The Relationship of Uterine Leiomyomata and Genetic Polymorphisms of *Cytochrome P-450 1A1, Cytochrome P-450 1B1*, and *Catechol-O-Methyltransferase*

Kyna McCullough Gooden

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Approved by
Chair: Dr. Jane C. Schroeder
Dr. Donna D. Baird
Dr. Marilie D. Gammon
Dr. Katherine M. Hartmann
Dr. Kari E. North

ABSTRACT

Kyna M. Gooden: The Relationship between Uterine Leiomyomata and Polymorphsims of *Cytochrome P-450 1A1, Cytochrome P-450 1B1*, and *Catechol-O-Methyltransferase*
(Under the direction of Jane C. Schroeder)

Uterine leiomyomata, or fibroids, are one of the most common neoplasms in women of reproductive age. They occur more often and are larger in African American women compared to White women. Although benign, the etiology of fibroids is largely unknown, however they are hormonally dependent. The biologic effect of estrogen is influenced by estrogen metabolism; therefore estrogen metabolism enzymes may influence fibroid development. *Cytochrome P-450 1A1 (CYP1A1)*, *Cytochrome P-450 1B1 (CYP1B1),* and *Catechol-O-Methyltransferase (COMT)* are polymorphic genes that encode key enzymes in the estrogen metabolism pathway. Four single nucleotide polymorphisms (SNP) of *CYP1A1*, 2 SNPs of *CYP1B1*, and 1 SNP of *COMT* were evaluated for associations with fibroid prevalence and size in a cross-sectional sample of premenopausal African American (n=583) and White (n=404) women from the National Institute of Environmental Health Sciences Uterine Fibroid Study. Participants provided DNA samples and completed telephone interviews and questionnaires. Race-specific prevalence ratios (PR) and 95% confidence intervals (CI) were estimated from log-risk regression models; prevalence differences (PD) and 95% CI were estimated from linear-risk regression models. Haplotypes and diplotypes were inferred for *CYP1A1* and *CYP1B1*. Genotype distributions varied by race. African Americans were more likely to have fibroids (72% vs 50%) and to have large fibroids (24% vs 11%) than Whites. The *CYP1A1*3*allele was associated with fibroids among African Americans (PR=1.14; 95%CI: 1.02, 1.28; PD=0.10; 95%CI: 0.01, 0.19). The *CYP1A1*4* allele was positively associated with fibroids among both Whites (PR=1.20; 95%CI: 0.90, 1.61;

PD=0.10; 95%CI: -0.07, 0.27) and African Americans (PR=1.16; 95%CI: 0.92, 1.48; PD=0.12; 95%CI: -0.08, 0.32). Haplotypes and diplotypes that included CYP1A1*3 and CYP1A1*4 showed similar results. Estimates for *CYP1B1* and *COMT* alleles were close to the null. Analyses of effect measure modification by age, body mass index, smoking status, alcohol use, oral contraceptive use, and number of fullterm births did not show deviations from additive or multiplicative expectations. Our results reveal possible relationships between *CYP1A1*3* and **4* polymorphisms and fibroid prevalence. These results must be confirmed in other populations, and consider additional genes and variants within the estrogen metabolism pathway.

To Taylor and Jasmine. When you grow up to be young women, I hope that this document will serve as proof that with faith and perseverance, you can attain absolutely anything.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

16α-OH	16α-hydroxyestradiol
2-OH	2-hydroxyestradiol
4-OH	4-hydroxyestradiol
A	Adenine
BMI	Body Mass Index
C	Cytosine
C-16α	16α Carbon position
C-2	2 Carbon position
C-4	4 Carbon position
CI	Confidence interval
Cm	Centimeter
COMT	Catechol-O-Methyltransferase
CYP1A1	Cytochrome P-450 1A1
CYP1A2	Cytochrome P-450 1A2
CYP1B1	Cytochrome P-450 1B1
CYP1B1AS	Cytochrome P-450 1B1 Ala119Ser
CYP1B1LV	Cytochrome P-450 1B1 Leu432Val
CYP3A4	Cytochrome P-450 3A4
DNA	Dexoyribonucleic Acid
E1	Estrone
E2	Esradiol
ER	Estrogen receptor
G	Guanine

HMGI	High Mobility Group Protein
HWE	Hardy Weinberg Equilibrium
ICR	Interaction Contrast Ratio
Kg	Kiligrams
LRT	Liklihood Ratio Test
MALDI-TOF	Matix-assisted laser desorption/ionization time-of-flight mass
MS	spectrometry
Mm	Millimeter
mRNA	Messenger ribonucleic Acid
NIEHS	National Institute of Environmental Health Sciences
OR	Odds ratio
PD	Prevalence difference
PR	Prevalence ratio
SAS	Statistical Analysis Systems
SNP	Single Nucleotide Polymorphism
SULT	Sulfotransferase
T	Thymine
UFS	Uterine Fibroid Study
UGT	UDP-glucuronosyltransferases
Var	Variant
Wt	Wildtype

I. INTRODUCTION

A. Epidemiology of uterine leiomyoma

Uterine leiomyomas, commonly called uterine fibroids, are the most common reproductive tract neoplasm in women[1]. They are firm, benign tumors of smooth muscle origin[2]. They can occur singly or in multiples[1], where multiple fibroids are clonally distinct from one another[3,4]. Fibroids can be detected by ultrasound when they are as small as 2-4 mm in diameter[5], and have been documented to be as large as 72.39 cm in diameter[1]. They are classified into three groups: subserous, mural, and submucous[1]. Subserous tumors project out from the peritoneal surface of the uterus, mural tumors are within the uterine wall, and submucous tumors often project into the uterine cavity. Some submucous or subserous fibroids are attached to the uterus by stalks (pedunculated fibroids) and, although infrequent, the submucous fibroids may protrude through the cervix and into the vagina[1].

Since the majority of tumors are asymptomatic, many remain undiagnosed[5]. Fibroids may be diagnosed during vaginal or abdominal examination, dilatation and curettage, hysteroscopy, laparoscopy, or transvaginal or abdominal ultrasound examination[6,7]. The most widely accepted non-invasive method of diagnosis is transvaginal ultrasound, which has an estimated sensitivity of 67-87% and specificity of 89-91%, using operative hysteroscopy and histological classification as the reference procedure[6]. Symptoms of fibroids include frequent urination, backache, constipation, abnormal bleeding, anemia, and pelvic/abdominal pain[1,8-11]. Severity of symptoms is generally associated with the number, size, and location of the tumors[12].

Treatment may include hormone therapy (anti-estrogen or anti- progesterone), observation, myomectomy (the removal of the fibroid), hysterectomy (the removal of the entire uterus), or embolization (the blockage of blood flow to the fibroid).[1] Uterine leiomyomas are the leading indication for hysterectomies that occur in the United States, accounting for up to 40% of all hysterectomies[13,14]. Choice of treatment is dependent on size of tumor, severity of symptoms, and rate of growth, as well as the woman's desire to have children[12].

