Published in final edited form as:

Maturitas. 2007 June 20; 57(2): 120–131.

Genetic Polymorphisms, Hormone Levels, and Hot Flashes in Midlife Women

Chrissy Schilling^a, Lisa Gallicchio^b, Susan R. Miller^c, Patricia Langenberg^a, Howard Zacur^c, and Jodi A. Flaws^{a,d}

^aDepartment of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland ^bPrevention and Research Center, Weinberg Center for Women's Health & Medicine, Mercy Medical Center, Baltimore, Maryland ^cDepartment of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Maryland ^dDepartment of Veterinary Biosciences, University of Illinois, Urbana, Illinois

Abstract

Objective—Hot flashes disrupt the lives of millions of women each year. Although hot flashes are a public health concern, little is known about risk factors that predispose women to hot flashes. Thus, the objective of this study was to examine whether sex steroid hormone levels and genetic polymorphisms in hormone biosynthesis and degradation enzymes are associated with the risk of hot flashes.

Methods—In a cross-sectional study design, midlife women aged 45 to 54 years (n=639) were recruited from Baltimore and its surrounding counties. Participants completed a questionnaire and donated a blood sample for steroid hormone analysis and genotyping. The associations between genetic polymorphisms and hormone levels, as well as the associations between genetic polymorphisms, hormone levels, and hot flashes were examined using statistical models.

Results—A polymorphism in *CYP11B* was associated with lower dehydroepiandrosterone-sulfate (DHEA-S) and progesterone levels, while a polymorphism in *CYP19* (*aromatase*) was associated with higher testosterone and DHEA-S levels. Lower progesterone and sex hormone binding globulin levels, lower free estradiol index, and a higher ratio of total androgens to total estrogens were associated with the experiencing of hot flashes. A polymorphism in *CYP11B* and a polymorphism in *3βHSD* were both associated with hot flashes.

Conclusion—Some genetic polymorphisms may be associated with altered levels of hormones in midlife women. Further, selected genetic polymorphisms and altered hormone levels may be associated with the risk of hot flashes in midlife women.

Keywords

Hot flashes; genetic polymorphisms; sex hormones

Corresponding Author: Jodi A. Flaws, Ph.D., University of Illinois, Department of Veterinary Biosciences, 3223 VMBS Building, 2001 S. Lincoln Ave., Urbana, IL 61802, Telephone (217) 333-7933, Email jflaws@uiuc.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Hot flashes, generally defined as periods of intense heat in the upper torso, neck, and face, can seriously disrupt a woman's quality of life [1]. Many women feel tired as a result of poor sleep quality resulting from being awakened during the night by their hot flashes [2,3]. For these reasons, hot flashes are a major reason that women seek medical care during the menopausal transition [3]. Even though more than 40 million women experience hot flashes each year [3], little is known about the factors that predispose women to hot flashes.

Previous studies indicate that some sex steroid hormones, but not all, may be associated with the risk of hot flashes [4-7]. For example, studies have shown that low endogenous total (bound and free) estradiol and estrone levels are associated with hot flashes [4,5], while other studies suggest that total androgen levels may not be associated with hot flashes [6,7]. In contrast, limited information is available on the association between free (amount unbound by sex hormone binding globulin (SHBG) and therefore thought to be biologically active) estrogen, free androgen, and progesterone levels and the risk of hot flashes. Thus, one purpose of this study was to examine the relation between free estrogen, free androgen, and progesterone levels and hot flashes.

Genetic polymorphisms may also be associated with the risk of hot flashes. For example, polymorphisms in *estrogen receptor-α* (*ERα*) have been associated with low response of tissues to estrogen, as well as the risk of hot flashes [8]. In addition, a polymorphism in an estrogen metabolizing enzyme, cytochrome P450 1B1 (*CYP1B1*), was found to be associated with the severity, frequency, and duration of hot flashes [5]. Polymorphisms in other estrogen synthesizing and metabolizing enzymes, *CYPc17α* and *CYP1A1*, however, have not been found to be associated with hot flashes [5]. To our knowledge, studies have not examined in detail the associations between other genetic polymorphisms and hot flashes. Therefore, the second purpose of this study was to examine the relation between genetic polymorphisms in other genes that encode estrogen synthesis and metabolizing enzymes and the risk of hot flashes.

The mechanism by which genetic polymorphisms may be associated with hot flashes is unclear, but it is likely that some genetic polymorphisms are associated with altered hormone levels. In turn, these altered hormone levels may be associated with the risk of hot flashes. While no studies have directly tested this association, a few studies have shown that polymorphisms in certain genes that encode estrogen synthesis and metabolizing enzymes are associated with altered hormone levels [9-15]. Specifically, a polymorphism in *CYPc17α* has been associated with higher estradiol and progesterone levels [9], higher estradiol only in women of low body mass index (BMI) [15], higher estrone levels [14], and higher SHBG levels [13]. A polymorphism in *CYP19* (*aromatase*) has been associated with lower estradiol [10,11] and estrone levels [10]. In contrast, polymorphisms in *CYPc17α* or *CYP19* have not been associated with estradiol levels [12], and a polymorphism in *catechol-o-methyl transferase* (*COMT*) has not been associated with estradiol or estrone levels [16]. Since no studies have examined the associations among genetic polymorphisms, sex steroid levels, and hot flashes in a single study, the third purpose of this study was to determine if polymorphisms in selected genes that encode estrogen synthesis and metabolizing enzymes are associated with altered sex steroid hormone levels and the risk of hot flashes.

Materials and Methods

Study population and design

Sample methods have been previously described in detail elsewhere [17-20]. Briefly, women aged 45 to 54 years were mailed an invitation to participate in a cross-sectional study of midlife

health. Interested participants contacted the clinic, and if the staff determined they were eligible to participate, a visit was scheduled. All clinic visits were scheduled in the morning and the women were instructed to fast overnight prior to the visit. At the clinic visit, participants were weighed, measured, and had their blood drawn for hormone assays and genotyping. Each woman was eligible for study participation if she was between 45 and 54 years of age and had intact ovaries and uterus. To ensure that women enrolled in the study were not post-menopausal, women were eligible only if they reported having at least 3 menstrual periods in the last 12 months. Women were excluded if they were pregnant, were taking hormone replacement therapy or hormonal contraception, or had a history of cancer. All participants in this study gave written informed consent according to procedures approved by the University of Maryland School of Medicine and Johns Hopkins University Institutional Review Boards.

