−	95.00 86.32, 104.55		126.65 116.50, 137.70		0.45 0.42, 0.48		0.74 0.63, 0.88		58.80 54.82, 63.07		0.59 0.53, 0.66		2.63 2.36, 2.93	
+/−,−/− and +/−, −/−	98.67 87.95, 110.71		133.04 120.34, 147.09		0.47 0.43, 0.51		0.79 0.65, 0.97		56.80 52.22, 61.79		0.64 0.56, 0.73		2.88 2.53, 3.28	

95% LL,UL= 95% confidence limits; lower limit, upper limit

SHBG= sex hormone binding globulin

SNP= single nucleotide polymorphism