Fibroids are common in reproductive age women[1]. Studies have reported prevalences ranging from 3.3% to as high as 87%[5,15-18]. Most cases are asymptomatic, yet symptomatic fibroids result in a significant amount of hospitalization[19]. Fibroids are more prevalent in African Americans than in Whites (16-87% among African Americans of reproductive age versus 9-78% among Whites of reproductive age)[16,20,21]. Diagnosis rates are also higher among African American women (30.6 per 1000 woman years) when compared to White women (8.9 per 1000 woman years) [18]. In addition, African American women are more likely to be diagnosed at earlier ages, report more severe symptoms, have a greater number of tumors, and have larger tumors than white women[9].

The etiology of fibroids remains poorly understood; however, several risk factors have been identified in epidemiologic studies. Factors affecting the hormonal milieu, such as pregnancy[22-24], parity[7,25], abortion[25], low body mass index (BMI), [7,26]and use of oral contraceptives[22 24,27] have been associated with a decreased risk of diagnosis. However, studies have also reported positive associations with increased BMI[22], abortions[22], and oral contraceptive use[21,23]. In addition to protection through the hormonal mileu, pregnancy may offer protection through apoptosis during post partum involution of the uterus[28]. Smoking was inversely associated with fibroids in several studies[21,23,29], but several other studies reported no association[26]. Other unconfirmed factors that have been associated with fibroids include hypertension, perineal talc use, history of pelvic inflammatory disease and chlamydial infection, and use of an intrauterine device[22,24,30].

The etiology of fibroids is unclear; however, genetic mutations and epigenetic mechanisms are the fundamental basis for the development of tumors, benign or metastatic. Evidence supports the role of clonal genetic abnormalities in the development of fibroid. The following chromosomal abnormalities are common among fibroids: translocation of (12;14)(q15;q23-24); deletion of (7)(q22q32); and rearrangements involving 6p21, 10q, trisomy 12. Early studies were small; however, they were consistent in finding chromosomal aberrations in approximately 30-50% of fibroids examined[31-37]. One study, examining 90 tumors, found clonal abnormalities in 34%; [31]another study examining 93 tumors found abnormalities in 50%. [32] The most common abnormality in both studies was del(7)(q21.2q31.2). Rein et al. (n=13) and Vanni et al. (n=40), found abnormalities in 54 and 32.5% of their samples (respectively), the majority of which involved the 12q14-15 and the 14q23-24 regions[36]. A later larger study examining 182 tumors, found abnormalities in 29%. [37]Chromosomal aberrations are tumor specific; their absence in normal uterine tissue suggests that they are nonrandom.

Research by Pandis et al. showed that cytogenetically abnormal leiomyomas (those with detectable chromosomal abnormalities) had a higher mitotic index than those that were cytogenetically normal[38], suggesting these abnormalities promote growth. A later study by Rein et al., examining 115 tumors, showed a relationship between chromosomal abnormalities and fibroid size; larger tumors were more likely to possess the t(12;14) abnormality, smaller tumors tended to possess the del(7), and middle sized tumors possessed a mosaic karyotype (composed of cells with and without abnormalities). [39] Consistent with these findings, another study examined 155 tumors and found those with translocations affecting 12q14-15 (but not deletions on chromosome 7) were significantly larger (8.9 ± 5.1 versus 3.4 ± 2.1 cm, $p<0.001$) than tumors with a normal karyotype (no chromosomal abnormalities)[40]. Translocations at the 12q14-15 may be associated with rearrangements of the high mobility group protein gene (HMGI) family; aberrations in the HMGI gene family have been found in various benign mesenchymal tumors

(e.g. pulmonary chondroid hamartomas, endometrial polyps, breast hamartomas, and lipomas) in addition to fibroid[41-43]. This gene is often truncated and joined to ectopic DNA sequences, leading to fusion genes that result in increased expression[44]. This suggests that *HMGI* may be a critical gene in the pathogeneisis of fibroid tumors. *HMGI* aberrations may even be related to the location of fiborids. Bronsens et al. examined 182 fibroids and found that intramural and subserous tumors had more chromosomal abnormalities (35% and 23% respectively) than submucous tumors (12%)[37].

B. Uterine leiomyoma and hormones

Known risk factors for fibroid tend to parallel those of other hormonally induced tumors, such as endometrial cancer. Although inconsistent, risk factors (e.g. BMI, oral contraception use, and parity as described in the section above) associated with the presence of fibroids support the influence of hormones on their development. Clinical observations lend further support; for example, fibroids are only diagnosed after menarche[45]. After menopause, they diminish in size and/or disappear[1,17].

Studies have found molecular evidence that fibroids are progesterone related. The addition of progesterone to cultured leiomyoma cells increased expression of an apoptosis inhibition protein (BCL-2) relative to control cultures[46]. Brandon et al. demonstrated increased expression of progesterone receptor mRNA and protein in 29 leiomyoma compared to adjacent myometrium[47].

Evidence also suggests fibroids are estrogen related. Andersen et al. found that they were hypersensitive to estrogen compared to normal myometrium, and that estrogen receptor levels in leiomyoma tissue were significantly elevated during the follicular phase[48]. Another study of 8 cases reported elevated expression of 4-hydroxyestradiol in fibroid tissue compared to normal

surrounding tissue[49]. The effect (if any) of these hormones on fibroid development or natural history remains unclear.

C. Estrogen and Estrogen Metabolism

Estrogen is a steroid hormone chiefly produced by the ovaries prior to menopause; after menopause, estrogen is primarily produced in the peripheral tissues (mostly adipose) by the conversion of androstenedione to estrogen by aromatase. A normal premenopausal adult female produces about 70 to 500 ug of estradiol per day, depending on the phase of the menstrual cycle. Regulation of biosynthesis and secretion is controlled by gonadotropins[50]. Estrogens affect almost all systems in the body, including influence on bone formation and maintenance, as well as behavior and mood[50]. In the uterus, estrogens increase cell progesterone receptors. They also stimulate mucus production in the cervix, and mammary gland duct growth[51].

Endogenous estrogen exists in three main forms: estradiol, estrone, and estriol. The most common circulating form is estradiol in premenopausal women, and estrone in postmenopausal women. Estradiol can be converted to estrone, and visa versa. Either estradiol or estrone can be converted to estriol[50].