The participants also completed the study survey, which included questions regarding demographic information, reproductive history, menstrual cycle characteristics, hormonal contraceptive use, menopausal symptoms, hormone replacement therapy use, medical and family history, and health behaviors (smoking, vitamin use, and eating habits). Women were considered pre-menopausal if they reported experiencing their last menstrual period in the previous 3 months and 11 or more periods within the previous year. Women were considered peri-menopausal if they reported their last menstrual period was within the previous year, but not within the previous 3 months, or their last menstrual period was within the previous 3 months, but they experienced 10 or fewer periods in the previous year. Women were considered post-menopausal, and excluded from the study, if they had not had a period in the previous 12 months.

Hormone assays

Serum concentrations of sex hormone binding globulin (SHBG), estradiol, estrone, testosterone, androstenedione, progesterone, and dehydroepiandrosterone sulfate (DHEA-S) were measured using enzyme-linked immunosorbent assays (ELISA). ELISA kits for estradiol, testosterone, androstenedione, and DHEA-S were obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX). ELISA kits for estrone, progesterone, and SHBG were obtained from American Laboratory Products Company (Windham, NH). The assays were run using the manufacturers' instructions [18]. 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 analysis. The laboratory personnel were blind with respect to any information concerning study subjects. For quality control purposes, samples from both women with hot flashes and women without hot flashes were run within the same laboratory batches. In addition, positive controls containing known amounts of estradiol, estrone, testosterone, androstenedione, progesterone, DHEA-S, 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.

The minimum detection limits and intra-assay coefficients of variation were as follows: estradiol 7pg/ml, $3.3 \pm 0.17\%$; estrone 10pg/ml, $4.8 \pm 0.25\%$; testosterone 0.04ng/ml, $2.2 \pm 0.56\%$; androstenedione 0.03ng/ml, $2.5 \pm 0.60\%$; DHEA-S 15ng/ml, $1.9 \pm 0.63\%$; progesterone 0.1ng/ml, $2.1 \pm 0.65\%$; and SHBG 0.1nmol/L, $2.4 \pm 0.67\%$. No estradiol, estrone, testosterone, androstenedione, DHEA-S, or SHBG samples were below the limit of detection. For progesterone samples that were below the limit of detection ($n=66$), the value was set at the limit of detection (0.1ng/ml). The average inter-assay coefficient of variation for all assays was less than 5%.

To estimate the amount of estradiol unbound by SHBG (free estradiol index, FEI), and therefore thought to be biologically active, a ratio of total estradiol to SHBG was calculated using a conversion factor to change pg/ml of estradiol to nmol/L: $100 \times (\text{total estradiol} \times 0.003671) /$

SHBG [21]. The free testosterone index (FTI) was also estimated using a conversion factor to change ng/ml of testosterone to nmol/L: $100 \times (\text{total testosterone} \times 3.467) / \text{SHBG}$ [21]. Other hormones measured do not bind appreciably to SHBG [22,23]; therefore free index calculations were not performed. The ratio of total androgens (androstenedione plus testosterone) to total estrogens (estradiol plus estrone) was also calculated as a measure of the degree of aromatization.

Genotyping

Genomic DNA was isolated from whole blood using the GenElute Blood Genomic DNA kit (Sigma, St. Louis, MO). All enzymes were obtained from New England BioLabs (Ipswich, MA). All primers were synthesized by the Biopolymer Lab at the University of Maryland (Baltimore, MD). Detection of the *CYPc17a* polymorphism was carried out using published methods and primers used were: forward 5'-CATTCGCACCTCTGGAGTC-3', reverse 5'-GGCTCTTGG GGTACTTG-3'. One band at 459 base pairs (bp) indicated the subject was homozygous for the wild-type allele (+/+). One band at 459 bp, one band at 335 bp, and one band at 124 bp indicated heterozygosity (+/-). Bands located at 335 bp and 124 bp indicated homozygosity for the mutant allele (-/-) [5,24]. Detection of the *CYP11B1* polymorphism was carried out using published methods and primers used were: forward 5'-CTGCCA ACACCTCTGTC TTG-3', reverse 5'-CTGAAATCGCACTGGTGAGC-3'. One band at 271 bp indicated the subject was homozygous for the wild-type allele (+/+). One band at 271 bp, one band at 166 bp, and one band at 105 bp indicated heterozygosity (+/-). Bands located at 166 bp and 105 bp indicated homozygosity for the mutant allele (-/-) [5,25-27]. Detection of the *CYP11A1* polymorphism was carried out using published methods and primers used were: forward 5'-CAGTGAAGAGGTGTAGCCGCT-3', reverse 5'-TAGGAGTCTTGTCTCATGCCT-3'. One band at 340 bp indicated the subject was homozygous for the wild-type allele (+/+). One band at 340 bp, one band at 200 bp, and one band at 140 bp indicated heterozygosity (+/-). Bands located at 200 bp and 140 bp indicated homozygosity for the mutant allele (-/-) [5,28,29]. Detection of the *COMT* polymorphism was carried out using published methods and primers used were: forward 5'-TACTGTGGCTACTCAGCTGTGC-3', reverse 5'-GTGA ACGTGGTGTGAACACC-3'. One band at 114 bp indicated the subject was homozygous for the wild-type allele (+/+). One band at 114 bp and one band at 96 bp indicated heterozygosity (+/-). One band located at 96 bp indicated homozygosity for the mutant allele (-/-) [16,30,31]. Detection of the *3β-hydroxysteroid dehydrogenase (3βHSD)* polymorphism was carried out using published methods and primers used were: forward 5'-AAGTGTGAAAGT TCTCCACTGTG-3', reverse 5'-GTGCCCTT GTCACCTTCTGTATGAG-3'. One band at 575 bp indicated the subject was homozygous for the wild-type allele (+/+). One band at 575 bp, one band at 371 bp, and one band at 204 bp indicated heterozygosity (+/-). Bands located at 371 bp and 204 bp indicated homozygosity for the mutant allele (-/-) [32,33]. Detection of the *CYP19* polymorphism was carried out using published methods and primers used were: forward 5'-AGTAACACAGA ACAGTTGCA-3', reverse 5'-TCCAGACTCG CATGAATTCTCCGTA-3'. One band at 188 bp indicated the subject was homozygous for the wild-type allele (+/+). One band at 188 bp and one band at 164 bp indicated heterozygosity (+/-). One band located at 164 bp indicated homozygosity for the mutant allele (-/-) [11].