When estrogens circulate the body, they are primarily bound to sex hormone binding globulin and albumen. It is believed that only unbound estrogens can enter target-tissue cells and induce biological activity[50]. Estrogen enters the cell through passive diffusion, then binds nuclear estrogen receptors (ER). There are two forms of the estrogen receptor, ERα and ERβ[52]. The two forms differ in tissue distribution, binding affinity, and biological function[53]. The ratio of expression of the two receptor types determines the biological response to estrogen. The ER is primarily an intranuclear binding protein[54], but it can shuttle between the nucleus and cytoplasmic compartments[55]. Once estrogen binds to the ER, the ER changes its conformation, which allows it to activate the transcription of estrogen-responsive genes[50]. Overall, estrogen

receptor levels are higher in leiomyomata tissue than in homologous myometrium[56,57]. Radioimmunoassays and immunocytochemical assays show a higher ER content in the subendometrial region than in the midmyometrial or the subserosal regions of the uterus[58].

The biologic effect of estrogen depends, in part, on how it is metabolized. Metabolism of estrogen primarily occurs in the liver through two phases, but can also occur locally in other tissues. Phase I metabolism creates an active site, primarily via hydroxylation, while Phase II conjugation reactions (methylation, glucuronidation, and sulfation) produce estrogen metabolites that may be excreted through the urine and feces, or be maintained in circulation. This process is not always linear; Phase II metabolites can be de-conjugated back to an active intermediate.

Phase I hydroxylation of estradiol (E_2) and estrone (E_1) mainly takes place at the 2 carbon (C-2) position or at the 16α carbon (C-16α) position, and is catalyzed by cytochrome P450 (CYP) enzymes. More specifically, hydroxylation at the C-2 position is catalyzed primarily by CYP1A1 and CYP1A2 enzymes, and hydroxylation at the C-16α position is catalyzed primarily by CYP3A4. Hydroxylation at the C-2 position yields the catechol estrogens, 2-hydroxyestrone and 2-hydroxyestradiol (2-OH); likewise, hydroxylation at the C-16α position yields 16α-hydroxyestrone and 16-hydroxyestradiol (16α-OH). A smaller amount of hydroxylation occurs at the 4 carbon (C-4) position, catalyzed primarily by CYP1B1 and CYP3A4, which yields the catechol estrogens, 4-hydroxyestrone and 4-hydroxyestradiol (4-OH). The 16α-OH and 4-OH metabolites are more estrogenic than 2-OH[59,60].

Catechol estrogens are easily oxidized to highly reactive semi-quinones and quinones that generate reactive oxygen species through redox cycling, which may damage DNA and promote carcinogenesis. Phase II methylation of 2-OH and 4-OH metabolites to less reactive metabolites (2-methoxyestrone and 2-methoxyestradiol and 4-methoxyestrone and 4-methoxyestradiol, respectively) reduces quinone formation. This process is catalyzed by the catechol-*O*-methyltransferase (COMT) enzyme. During glucuronidation, another element of Phase II

metabolism, UDP-glucuronosyltransferases (UGTs) conjugate glucuronic acid with the estrogen to aid in deactivation. Finally, sulfation (catalyzed by members of a superfamily of cytosolic sulfotransferase (SULT) enzymes) yields estrone sulfate or estradiol sulfate—water soluble conjugates that may be excreted in the urine. Estrone sulfate is the most abundant form of estrogen in circulation.

Figure A.1 presents a simplified version of the catechol estrogen metabolism process. In summary, estrogen metabolism may lead to the formation of metabolites that vary with regard to their ability to stimulate estrogen receptors and/or damage DNA. The net result of this process will depend in part on the activity of Cytochrome P-450 (CYP) and COMT enzymes that may favor different metabolic pathways. Therefore, polymorphisms that influence the activity of these enzymes may influence estrogen-mediated tumorigenesis processes.

D. Carcinogenic effects of estrogen

Animal studies have shown estrogen to be carcinogenic[61]. For example, administration of estrogen to rodents increases the incidence of tumors of the mammary and pituitary glands, as well as the uterus, cervix, vagina, testicles, and bone[62-65]. Although the majority of studies to date have looked primarily at breast cancer, there is adequate epidemiological evidence that estrogen is carcinogenic in humans[66-69]. Consequently, it has been listed as a carcinogen by the International Agency for Research on Cancer[70]. Little to no research to date has examined the relationship between fibroid and estrogen regulating polymorphisms.

Estrogen promotes cell proliferation, which may increase the likelihood of spontaneous mutations caused by errors during DNA replication. Once these mutations are present within the genome, continued replication would create clones of the mutations or perhaps more mutations. If these errors are uncorrected (either through repair or apoptosis) they can lead to a carcinogenic phenotype. It appears that the metabolites of estrogen have specific and distinct activities in

estrogen-sensitive tissue. The estrogen metabolite 4-OH was shown to be estrogenic in rats—promoting uterine weight gain and mammary gland and bone proliferation[71,72]. Conversely, 2-OH metabolite had little to no estrogenic activity in these tissues[71,72]. Another study found that 2-OH metabolite was a weak estrogen agonist with respect to bone formation in rats[73]. 2-OH metabolite, however, had no estrogenic activity in human osteoblastic cells[74]. Schneider and colleagues experimented with human breast cancer cells and found 2-OH metabolite to be antiestrogenic, suppressing cell growth and proliferation, but only in ER positive cell lines[75]. It is important to note that activities of estrogen metabolites are due in part to the type of estrogen receptors present within a specific tissue[53]; the lack of a suitable receptor would mean less opportunity for estrogenic action.

Estrogen metabolites may also be directly genotoxic. Quantitatively, 4-OH is a less common metabolite than 16-OH and 2-OH; however it may be very critical to the genotoxic pathway. 4-OH metabolite further metabolizes to 3,4-semi-quinone, which in turn is metabolized to 3,4-quinone. Quinones are recognized tumor initiators[76]. Redox cycling between semi-quinones and quinones produces free radicals that may damage DNA. In addition, the 3,4-quinone can form depurinating DNA adducts that increase the likelihood of DNA mutations[76]. 2-OH metabolite also metabolizes to 2,3-semi-quinone and later 2,3-quinone, which produces superoxide free radicals. However, in contrast with 4-OH quinone metabolites, 2,3-quionone metabolites form stable DNA adducts that remain covalently bonded to DNA unless removed during repair. Overall, evidence suggests that the 4-OH catechol estrogen is both more estrogenic and more carcinogenic than 2-OH catechol estrogen[72,77,78]. Rogan and collegues found four times the amount of 4-OH metabolite in the breast tissue of breast cancer cases than in controls. Likewise, more 2-OH metabolite was found in breast tissue controls than cases[91].

Relevant polymorphic enzymes

CYP1A1 and CYP1B1 are both key polymorphic enzymes in estrogen metabolism, triggering hydrocarbon hydroxylase activity. COMT, another key polymorphic enzyme, is involved in *O*-methylation. They are expressed in a variety of tissues, including the liver, breast, lung, uterus, and kidneys. [79-84] Table A.1 details *CYP1A1,* CYP1B1*,* and *COMT* polymorphisms described below.