In all genotyping assays, laboratory personnel were blinded to hot flash status and the polymorphic status of each sample was determined by at least two investigators. In approximately 98% of the readings, there was complete agreement between the two investigators. In the rare instances that there was not agreement, the samples were rerun and agreement was reached between the investigators. Each gel contained samples from women with hot flashes and women without hot flashes as well as samples without DNA (negative controls) and samples with known polymorphisms (positive controls).

Statistical analyses

Characteristics of women with hot flashes and women without hot flashes were compared using chi-square analyses.

Associations between hormone levels and genotype, and associations between hormone levels and hot flash variables, were examined using general linear models. In addition, associations between free estradiol index (FEI), free testosterone index (FTI), the ratio of total androgen to total estrogen, and hot flash variables were examined using general linear models. In adjusted analyses, factors were considered to be potential confounders if they were associated ($p < 0.1$) with genotype, FEI, FTI, or hormone levels. Variables used in the final confounder-adjusted model were age, race, smoking status, BMI, and number of days since last menstrual period.

Polymorphisms were categorized as wild type (+/+), heterozygous (+/-), or homozygous (-/-). The +/+ group served as the reference group for the homozygous or heterozygous variants. The analyses were completed treating each group separately as well as combining the homozygous and heterozygous variants. Risk ratios (RR) and 95 % confidence intervals (95% CI) were calculated using modified Poisson models [34] to assess the association between specific *CYP450* polymorphisms and the occurrence, severity, frequency, and duration of hot flashes controlling for potential confounders. Categories for the hot flash variables were created based on distributions among participants and clinical relevance. Each outcome was assessed using a separate model, and analyses were adjusted for race. In addition, hormones that were determined to be associated with hot flashes were added to the polymorphism and hot flash model. To examine dose-response associations with multiple polymorphisms, a trend test was performed across all levels of exposure in a logistic regression model.

In all analyses, hormone variables, BMI, and number of days since last menstrual period were log transformed, as none were normally distributed. Analyses were performed using SPSS Version 11.0 (Chicago, IL) and SAS Version 9.1 (Cary, NC). A p-value of less than 0.05 was considered to be statistically significant.

Results

Characteristics of women with hot flashes and women without hot flashes are presented in Table 1. Women with hot flashes were more likely to be older, of black race, current or former smokers, and have a higher body mass index compared to women without hot flashes. Although women with hot flashes were more likely to be of black race, the association between black race and hot flashes was not due to racial differences in socioeconomic status between black and white women in our study population (data not shown) [19]. The percentage of women with individual genetic polymorphisms was not significantly different between women with and without hot flashes.

The association between genetic polymorphisms and sex steroid hormone levels is presented in Table 2. Polymorphisms in *CYPc17a*, *CYP11A1*, *COMT*, or *3βHSD* were not associated with any of the selected hormones. In contrast, a polymorphism in *CYP11B1* was associated with lower levels of DHEA-S and progesterone, although the associations were of borderline significance. A polymorphism in *CYP19* was associated with a higher DHEA-S level ($p = 0.006$) and a higher testosterone level ($p = 0.06$).

Sex steroid hormone levels and their association with hot flash variables are shown in Table 3. Testosterone, androstenedione, and DHEA-S levels were not associated with recent hot flashes, severity of hot flashes, frequency of hot flashes, or duration of hot flashes. Progesterone levels were significantly lower in women with hot flashes in the past 30 days ($p = 0.001$), moderate or severe hot flashes ($p = 0.003$), hot flashes that occur at least weekly ($p = 0.001$), and

hot flashes that occurred for less than one year ($p=0.002$), compared to women who never had hot flashes. To determine if the association between higher progesterone levels and never experiencing hot flashes was due to a corresponding increase in estrogen levels, estradiol, estrone, and the ratio of total androgen to total estrogen were independently added to the progesterone and hot flashes model. Adding estradiol, estrone, or the ratio of total androgen to total estrogen to the progesterone and hot flashes model did not change the associations reported (data not shown). SHBG levels were significantly lower in women with hot flashes in the past 30 days ($p=0.03$) and in women with hot flashes that occur at least weekly ($p=0.003$), compared to women who have never had hot flashes. SHBG levels were not associated with moderate or severe hot flashes or duration of hot flash occurrence. Similar to our previous study, lower total estradiol and estrone levels were associated with hot flashes (data not shown) [5].

The association between the free estrogen index (FEI), the free testosterone index (FTI), and the total androgen to total estrogen ratio and hot flashes is shown in Table 4. The FTI was not associated with hot flashes in the past 30 days, or the severity, frequency, or duration of hot flashes. The FEI, however, was significantly lower in women who experienced hot flashes within the past 30 days ($p=0.04$), women with moderate or severe hot flashes ($p=0.04$), women who experienced hot flashes at least weekly ($p=0.02$), and women who have experienced hot flashes for less than one year ($p=0.04$), compared to women who have never experienced hot flashes. Conversely, the ratio of total androgens to total estrogens was higher in women who had hot flashes in the past 30 days ($p=0.002$), had severe hot flashes ($p=0.007$), had frequent hot flashes ($p=0.001$), or had experienced hot flashes for less than one year ($p=0.002$), compared to women who had not experienced hot flashes.