CYP1A1

The *CYP1A1* gene is located on chromosome 15, and is induced by polycyclic aromatic hydrocarbons. At least seven polymorphisms have been identified[85], but most do not appear to alter the function of *CYP1A1*[85].

The polymorphism that was first detected (*CYP1A1*2A,* also known as *m1*) is a T→C transition that creates a new MspI cleavage site located 1194 bp downstream of exon 7 in the 3' non-coding region[86]. One or more of this common allele have been found in up to 16% of healthy White and 44.1% of healthy African Americans[87]. *CYPA1A1*2A* does not seem to alter the expression of CYP1A1; however, it is in linkage disequilibrium with the *CYP1A1*2C* (also known as *m2*) [88 92]polymorphism that may increase expression[85]. The *CYPA1A1*2C* polymorphism is an A→G transition located at nucleotide 4889 (exon 7), which results in an amino acid substitution of Isoleucine to Valine in the heme-binding region of the CYP1A1 enzyme[88]. One or more *CYPA1A1*2C* alleles were found in 7.4% of healthy Caucasians[87,92], but no *CYPA1A1*2C* alleles were found in healthy African Americans[87]. This polymorphism has been associated with increased inducibility of CYP1A1, as measured by the ethoxyresorufin O-deethylase (EROD) assay[85]. One animal study found that this mutation was not functionally important[93]. The *CYP1A1*3*, allele, also known as *m3*, is in the 3' non-coding region like *CYP1A1*2A*[94]. This variant results from a T→C transition. It is unclear whether the CYP1A1*3 allele affects function. This allele is common among African Americans (23.7%); [87]several

studies did not find it in Caucasians[87,92,94]. Another polymorphism, *CYP1A1*4* (*m4*), is two bases upstream of the *CYP1A1*2C* site, also in the heme-binding region of *CYP1A1*[91]. The variant allele has a C→A substitution located at the 4887 position of exon 7, resulting in a threonine to asparagine substitution. This allele is found more frequently in Caucasians (~4%) than African Americans (~0.4%)[87].

CYP1A1 has been examined in relation to a number of cancers. Details on previous studies can be found in Table A.2. *CYPA1A1*2A* was associated with a greater risk of lung cancer in a study of 45 Japanese subjects[95], but has not been associated with lung cancer among Caucasians[81,90,96]. Drakoulis et al. reported an increased risk of lung cancer among those with the *CYPA1A1*2C* allele (OR=2.16; 95% CI 0.96-5.11, p=0.03). Lung cancer was also not related to the *CYP1A1*4* allele. Cascorbi et al. reported its' frequency in 576 lung cases and 304 controls was identical (case frequency = 2.87%, 95% CI = 1.32%-5.37%; control frequency = 2.87%, 95% CI = 1.71%-4.47%)[91]. The relationship of CYP1A1 alleles with breast cancer is conflicting as well. The *CYPA1A1*2A* variant was associated with breast cancer in a case-control study of 932 women (>97% White), but only among women who initiated smoking before age 18 (RR=5.65, 95% CI=1.11-11.7). [97] Another study found an independent association (OR=9.7, 95% CI=2.0-47.9) between the *CYPA1A1*2A* polymorphism and breast cancer among African American women (21 cases, 86 controls), but not Caucasian women (30 cases, 183 controls). [98] Other studies have not found a clear association of *CYP1A1*2A* with breast cancer[87,99,100]. Some studies have found the *CYPA1A1*2C* variant to be independently associated with an increase in breast cancer risk[80], while other studies have not[87,101]. Still others have found a higher risk of breast cancer associated with this mutation only in the presence of environmental factors such as smoking[80] or polychlorinated byphenols. [100] Bailey and colleagues found no association between

the *CYP1A1*3* or *CYP1A1*4* alleles and breast cancer in a case-control study (n=223 cases, 221 controls). [87]

CYP1B1

CYP1B1 is another enzyme key to the metabolism of estrogen. The *CYP1B1* gene was first identified by Sutter and colleagues and is located on chromosome 2 at the 2p21-22 region. It contains three exons and two introns[102]. CYP1B1 is expressed in a variety of tissues, including liver, lymphocytes, kidneys, testis, breast, colon, bladder, and uterus[82-84]. There are at least six single nucleotide polymorphic sites in this gene, four of which cause amino acid substitutions[103,104].

One *CYP1B1* polymorphism, *Ala119Ser*, causes an amino acid change from alanine to serine at codon 119 in exon 2. This polymorphism results from a G to T substitution. Another polymorphism, Leu432Val, is located at codon 432 in exon 3. *Leu432Val* is a G to C substitution, resulting in an amino acid change from leucine to valine. The *Ala119Ser* variant is in complete linkage disequilibrium with another CYP1B1 variant, *Arg48Gly*. *Ala119Ser* but does not alter enzyme function[105]. Conversely, *Leu432Val* was shown to increase 2 and 4-hydroxylation of estradiol by at least three-fold. [106]Both of these SNPs are common, however the *Leu432Val* is more common among African Americans (~75%) than in Caucasians (43%)[107,108].

In general, *CYP1B1* expression is increased in human breast[109,110], colon, lung, esophagus, skin, [110]and uterine[49] cancers relative to normal tissues. Watanabe and colleagues examined a Japanese population (n=990) and reported that Ala119Ser was associated with both breast (χ^2 = 8.32; d.f. = 2; p = 0.016) and lung cancers (χ^2 = 7.02; d.f. = 2; p = 0.03). [111] Goodman and colleagues studied 129 ovarian cancer cases and 144 controls, and found an increased risk of ovarian cancer associated with the *Leu432Val* variant (OR=3.8; 95% CI = 1.2-11.4). [112] Thirty-four percent of White prostate cancer cases studied by Tang et al. were homozygous for this

polymorphism, while only 12% of controls had this genotype[107]. Another study (Fritsche et al.) examined 101 and 187 German cases and controls, respectively, and found an association with colorectal cancer (OR=1.93; 95% CI = 1.15-3.24). [113] Zheng et al. found a 2.3 fold elevated risk of breast cancer among women homozygous for the variant compared to women with the wildtype genotype. Conversely, this variant was not associated with breast cancer in two larger case-control studies: De Vivo and colleagues' analysis of data from the Nurses' Health Study (n=909), [114]and Watanabe and colleagues' study of a Japanese population ($\chi^2 = 0.43$; d.f. = 2; p = 0.808). [111]

COMT

The catechol-*O*-methyltransferase (*COMT*) gene was first discovered by Axelrod and colleagues[115]. Located on chromosome 22, it can exist in either a membrane bound form (M-COMT) or a soluble form (S-COMT). COMT catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, inactivating biologically active catechols. Like the CYP genes, COMT is expressed in a variety of tissues including brain, liver, kidney, uterus, and breast[116].

Val158Met is a common SNP in exon 4 of the COMT gene. This polymorphism results from a G to A at codon 158 of M-COMT and codon 108 of S-COMT. This substitution causes an amino acid change of valine to methionine. The methionine protein has lower enzymatic activity than valine.

The low activity variant genotype has been associated with breast cancer in a number of studies. Huang et. al. observed a 4 fold increase in risk for breast cancer among women with two variant alleles compared to women with one or less allele (n=150 cases, 150 controls) [117]. Thompson et. al. examined 570 women and found a 2 fold risk in premenopausal women with two variant alleles. In postmenopausal women, however, there was an inverse relationship[118].

Conversely, Lavigne et. al. found the exact opposite. Premenopausal women with 2 variant alleles were inversely associated while postmenopausal women with 2 variant alleles had an increase risk[119]. These contradictory findings suggest that the variation in effect of the *COMT* Val158Met polymorphism may be due to factors beyond menopausal status. For example, Mitrunen et. al. found an inverse association with breast cancer among postmenopausal women with 2 variant alleles and low body mass index[120]. Mitrumen et. al. also found that postmenopausal women with this genotype and early age at menarche had an 8-fold increase in risk; postmenopausal women with this genotype and long term use of estrogen had a 4-fold increase in risk. This polymorphism has also been linked to neurological conditions such as Parkinson's disease[121] and attention deficit hyperactivity disorder[122], as well as psychological conditions such as suicidal behavior[123,124] and alcoholism[125-127].

Research suggests that estrogen and estrogen metabolites are carcinogenic and/or tumor promoters. Polymorphisms that control the production of estrogen metabolites may influence the initiation and growth of fibroids, which would explain the evidence supporting estrogen's link to fibroids.

E. Potential modifiers

Several risk factors for leiomyoma, specifically BMI, smoking, and alcohol use, may be associated with the tumors through their effects on estrogens.

BMI

Obesity (BMI $\geq$ 30 kg body weight/m^2 height) is associated with altered hormone levels and ovarian function. A small study following 20 obese women found that weight loss improved menstrual cyclicity[128]. One cross-sectional study by Dorgan et al. (N=107) showed that heavier women had significantly lower plasma sex-hormone binding globulin levels (percent difference/kg = 1.2; 95% CI 0.6-1.9). [129] A cross-sectional study by Boyapati et al. also found an

inverse association between BMI and sex hormone binding globulin[130], which may result in a higher level of circulating free estrogens. Likewise, this same study, along with a previous study by Sheinder et al. (N=22, pre-menopausal women), found estradiol and estrone levels to be positively correlated with body size[130,131]. Similarly, a case-control study conducted by Bruning et al. (n=225 cases, 44 controls) demonstrated that BMI was positively correlated with serum estrogen levels in women aged 38-75 years[132]. In contrast, a prospective study by Westhoff et al. (N=175) did not find an association between body weight and estrogen levels, but did find an inverse association with progestin levels[133].

Studies have shown that BMI is associated with an increased risk of being diagnosed with fibroids. A study of Finnish twins found that women with fibroids had a higher mean BMI (23.7 vs. 21.7, p=0.009) than women without fibroids[7]. A large prospective study of 94,095 multiethnic women found a modest association between BMI and fibroids (RR for BMI $\geq$ 30 compared to BMI between 20.0-21.9 = 1.48, 95% CI 1.15 – 1.91)[26]. Race did not appear to modify the association between BMI and fibroids. In contrast to these studies, a hospital-based case-control study (n=275 cases, n=722 controls) of Italian women by Parazzini et al. did not find an association with fibroids and BMI.

Smoking

Smoking has been implicated in a number of cancers, yet antiestrogenic effects may be protective in cancers that are estrogen dependent. Animal studies have shown that a chief constituent of cigarette smoke, nicotine, increases the number of regressing follicles in the ovary and blocks the aromatase enzyme, inhibiting ovulation and estradiol production, both *in vivo* and *in vitro*[134]. Likewise, human studies have found lower estrogen levels associated with cigarette smoking. Westhoff et al. found cigarette smoking was associated with decreased estradiol levels during the midcycle and luteal-phase of the menstrual cycle[133]. Another study examined 197 infertile women and found a strong negative correlation between a metabolite of cigarette

smoking, cotinine, and estradiol levels (r=-0.65)[135]. Predictably, smoking appeared to reduce the effectiveness of hormone replacement therapy. Smokers on oral hormone replacement therapy had estrogen levels that were 40-70% lower than that of non-smokers[136].

Several studies have found smoking to be inversely associated with fibroids. A small case-control study by Cramer et al. showed a negative association between pathologically confirmed leiomyomas and amount of cigarettes smoked per day, with 22 controls averaging 11 cigarettes per day while 22 cases averaged 7 per day[29]. A larger case-control study (n=535 cases, n=535 controls) found that smokers of 20 cigarettes a day had approximately two thirds the risk of developing pathologically confirmed fibroids than nonsmokers[23]. Faerstein et al. found an association between fibroids (pathologically or sonographically confirmed) and duration of smoking in their case-control study (n=318 cases, n=394 controls), with women who smoked 19 or more years being less likely to be diagnosed with fibroids than women who never smoked (OR=0.6, 95% CI 0.4-1.1). [21] No association was found between smoking and fibroids in the Nurses' Health Study II[26], or the Black Women's Health Sutdy[137], which are both prospective studies. Nonetheless, on balance, epidemiologic and biologic data suggest that smoking may be protective against fibroids through its antiestrogenic effects.

Alcohol Use

Alcohol consumption is associated with increased levels of endogenous estrogen. Results from the Nurses Health Study indicated that alcohol use was positively associated with estrone sulfate concentrations[138]. A cross-sectional study by Onland-Moret showed that Dutch women who consumed more than 25g of alcohol per day had higher levels of estrone and estradiol than nondrinkers[139]. A clinical trial conducted by Reichman and colleagues show that women who consumed 30g of alcohol per day had higher total estrogen concentrations than nondrinkers[140]. Conflicting results have also been presented by studies with smaller population sizes and inferior study designs. A study by Cauley et al. examining 176 Caucasian women found estrogen levels

to decline with increasing alcohol consumption[141]. London and colleagues found no association between alcohol intake and estrogen levels in their study of 325 healthy pre-menopausal women[142], and Dorgan's cross-sectional study (N=107) showed no association either[143].

Alcohol use has been implicated as a modifiable risk factor for several cancers. Several large population based studies have also found an association between alcohol use and fibroids. The Nurses' Health Study found an association between fibroids and current alcohol consumption among pre-menopausal women[18]. Wise et al. studied participants from the Black Women's Health Study and found a positive association between fibroids and alcohol use, particularly beer[137]. Alcohol's potential relationship with estrogen may explain its positive association with fibroids.