The risk ratios (RR) of experiencing hot flashes by *3βHSD*, *CYP19*, and *COMT* genotype are shown in Table 5. Polymorphisms in *CYP19* and *COMT* were not significantly associated with any of the hot flash variables examined. In contrast, having a polymorphism in *3βHSD* was associated with a significantly higher risk of experiencing moderate or severe hot flashes compared to not having the polymorphism (RR: 1.38, 95% Confidence Interval (95% CI): 1.00, 1.90). A polymorphism in *3βHSD* was also associated with a higher risk of experiencing hot flashes for a year or more compared to not having the polymorphism, although the RR was of borderline statistical significance (RR: 1.36, 95% CI: 0.99, 1.86). In agreement with our previous report, a polymorphism in *CYP1B1* was associated with a significantly higher risk of experiencing any hot flashes (RR: 1.18, 95% CI: 1.00, 1.40), moderate or severe hot flashes (RR: 1.33, 95% CI: 1.04, 1.71), hot flashes that occur at least weekly (RR: 1.37, 95% CI: 1.02, 1.84), and hot flashes that occur for a year or more (RR: 1.33, 95% CI: 1.05, 1.69), compared to not having the polymorphism [5].

A model was generated to determine if women who carry a polymorphism in both genes related to hot flashes (*3βHSD* and *CYP1B1*) would have a higher risk of experiencing hot flashes than women who do not carry a polymorphism or only carry one polymorphism (Table 5). Women who carried a polymorphism in both *3βHSD* and *CYP1B1* had a higher risk of experiencing hot flashes that occur for a year or more (RR: 2.77, 95% CI: 0.99, 7.80) compared to women who did not carry either of the polymorphisms. In addition, there was a statistically significant trend for a higher risk of hot flashes with the number of polymorphisms carried. Women who carried polymorphisms in both *3βHSD* and *CYP1B1* had a higher risk of experiencing any hot flashes ($p\text{-for-trend}=0.02$), moderate or severe hot flashes ($p\text{-for-trend}=0.006$), or hot flashes that occur at least weekly ($p\text{-for-trend}=0.02$) compared to women who do not carry the polymorphisms.

In addition, the hormones that were associated with hot flashes (progesterone and SHBG) were independently added to the polymorphism and hot flashes models. The RRs for hot flashes did

not change when SHBG was added to any of the models (data not shown). When progesterone was added to the model examining the association between a polymorphism in *CYP11B1* and moderate or severe hot flashes, the point estimate decreased slightly and became non-significant (RR: 1.16, 95% CI: 0.98, 1.37), indicating progesterone may explain a small part of the association. RRs for the other models examining the role of progesterone in the association between polymorphisms and hot flashes did not change.

Discussion

Collectively, the data from this study indicate that some sex steroid hormones (progesterone, FEI, ratio of total androgens to total estrogens, and SHBG) and certain polymorphisms in genes that encode estrogen synthesis and metabolizing enzymes (*3βHSD* and *CYP11B1*) are associated with an increased risk of hot flashes in midlife women. In addition, we have shown that women who carry polymorphisms in both genes related to hot flashes (*3βHSD* and *CYP11B1*) have a higher risk of experiencing hot flashes compared to women who do not carry a polymorphism in either gene, or only carry a polymorphism in one gene. Further, some genetic polymorphisms in estrogen synthesis and metabolizing enzymes (*CYP11B1* and *CYP19*) may be associated with altered hormone levels (progesterone, DHEA-S, and testosterone).

Consistent with other reports, we observed no difference in the levels of testosterone or its precursors, androstenedione and DHEA-S, between women with hot flashes and women without hot flashes [6,7]. Although our study suggests that SHBG levels are lower in women with hot flashes than in women who have never experienced hot flashes, this is different from two previously published studies that reported no change in SHBG levels with hot flashes [4, 7]. These conflicting observations may be due to differences in study sample, data analysis, or categorization of hot flash variables. The FEI and FTI data in our study, however, which are estimated by using SHBG levels, are consistent with previous reports of lower FEI levels [4, 7] and no change in FTI levels [7] in women with hot flashes compared to women without hot flashes. The ratio of total androgens (androstenedione plus testosterone) to total estrogens (estradiol plus estrone) was significantly higher in women with hot flashes compared to women without hot flashes. This association has not been examined previously, but the result was somewhat expected, as women with hot flashes have been reported to have lower estrogen levels than women without hot flashes [4-7,17,18].

This is the first study we are aware of to examine the association between progesterone levels and the risk of hot flashes. The mean progesterone levels in this study were between 0.5 and 1.0ng/ml, which is consistent with the levels expected for women in the age range examined in the study [35]. Although the progesterone levels were low overall, there were significantly lower levels in women experiencing recent, severe, frequent, and shorter duration hot flashes, compared to women who have not experienced hot flashes. Progestins and estrogens are thought to interact with several neurotransmitter pathways in the hypothalamus responsible for central thermoregulation [36]. Therefore, this association between progesterone levels and hot flashes may be an important one. Previous studies have shown that women given progesterone for treatment of hot flashes experience significantly fewer hot flashes compared to women given placebo [reviewed in 36,37]. Our data support this finding and suggest that low doses of progesterone may be enough to reduce vasomotor symptoms. However, this hypothesis needs to be tested directly.

An area of emerging interest is the association between genetic polymorphisms and hot flashes. A previous study showed an association of polymorphisms in *ERα* with hot flashes [8]. Our previous study showed an association between a polymorphism in *CYP11B1* and hot flashes [5], although two subsequent studies did not observe this association in other populations [38,39]. The link between polymorphisms and hot flashes may be due to the polymorphism

altering sex steroid hormone levels. Current data regarding the effects of genotype on hormone levels, however, are limited. Consistent with previous reports, we showed no difference in estradiol levels with a polymorphism in *CYPc17a* [12] and no difference in estradiol or estrone levels with a polymorphism in *COMT* [16,40], *CYP19* [40], or *CYP1B1* [40]. Other studies, however, have shown lower estradiol [10,11] and estrone [10] levels with a polymorphism in *CYP19*. We observed higher hormone levels with a polymorphism in *CYP19*, but only the higher testosterone and DHEA-S levels were statistically significant. Differences in findings may be due to our study consisting of pre- and peri-menopausal Caucasian and African-American women, while the Dunning *et al* [10] and Somner *et al* [11] studies consisted of post-menopausal Caucasian women only. We also showed that progesterone levels may explain a small part of the association between a polymorphism in *CYP1B1* and the risk of moderate or severe hot flashes. This indicates that some of these polymorphisms may have functional significance.