F. Significance

Fibroids are the most common reproductive tract neoplasm in all women (up to 87%), and they substantially affect higher proportions of African American women for more of their reproductive lifetime compared to White women. Although this smooth muscle tumor is benign, it is associated with significant morbidity and is the leading cause for hysterectomy (> 200,000 a year) in the United States. Fibroids also cause dysmenorrhea, menorrhagia, and possibly infertility. The causes of fibroids are unknown; however, uterine fibroids are influenced by the presence or absence of hormones, notably estrogen. Uterine fibroids are primarily diagnosed during the reproductive years--after menarche and before menopause. Estrogen metabolism may influence fibroid development by influencing exposure to estrogen and metabolites with estrogenic activity, and/or by affecting the formation of reactive catechol estrogens that may directly or indirectly induce DNA damage.

All women are exposed to estrogen and therefore catechol estrogen. If more metabolites are being created than can be conjugated, then metabolites have the potential of causing both

estrogenic effects and genotoxic damage. Polymorphisms of estrogen metabolism genes may affect how estrogen is metabolized, which consequently may determine which predominant conjugates are created. Polymorphisms in genes that encode three of these enzymes, *CYP1A1, CYP1B1*, and *COMT* may influence the rate of estrogen metabolism and the proportion of estrogenic and reactive metabolic byproducts formed locally in uterine smooth muscle. These differences in estrogen metabolism could partly account for differences in risk of fibroid development.

Since the etiology of fibroids is unknown, discovering genetic variants associated with the disease would add to our understanding of its pathogenesis and could potentially lead to new tailored medical treatments. The absence of an association with *CYP1A1, CYP1B1,* and *COMT* polymorphisms would not rule out the importance of estrogen metabolism genes in the development of fibroids, since the polymorphisms examined in this study represent only a small sample of the gene variants in this pathway. There are also additional variants which may be more informative. More studies will need to be conducted, considering a much broader range of variants in these genes and other genes in the same pathway before any conclusions can be made.

G. References

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II. STATEMENT OF SPECIFIC AIMS

A. Study questions

The aim of this dissertation is to examine the relations between uterine leiomyomata (fibroids) and estrogen metabolism gene polymorphisms that may influence estrogen activity and catechol estrogen formation. Our first hypothesis is that women with one or more variant alleles will be more likely to have uterine fibroids, and their tumors will be larger. We also hope to better understand ethnic differences (African American versus White) in the prevalence of uterine fibroids by examining associations between estrogen metabolism gene polymorphisms and uterine fibroids within ethnic strata. We hypothesize ethnic differences in the prevalence of polymorphisms may explain some of the ethnic differences in the prevalence of uterine fibroids between African Americans and Whites. As a secondary aim, we propose to evaluate the relationship between gene-environment interactions and uterine fibroids. The hypothesis is that women with one or more variant alleles and selected environmental factors will be more likely to have uterine fibroids than women without a variant allele and environmental factor.

B. Specific Aims

Primary Aims are as follows:

1. To determine if there is a positive association between the presence or size of uterine fibroids and one or more of the following polymorphisms for *CYP1A1*: **2A, *2C, *3, *4.*

2. To determine if there is a positive association between the presence or size uterine fibroids and one or both of the following polymorphisms for *CYP1B*1: *Ala119Ser* and *Leu432Val*.
3. To evaluate whether associations with the *CYP1A1* and *CYP1B1* variants noted above are modified by race.
4. To infer haplotypes for *CYP1A1* and *CYP1B1* and evaluate whether the least common inferred haplotypes are positively associated with the presence or size of uterine fibroids.
5. To determine whether there is a positive association between the Val158Met polymorphism of *COMT* and uterine fibroids.

Exploratory Aim:

1. To evaluate whether associations with the *CYP1A1* and *CYP1B1* variants noted above are modified by smoking status, age, alcohol use, or BMI.

III. METHODS

A. Overview of methods

The present study is based upon the National Institute of Environmental Health Sciences (NIEHS) Uterine Fibroid Study, a cross-sectional study of urban women aged 35-49 (Donna Baird, PI). Our primary aim is to determine whether polymorphisms of *CYP1A1, CYP1B1,* and *COMT* are associated with the prevalence of uterine leiomyomas in a sample population of African American and White women from a prepaid urban health plan. Standard analysis techniques will be conducted to determine prevalence risk ratios.

B. Study Population

The data used for this study are from the National Institute of Environmental Health Sciences Uterine Fibroid Study (UFS), in collaboration with the George Washington University Medical Center. The UFS was designed to determine the prevalence of leiomyoma in African American and White women[1]. Participants in the parent study were randomly sampled from female members of a prepaid urban health plan with approximately 50% black membership and a broad socioeconomic base. To be eligible for the parent study, participants had to be female, aged 35-49 years, English speaking, and a member of the health plan's Washington, DC site. This age range was chosen because it precedes natural menopause for the majority of the women, and would capture women with the highest prevalence of uterine fibroids. In addition, ultrasound screening for fibroids is more sensitive among women in this age range, since fibroids may regress in size after menopause and are therefore more difficult to detect.

Women included in the sample (N=2,384) were sent a letter that described the study, and were later telephoned to confirm eligibility and obtain informed consent. Of the 2,384 women that were randomly sampled, 129 (5%) could not be contacted, 150 (6%) declined screening for eligibility, and three (0.1%) were not contacted because of screener error. Of the 2,102 women screened, 316 (16%) were ineligible, mostly because they were no longer receiving care at the Washington, DC site. Nineteen percent of eligible women (N = 335) declined participation. In addition, four women who were unsure about participating and 17 women who agreed to participate could not be recontacted for the telephone interview. In total, 80% of eligible women (N=1,430) participated in the parent study.

The present study only included women from the parent study who were premenopausal and had DNA available for genotyping. Of the 1,430 women in the parent study, 1,245 (87%) were premenopausal, and 1,064 of these had DNA available for genotyping. The majority of the final sample was African American (n=583), followed by White (n=404). The number of women who were neither African American nor White was small (n=77) and therefore not included in the analysis sample.

Preliminary Data

Table A.3 shows the distribution of exposure and outcome characteristics of the study population by race. There were 404 White women, 583 African American women, and 77 women of other races. Fibroids were detected disproportionately in African American women (73%), compared with 51% of White and 57% of women classified as 'other' race. The majority of women with ultrasound detectable fibroids had fibroids that were between 2-4 cm in diameter. Only one quarter of African American women had a BMI in the normal range(<25). The other 75% were overweight or obese (BMI=25-29.9 and BMI $\geq$30, respectively). Approximately half of White women and women of other races had normal BMIs. The age distribution of study

participants was similar across races, with approximately one third aged 35-39, 40-44, and 45 and older, respectively. African American participants were more likely to be current smokers (29%) than White participants (8%) or participants of other races (16%). The majority of women had a history of oral contraceptive use (92% of African American, 83% of White, and 78% of women of other races).

Approximately 60% of African American and White women and 50% of 'other race' women were homozygous wildtype for *CYP1A1*2A*. Only 4% (n=23) of African Americans and 2% (n=9) of Whites were homozygous variant (C/C) for *CYP1A1*2A*, compared with 16% (n=12) of women of other races.

The overwhelming majority of African American and White women were homozygous wildtype for *CYP1A1*2C* (98% and 90% respectively), compared with 67% of women of other races. Only one white woman and no African American women were homozygous variant (G/G) for *CYP1A1*2C*, in contrast with 9% (n=7) of women of other races.

Previous studies have found the variant *CYP1A1*3* allele (C/C) primarily in African Americans[2-4]. Our population is similar. Approximately 86% of African American participants were homozygous wildtype, 14% were heterozygous, and <1% (n=4) were homozygous variant for *CYP1A1*3*. In contrast, over 99% of White participants and 95% of other race participants were homozygous wildtype for *CYP1A1*3*.

The prevalence of *CYP1A1*4* was similar across racial categories. Over 90% of participants within each race possessed the wildtype genotype, and only one 'other race' participant was homozygous for the *CYP1A1*4* variant.

About one quarter of African American participants were homozygous wildtype for the *CYP1B1* Ala119Ser polymorphism, while 50% were heterozygous. In contrast, about half of White and 'other race' participants were homozygous wildtype for Ala119Ser, and only a third was heterozygous.

Half of African American participants were homozygous wildtype for the *CYP1B1* Leu432Val polymorphism. The other half possessed one or more of the variant alleles (37% heterozygous, 7% homozygous variant). Only 17% of White participants had the homozygous wildtype genotype, while the majority had one or more variant alleles (51% heterozygous, 33% homozygous variant). Likewise, the majority of participants of other races had one or more variant alleles (25.97% wildtype, 40.26% heterozygous, 33.77% homozygous variant).

Almost half of the African American participants possessed the homozygous wildtype genotype for the *COMT* Val158Met polymorphism. Forty-three percent were heterozygous and 9.62% were homozygous recessive. Approximately one quarter of White participants were homozygous wildtype for this polymorphism. Half of the White participants possessed the heterozygous genotype, while the quarter possessed the homozygous variant genotype.

The distributions of all of the *CYP1A1, CYP1B1,* and *COMT* genotypes to be analyzed in the proposed study were evaluated for consistency with Hardy Weinberg equilibrium (HWE). HWE was calculated using the following formula:

$$(p + q)^2 = p^2 + 2pq + q^2,$$

where p equals the proportion of one of the alleles, and q equals the proportion of the other allele. The above equation gives the expected genotypic frequencies of p^2, 2pq, and q^2, and a chi-square test was used to determine whether the difference between observed and expected genotypic frequencies was statistically significant. HWE was calculated among controls. It was also evaluated among the racial groups separately (White, African American), since allele frequencies varied by race. The distribution of all genotypes within racial groups, were consistent with HWE (df=1, p=0.05, critical value=3.84). Table A.4 shows the expected and observed frequencies of each genotype, and chi-square results.

C. Data Collection and Classification

Overview

Demographic data for the current study were collected by a self-administered questionnaire, and by a telephone interview (for reproductive and gynecologic history data) conducted by trained staff. Ultrasound examinations (to detect uterine leiomyomas and determine their size), blood collection (for DNA) and weight measurements were performed during a clinic visit[1]. DNA was genotyped using matix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), an accurate and efficient technique that permits simultaneous identification of multiple single nucleotide polymorphisms (SNPs) in a single reaction[5].

Outcome Assessment

Transabdominal and transvaginal ultrasounds to detect uterine fibroids were performed on premenopausal participants by sonographers who were certified by the American Registry of Diagnostic Medical Sonographers, under the supervision of a radiologist trained in ultrasonography[1]. If a premenopausal woman had a pelvic ultrasound examination recently, the radiology records from that examination were used to assess fibroid status. Women were classified as positive for uterine leiomyoma if the ultrasound examination associated with the clinic visit identified fibroids; they were classified as negative if the sonogram evidence confirmed the absence of fibroids. The ultrasound examination was also used to determine the size of the largest fibroid. Fibroid size was categorized into four levels: no detectable fibroids, largest fibroid 2 cm or less in diameter (small), largest fibroid 2-4 cm in diameter (medium), and largest fibroid 4 cm or larger in diameter (large).

Genotype Assessment

Fasting blood samples were collected during the clinic visit. After preparation (aliquotion and centrifugation), blood samples were sent to an outside laboratory (BioServe Biotechnologies,

Ltd.) for DNA extraction and genotyping. DNA was extracted from whole blood using a modified salt precipitation DNA extraction kit, GenQuick (manufactured by BioServe Biotechnologies). Polymorphisms of *CYP1A1, CYP1B1,* and *COMT* were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectometry (MALDI-TOF MS) technique.

The MALDI-TOF system is a high throughput, cost-effective, and accurate technique that simultaneously performs multiple genotyping analysis on very small quantities of DNA[5]. The process begins with polymerase chain reaction (PCR) amplification of a 60 to 200 base pair region surrounding the SNP of interest. The corresponding MassEXTEND primer extension reaction generates allele-specific products that are applied to a SpectroChip array. The SpectroChip is placed in the MALDI-TOF spectrometer where a laser beam causes desorption and ionization. The product then travels towards a detector. The time that it takes for the product to reach the detector determines the product's unique mass. SpectroTYPER software was used to analyze the results. Assays failed for less than 2% of all participants for each SNP. To ascertain genotying error from genotyping, repeat samples were run for 30 participants. All but one of the repeat sample pairs were in agreement with one another. The exception was discordant for *Val158Met* of the *COMT* gene.

The SNPs (CYP1A1*2A, CYP1A1*2C, CYP1A1*3, CYP1A1*4, Ala119Ser, Leu432Val, and Val158Met) were chosen based on their associations with estrogen mediated tumors in previous research. The primers used for each SNP are listed in Table A.5. For each SNP, participants were classified as homozygous wildtype if they had two copies of the wildtype allele (referent group), homozygous variant if they had two copies of the variant allele, and heterozygous if they had one wildtype allele and one variant allele. Table A.3 details the prevalence of each SNP in the study population.

Covariates

A self-administered questionnaire was used to collect demographic data. Telephone interviews were used to gather reproductive and gynecologic history data. Weight was measured during a clinic visit.