The presented results must be taken in context of their limitations. The study design does not allow us to determine the temporality of the association between hormone levels and hot flashes, or the prevalence of hot flashes in the population. Blood samples were not collected during the same day of the menstrual cycle for pre-menopausal women and samples were obtained only once. Further, the assays used to measure hormone levels may not be as sensitive and specific as newly developed assays such as sequential mass spectrometry [41]. To attempt to minimize the daily and monthly variability of hormone levels, samples were collected between 8 and 10 AM after an overnight fast, and statistical analyses were adjusted for body mass index and number of days since the beginning of the last menstrual period. Despite these limitations, there were several strengths to this study. Hot flash status was assigned at the clinic visit based on information provided on the questionnaire. Genotyping and hormone assays were performed by a single investigator. Also, to our knowledge, this is one of the first studies to examine the relation of genotype to hot flashes, in addition to the association with hormone levels in midlife women.

In this study, women were more likely to experience recent, severe, frequent, and a shorter duration of hot flashes if they had low progesterone levels. Women who carried a polymorphism in the estrogen metabolizing enzyme *CYP1B1* had lower progesterone levels and were also more likely to experience any, severe, frequent, and shorter duration hot flashes than women who did not carry a polymorphism. Further, progesterone levels explained part of the association between a polymorphism in *CYP1B1* and the risk of moderate or severe hot flashes. This suggests that progesterone levels may be an important indicator of the risk of hot flashes and that in addition to low hormone levels (progesterone and estrogen), genetic polymorphisms may be involved in the mechanism of hot flashes. Future longitudinal and mechanistic studies need to be performed to understand the functional significance of the *CYP1B1* polymorphism and low progesterone levels on hot flashes.

Acknowledgements

This work was supported by NIH AG18400, NIEHS/NIH Training Grant T32 ES07263, and the Women's Health Research Group at the University of Maryland. The authors thank Dr. Christina Borgeest, Ms. Lynn Lewis, and Ms. Janice K. Babus for their contributions to this work.

References

1. Kronenberg F, Downey JA. Thermoregulatory physiology of menopausal hot flashes: a review. *Can J Physiol Pharmacol* 1987;65:1312–24. [PubMed: 3304594]
2. Hollander L, Freeman EW, Sammel MD, Berlin JA, Grisso JA, Battistini M. Sleep quality, estradiol levels, and behavioral factors in late reproductive age women. *Obstet Gynecol* 2001;98:391–7. [PubMed: 11530118]

3. Kronenberg F. Hot flashes: epidemiology and physiology. *Ann NY Acad Sci* 1990;592:52–86. 123–33. [PubMed: 2197954]
4. Erlik Y, Meldrum DR, Judd HL. Estrogen levels in postmenopausal women with hot flashes. *Obstet Gynecol* 1982;59:403–7. [PubMed: 7078891]
5. Visvanathan K, Gallicchio L, Schilling C, et al. Cytochrome gene polymorphisms, serum estrogens, and hot flushes in midlife women. *Obstet Gynecol* 2005;106:1372–81. [PubMed: 16319265]
6. Overlie I, Moen MH, Holte A, Finset A. Androgens and estrogens in relation to hot flashes during the menopausal transition. *Maturitas* 2002;41:69–77. [PubMed: 11809345]
7. Randolph JF Jr, Sowers MF, Bondarenko I, et al. The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. *J Clin Endocrinol Metab* 2005;90:6106–12. [PubMed: 16144949]
8. Malacara JM, Perez-Luque EL, Martinez-Garza S, Sanchez-Marin FJ. The relationship of estrogen receptor- α polymorphism with symptoms and other characteristics in post-menopausal women. *Maturitas* 2004;49:163–9. [PubMed: 15474761]
9. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17 α gene (*CYP17*) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Research* 1998;58:585–7. [PubMed: 9485002]
10. Dunning AM, Dowsett M, Healey CS, et al. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 2004;96:936–45. [PubMed: 15199113]
11. Somner J, McLellan S, Cheung J, et al. Polymorphisms in the P450c17 (17-hydroxylase/17,20-lyase) and P450c19 (aromatase) genes: association with serum sex steroid concentrations and bone mineral density in postmenopausal women. *J Clin Endocrinol Metab* 2004;89:344–51. [PubMed: 14715870]
12. Travis RC, Churchman M, Edwards SA, et al. No association of polymorphisms in *CYP17*, *CYP19*, and *HSD17-B1* with plasma estradiol concentrations in 1,090 British women. *Cancer Epidemiol Biomarkers Prev* 2004;13:2282–4. [PubMed: 15598795]
13. Tworoger SS, Chubak J, Aiello EJ, et al. Association of *CYP17*, *CYP19*, *CYP11B1*, and *COMT* polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2004;13:94–101. [PubMed: 14744739]
14. Haiman CA, Hankinson SE, Spiegelman D, et al. The relationship between a polymorphism in *CYP17* with plasma hormone levels and breast cancer. *Cancer Research* 1999;59:1015–20. [PubMed: 10070957]
15. Small CM, Marcus M, Sherman SL, Sullivan AK, Manatunga AK, Feigelson HS. *CYP17* genotype predicts serum hormone levels among pre-menopausal women. *Hum Reprod* 2005;20:2162–7. [PubMed: 15878919]
16. Worda C, Sator MO, Schneeberger C, Jantschev T, Ferlitsch K, Huber JC. Influence of the catechol-O-methyltransferase (COMT) codon 158 polymorphism on estrogen levels in women. *Hum Reprod* 2003;18:262–6. [PubMed: 12571159]
17. Gallicchio L, Miller SR, Visvanathan K, et al. Cigarette smoking, estrogen levels, and hot flashes in midlife women. *Maturitas* 2005;53:133–43. [PubMed: 16368467]
18. Gallicchio L, Visvanathan K, Miller SR, et al. Body mass, estrogen levels, and hot flashes in midlife women. *Am J Obstet Gynecol* 2005;193:1353–60. [PubMed: 16202725]
19. Miller SR, Gallicchio LM, Lewis LM, et al. Association between race and hot flashes in midlife women. *J Gen Intern Med*. 2005
20. Schilling C, Gallicchio L, Miller SR, et al. Current alcohol use is associated with a reduced risk of hot flashes in midlife women. *Alcohol Alcohol* 2005;40:563–8. [PubMed: 16087658]
21. Vankrieken, L. Testosterone and the free androgen index. Diagnostic Products Corporation; 1997.
22. Cunningham SK, Loughlin T, Culliton M, McKenna TJ. The relationship between sex steroids and sex-hormone-binding globulin in plasma in physiological and pathological conditions. *Ann Clin Biochem* 1985;22:489–97. [PubMed: 4062218]
23. Cunningham GR, Tindall DJ, Lobl TJ, Campbell JA, Means AR. Steroid structural requirements for high affinity binding to human sex steroid binding protein (SBP). *Steroids* 1981;38:243–62. [PubMed: 7197818]
24. Feigelson HS, Coetzee GA, Kolonel LN, Ross RK, Henderson BE. A polymorphism in the *CYP17* gene increases the risk of breast cancer. *Cancer Research* 1997;57:1063–5. [PubMed: 9067272]

25. Fukatsu T, Hirokawa Y, Araki T, et al. Genetic polymorphisms of hormone-related genes and prostate cancer risk in the Japanese population. *Anticancer Research* 2004;24:2431–8. [PubMed: 15330195]
26. Tang YM, Green BL, Chen GF, et al. Human CYP1B1 Leu⁴³²Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. *Pharmacogenetics* 2000;10:761–6. [PubMed: 11221602]
27. Thyagarajan B, Brott M, Mink P, et al. CYP1B1 and CYP19 gene polymorphisms and breast cancer incidence: no association in the ARIC study. *Cancer Lett* 2004;207:183–9. [PubMed: 15072827]
28. Taioli E, Trachman J, Chen X, Toniolo P, Garte SJ. A *CYP1A1* restriction fragment length polymorphism is associated with breast cancer in African-American women. *Cancer Research* 1995;55:3757–8. [PubMed: 7641189]
29. Fontana X, Peyrottes I, Rossi C, et al. Study of the frequencies of *CYP1A1* gene polymorphisms and glutathione S-transferase *mu1* gene in primary breast cancers: an update with an additional 114 cases. *Mutation Research* 1998;403:45–53. [PubMed: 9726005]
30. Lavigne JA, Helzlsouer KJ, Huang HY, et al. An association between the allele coding for a low activity variant of catechol-O-methyltransferase and the risk for breast cancer. *Cancer Research* 1997;57:5493–7. [PubMed: 9407957]
31. Goodman JE, Lavigne JA, Hengstler JG, Tanner B, Helzlsouer KJ, Yager JD. Catechol-O-methyltransferase polymorphism is not associated with ovarian cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000;9:1373–6. [PubMed: 11142424]
32. Rosmond R, Chagnon M, Bouchard C, Bjorntorp P. Polymorphism in exon 4 of the human β -hydroxysteroid dehydrogenase type I gene (*HSD3B1*) and blood pressure. *Biochem Biophys Res Commun* 2002;293:629–32. [PubMed: 12054649]
33. Chang B, Zheng SL, Hawkins GA, et al. Joint effect of HSD3B1 and HSD3B2 genes is associated with hereditary and sporadic prostate cancer susceptibility. *Cancer Research* 2002;62:1784–9. [PubMed: 11912155]
34. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;159:702–6. [PubMed: 15033648]
35. Trevoux R, De Brux J, Castanier M, Nahoul K, Soule JP, Scholler R. Endometrium and plasma hormone profile in the peri-menopause and post-menopause. *Maturitas* 1986;8:309–26. [PubMed: 3106758]
36. Berendsen HH. The role of serotonin in hot flushes. *Maturitas* 2000;36:155–64. [PubMed: 11063896]
37. Lucero MA, McCloskey WW. Alternatives to estrogen for the treatment of hot flashes. *Ann Pharmacother* 1997;31:915–7. [PubMed: 9220057]
38. 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:S52–60. [PubMed: 16949389]
39. Woods NF, Mitchell ES, Tao Y, Viernes HM, Stapleton PL, Farin FM. 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 Sept 12;[Epub ahead of print]
40. Lurie G, Maskarinec G, Kaaks R, Stanczyk FZ, Le Marchand L. Association of genetic polymorphisms with serum estrogens measured multiple times during a 2-year period in premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2005;14:1521–7. [PubMed: 15941966]
41. Wierman ME, Basson R, Davis SR, Khosla S, Miller KK, Rosner W, Santoro N. Androgen therapy in women: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2006;91:3697–3710. [PubMed: 17018650]

Table 1

Characteristics of Study Sample

Variable	Women with hot flashes		Women without hot flashes		p-value
	n	%*	n	%*	
Sample Size	372	58.2	267	41.8	
Participant Characteristics					
Age (years)					
45 to 49	219	58.9	194	72.7	0.001
50 to 54	153	41.1	73	27.3	
Race					
White	302	81.2	230	86.1	0.006
Black	66	17.7	28	10.5	
Other	3	0.8	8	2.9	
Smoking Status					
Current	42	11.3	16	6.0	0.006
Former	153	41.1	94	35.2	
Never	176	47.3	157	58.8	
Body Mass Index (kg/m ²)					
≤24.9	146	39.2	127	47.6	0.002
25.0 to 29.9	94	25.3	81	30.3	
≥30.0	131	35.2	59	22.1	
Genotype					
<i>CYPc17a</i>					
+/+	134	36.0	96	36.0	1.0
+/-	193	51.9	137	51.3	
-/-	45	12.1	34	12.7	
<i>3βHSD</i>					
+/+	53	14.2	54	20.2	0.06
+/-	161	43.3	122	45.7	
-/-	150	40.3	88	33.0	
<i>CYP19</i>					
+/+	118	31.7	81	30.3	0.6
+/-	181	48.7	125	46.8	
-/-	69	18.5	59	22.1	
<i>CYP11A1</i>					
+/+	291	78.2	209	78.3	1.0
+/-	75	20.2	54	20.2	
-/-	6	1.6	4	1.5	
<i>CYP11B1</i>					
+/+	87	23.4	77	28.8	0.3
+/-	180	48.4	122	45.7	
-/-	103	27.7	67	25.1	
<i>COMT</i>					
+/+	98	26.3	67	25.1	0.1
+/-	205	55.1	131	49.1	
-/-	69	18.5	68	25.5	

* Due to missing information, some columns do not add up to the total n listed, and percentages do not add up to 100.

Association between genotype and sex steroid hormone levels

Genotype	n	Testosterone			Androstenedione			DHEA-S			Estradiol			Estrone			Progesterone		
		Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value	Geometric Mean (95% CI)	p-value		
<i>CYP11B1</i> +/+ +/	161	0.499 (0.454, 0.548)	0.1	2.109 (1.948, 2.280)	0.2	403.83 (369.08, 441.86)	0.08	99.98 (88.41, 112.96)	0.7	135.64 (122.12, 150.51)	0.6	0.960 (0.766, 1.202)	0.07						
	461	0.457 (0.433, 0.483)		1.986 (1.896, 2.077)		367.23 (348.63, 386.84)		97.13 (90.56, 104.27)		131.24 (123.59, 139.49)		0.752 (0.660, 0.857)							
	3	Missing																	
<i>CYPc17a</i> +/+ +/	224	0.472 (0.437, 0.511)	0.8	2.090 (1.960, 2.230)	0.2	382.22 (354.96, 411.58)	0.6	104.17 (94.16, 115.12)	0.1	134.42 (123.35, 146.50)	0.7	0.807 (0.670, 0.972)	1.0						
	401	0.467 (0.440, 0.495)		1.982 (1.890, 2.081)		373.53 (353.19, 394.65)		94.54 (87.71, 101.90)		131.63 (123.47, 140.33)		0.803 (0.698, 0.922)							
	487	0.471 (0.447, 0.497)	0.7	2.004 (1.917, 2.094)	0.4	378.80 (359.96, 398.22)	0.7	98.49 (91.93, 105.43)	0.7	133.09 (125.59, 141.17)	0.8	0.819 (0.722, 0.929)	0.5						
<i>CYP11A1</i> +/+ +/	138	0.460 (0.416, 0.508)		2.079 (1.913, 2.259)		369.44 (335.96, 406.26)		95.87 (84.27, 109.07)		130.97 (117.33, 146.20)		0.754 (0.594, 0.956)							
	193	0.437 (0.401, 0.476)	0.06	1.960 (1.824, 2.104)	0.3	342.41 (315.77, 371.67)	0.006	94.73 (84.86, 105.74)	0.4	127.74 (116.16, 140.47)	0.3	0.796 (0.648, 0.979)	0.9						
	426	0.483 (0.457, 0.512)		2.052 (1.958, 2.153)		393.86 (373.16, 415.72)		100.38 (93.32, 107.99)		135.64 (127.36, 144.46)		0.812 (0.708, 0.931)							
Missing <i>COMT</i> +/+ +/	6																		
	160	0.468 (0.427, 0.514)	1.0	2.050 (1.898, 2.217)	0.7	382.99 (350.72, 418.64)	0.7	99.78 (88.23, 112.84)	0.7	128.51 (115.82, 142.59)	0.5	0.786 (0.629, 0.982)	0.8						
	464	0.469 (0.444, 0.495)		2.012 (1.923, 2.104)		374.28 (355.31, 394.26)		97.22 (90.47, 104.48)		134.02 (126.09, 142.45)		0.807 (0.709, 0.919)							
Missing <i>βHSD</i> +/+ +/	1																		
	104	0.460 (0.409, 0.513)	0.7	1.964 (1.784, 2.164)	0.6	375.40 (336.30, 419.47)	0.9	105.32 (90.38, 122.73)	0.4	138.38 (121.63, 157.43)	0.6	0.942 (0.715, 1.242)	0.2						
	510	0.468 (0.444, 0.492)		2.018 (1.933, 2.106)		373.90 (356.02, 392.68)		97.51 (91.10, 104.38)		133.09 (125.71, 141.03)		0.775 (0.685, 0.876)							
Missing	11																		

* adjusted for age, race, smoking status, BMI, days since last menstrual period

Association between sex steroid hormone levels and hot flash variables

Variable	n	Testosterone * Geometric Mean (95% CI)	p- value	Androstenedione * Geometric Mean (95% CI)	p- value	DHEA-S * Geometric Mean (95% CI)	p- value	Progesterone * Geometric Mean (95% CI)	p- value	SHBG Geometric Mean * (95% CI)	p- value
Hot Flashes in the past 30 days											
Never	265	0.465 (0.433, 0.501)	0.9	2.026 (1.906, 2.155)	1.0	384.52 (358.17, 412.40)	0.8	1.017 (0.855, 1.210)	0.001	59.15 (55.48, 63.05)	0.03
No (with ever hot flashes)	115	0.463 (0.415, 0.516)		2.040 (1.863, 2.234)		374.28 (337.31, 415.30)		0.858 (0.663, 1.110)		62.18 (56.54, 68.37)	
Yes	242	0.475 (0.439, 0.513)		2.012 (1.885, 2.147)		369.81 (343.44, 398.62)		0.603 (0.501, 0.725)		53.62 (50.10, 57.40)	
Severity of Hot Flashes											
Never	265	0.465 (0.433, 0.501)	1.0	2.024 (1.904, 2.151)	0.9	384.14 (358.17, 411.99)	0.7	1.007 (0.846, 1.198)	0.003	58.97 (55.31, 62.93)	0.5
Mild	132	0.470 (0.425, 0.521)		1.980 (1.818, 2.155)		376.15 (341.38, 414.88)		0.718 (0.564, 0.914)		56.04 (51.21, 61.31)	
Moderate/Severe	227	0.467 (0.432, 0.505)		2.034 (1.904, 2.171)		366.87 (340.36, 395.44)		0.652 (0.541, 0.785)		56.20 (52.46, 60.28)	
Frequency of Hot Flashes											
Never	265	0.466 (0.433, 0.501)	0.5	2.030 (1.910, 2.158)	0.3	385.68 (359.60, 413.64)	0.6	1.020 (0.857, 1.213)	0.001	59.15 (55.48, 63.05)	0.003
Monthly	158	0.483 (0.440, 0.529)		2.083 (1.929, 2.252)		376.91 (344.81, 411.99)		0.791 (0.634, 1.015)		63.05 (58.15, 68.37)	
At least weekly	178	0.447 (0.408, 0.489)		1.919 (1.777, 2.071)		363.58 (332.95, 396.63)		0.574 (0.462, 0.712)		51.78 (47.85, 56.04)	
Duration of Hot Flashes											
Never	265	0.465 (0.432, 0.500)	0.9	2.026 (1.906, 2.153)	1.0	384.52 (358.53, 412.40)	0.2	1.005 (0.845, 1.196)	0.002	58.97 (55.32, 62.87)	0.2
≥ 1 year	233	0.463 (0.429, 0.501)		2.006 (1.879, 2.140)		359.24 (333.62, 386.84)		0.735 (0.611, 0.883)		57.69 (53.89, 61.74)	
< 1 year	117	0.480 (0.430, 0.534)		2.014 (1.839, 2.203)		400.61 (361.04, 444.08)		0.586 (0.453, 0.757)		53.20 (48.38, 58.44)	

* adjusted for age, race, smoking status, BMI, days since last menstrual period

Table 4
Association between FEI, FTI, androgen to estrogen ratio, and hot flash variables

Variable	n	Free Estradiol Index (FEI)		Free Testosterone Index (FTI)		Ratio of total androgens to total estrogens [†]	
		Geometric Mean [*] (95% CI)	p-value	Geometric Mean [*] (95% CI)	p-value	Geometric Mean [*] (95% CI)	p-value
Hot Flashes in the past 30 days							
Never	265	0.703 (0.628, 0.788)	0.04	2.729 (2.465, 3.019)	0.1	9.337 (8.611, 10.115)	0.002
No (with ever hot flashes)	115	0.574 (0.485, 0.680)		2.581 (2.219, 2.998)		10.268 (9.116, 11.565)	
Yes	242	0.577 (0.511, 0.652)		3.071 (2.757, 3.421)		11.658 (10.708, 12.692)	
Severity of Hot Flashes							
Never	265	0.702 (0.626, 0.787)	0.04	2.735 (2.472, 3.025)	0.7	9.365 (8.637, 10.145)	0.007
Mild	132	0.600 (0.512, 0.704)		2.912 (2.529, 3.353)		10.957 (9.796, 12.256)	
Moderate/Severe	227	0.564 (0.499, 0.637)		2.881 (2.583, 3.209)		11.257 (10.329, 12.268)	
Frequency of Hot Flashes							
Never	265	0.703 (0.627, 0.788)	0.02	2.729 (2.469, 3.019)	0.4	9.346 (8.619, 10.125)	0.001
Monthly	158	0.572 (0.495, 0.662)		2.656 (2.337, 3.016)		10.486 (9.459, 11.612)	
At least weekly	178	0.548 (0.476, 0.632)		2.989 (2.638, 3.387)		12.061 (10.903, 13.330)	
Duration of Hot Flashes							
Never	265	0.702 (0.626, 0.787)	0.04	2.732 (2.469, 3.022)	0.3	9.356 (8.645, 10.135)	0.002
≥ 1 year	233	0.594 (0.527, 0.670)		2.787 (2.504, 3.099)		10.633 (9.777, 11.565)	
< 1 year	117	0.551 (0.466, 0.652)		3.127 (2.694, 3.629)		11.989 (10.665, 13.491)	

* adjusted for age, race, smoking status, BMI, days since last menstrual period

[†] total androgens = androstenedione plus testosterone, total estrogens = estradiol plus estrone

Table 5

Risk ratios of experiencing hot flashes by genotype

	Women without hot flashes	Women with hot flashes	Any hot flashes RR* (95% CI)	Women with hot flashes	Moderate or severe hot flashes RR* (95% CI)	Women with hot flashes	At least weekly hot flashes RR* (95% CI)	Women with hot flashes	Hot flashes for ≥ 1 year RR* (95% CI)
3HSD	n	n		n		n		n	
+/+	54	53	1.00	28	1.00	25	1.00	30	1.00
+/- or -/-	210	311	1.18 (0.96, 1.46)	203	1.38 (1.00, 1.90)	158	1.29 (0.91, 1.83)	208	1.36 (0.99, 1.86)
Missing	3	8		5		5		4	
CYP19									
+/+	81	118	1.00	73	1.00	54	1.00	79	1.00
+/- or -/-	184	250	1.00 (0.86, 1.17)	162	1.03 (0.83, 1.28)	130	1.10 (0.85, 1.43)	161	0.99 (0.81, 1.22)
Missing	2	4		1		4		2	
COMT									
+/+	67	98	1.00	56	1.00	52	1.00	62	1.00
+/- or -/-	199	274	0.98 (0.84, 1.14)	180	1.06 (0.84, 1.34)	137	0.94 (0.73, 1.21)	180	1.00 (0.81, 1.25)
Missing	1	0		0		0		0	
CYP1B1									
+/+	77	87	1.00	50	1.00	39	1.00	53	1.00
+/- or -/-	189	283	1.18 (1.00, 1.40)	185	1.33 (1.04, 1.71)	148	1.37 (1.02, 1.84)	189	1.33 (1.05, 1.69)
Missing	1	2		1		1		2	
3HSD, CYP1B1									
+/+, +/+	14	10	1.00	6	1.00	4	1.00	3	1.00
+/- and +/- or -/-, or +/- or -/-									
- and +/+	100	119	1.23 (0.75, 2.01)	66	1.22 (0.60, 2.45)	56	1.50 (0.61, 3.67)	77	2.24 (0.79, 6.35)
+/- or -/- and +/- or -/-	149	233	1.42 (0.88, 2.30)	158	1.63 (0.82, 3.21)	122	1.91 (0.80, 4.60)	158	2.77 (0.99, 7.80)
Missing	4	10		6		6		4	
<i>p-for-trend</i>			0.02		0.006		0.02		0.003

* adjusted for race