Body Mass Index (BMI). BMI (kg/m^2) was determined based on weight recorded at the clinic visit by a trained researcher. Height was self-reported during the telephone interview. Previous Participants were then categorized as follows: normal (BMI below 25), overweight (BMI between 25 and 29.9), and obese (BMI = 30 or above).

Age. Age at enrollment (in years) was self-reported during the telephone interview. Age was categorized into five-year intervals: 35 – 39, 40 – 44, and 45 and above.

Smoking Status. During the telephone interview, participants were asked, "Have you ever smoked an average of at least one cigarette a day for six months or more?" Women who responded "no" were classified as non-smokers. Women who responded positively were asked additional questions to determine their current smoking status. Women were classified as former smokers if they answered negatively to the question, "Do you currently smoke even one cigarette per day?" Positive responders were classified as current smokers.

Race. Race and ethnicity were self-reported in the mailed questionnaire. Specifically, participants were asked, "Which category best describes you" and given the following options: "White/not Hispanic," "White/Hispanic," "Black/not Hispanic," "Black/Hispanic," "Asian/Pacific Islander," and "American Indian/Eskimo/Aleut." Respondents were also given the option of specifying other categories. If participants did not respond to the question on race/ethnicity in the mailed questionnaire, they were asked again during any subsequent telephone interview. Clinic records were used to determine race if it was not available from the mailed questionnaire or interview.

Participants were classified as African American if they self-identified themselves as "Black" in any way (e.g. Black/not Hispanic, Black and White, etc.). Participants were coded as

Caucasian if they self-identified themselves as "White" in any way, with the exception of "White" in combination with "Black." Only 77 of the 1,064 women included in the present study were classified as a race other than "Black" or "White." Therefore, these women were grouped together in an "other race" category.

Births after age 24. During the telephone interview, participants were asked, "Have you given birth to any children?" and "In what month and year?" The age of the mother at the time of birth was calculated based on her response and her birthday. Only deliveries occurring after the age of 24 were considered for the control of a parity effect. The protective effects were not seen for births at early ages in previous research[28].

Oral contraceptive use. Oral contraceptive use was recorded during the telephone interview. Participants were asked, "Have you ever used birth control pills?" A favorable response was categorized as positive for oral contraceptive use.

Alcohol use. Alcohol use was self-reported in the mailed questionnaire. Participants were asked how often they consume beer, wine, and liquor as separate questions. They were given the following options: never or less than 1 per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day, 4-5 per day, and 6+ per day. Next, they were asked how much they consumed (small, medium, or large serving size). As a reference, a medium size serving for beer, wine, and liquor was defined as a 12 ounce can or bottle, one medium glass, and one shot, respectively.

D. Analysis

Power Calculations

Power was calculated using the Power Program V3.0[6]. Calculations are based on the known prevalence of dichotomous genotypes (homozygous wildtype versus heterozygous and homozygous variant genotypes, grouped together) and uterine fibroids (73% and 51% among

African American women and White women, respectively) in the study population. Heterozygous and homozygous variant genotypes were grouped together to increase power. Power calculations are based on estimation of odds ratios instead of prevalence risk ratios; however the estimations should be roughly comparable. Tables A.6 and A.7 show the estimated power to detect statistically significant odds ratios of 1.5, 2.0, and 3.0 (where α=.05) given the distribution of genotypes and fibroids among White and African American participants, respectively. There is adequate power to detect an OR of 3.0 in both groups for the following SNPs: CYP1A1*2A, Ala119Ser, and Leu432Val. However, there may not be enough power to detect a OR of 3.0 for CYP1A1*2C and CYP1A1*4 for African Americans. Within the White group, there may not be enough power to detect a OR of 3.0 for CYP1A1*3.

Haplotyping

PHASE version 2.0.2[7,8] was used to statistically estimate the frequencies of various haplotype phases, using the Markov chain-Monte Carlo algorithm, given the distribution in the study population. A haplotype is a group of alleles on the same chromosome that tend to segregate together. The identification of a haplotype identifies all other polymorphic sites at a region, though the proposed study is limited in accomplishing this due to the small number of SNPs. To account for uncertainty, we limited the sample to include only those with data for 3 of 4 *CYP1A1* polymorphisms. For the *CYP1B1* haplotype analysis, we included those with data for both polymorphisms. To further account for uncertainty, the haplotype probability estimates $\leq$ 0.90 were excluded. Haplotypes were modeled with the most common haplotype as the referent. Since haplotype analysis was not the focus of the parent study, SNPs were not chosen to capture all of the variability of the gene. Unlike typical haplotype analyses, haplotype reconstruction were done with sparse coverage for this study.

Main effects of genotypes

When stratified by race, all genotypes were consistent with HWE. Each polymorphism and haplotype was analyzed singly (wildtype versus homozygous variant and heterozygous variant grouped together) to determine whether there is an association with the presence of fibroids. The main effects of the genotypes was determined using the following model for the dichotomous outcome of fibroid presence:

$$\text{Log } [p(\text{fibroids})] = \beta_0 + \beta_1(\text{polymorphism/haplotype}).$$

For the polytmous outcome of fibroid size, the following models was used with "no fibroids" as the common reference:

$$\log[p(\text{fibroids}<2\text{cm})] = \beta_0 + \beta_1(\text{polymorphism/haplotype})/1+\beta_0 + \beta_1(\text{polymorphism/haplotype}),$$

$$\log[p(\text{fibroids } 2\text{-}3.999\text{cm})] = \beta_0 + \beta_1(\text{polymorphism/haplotype})/1 + \beta_0 + \beta_1(\text{polymorphism/haplotype}), \text{ and}$$

$$\log[p(\text{fibroids} \geq 4\text{cm})] = \beta_0 + \beta_1(\text{polymorphism/haplotype})/1 + \beta_0 + \beta_1(\text{polymorphism/haplotype}).$$

All parameter estimates were obtained using SAS 8.0 (Statistical Analytic Systems, Version 8.0; SAS Institute, Inc., Cary, NC; 1999). The exponentiated parameter estimates represent prevalence risk ratios and is interpreted as the risk of having fibroids among those with the variant polymorphism (e.g. homozygous and heterozygous variant for *CYP1A1*2A*) or haplotype versus the risk among those with the wildtype polymorphism (e.g. homozygous wildtype for *CYP1A1*2A*). These models were analyzed again with fibroid size as the outcome.

Based on previous literature described earlier (Chapter 1), all genotype main effects were examined for effect modification/interaction by alcohol use (<0.5, 0.5-<3, 3-<7, and 7 or more drinks per week) BMI (<25, 25-29.99, and ≥30), smoking (never smoker, current smoker, and past smoker), oral contraception use (never used, current/past use), number of births after age 24

(none, 1, 2, 3 or more), and age (35-39, 40-44, >=45). Effect measure modification was assessed on the multiplicative and additive scales by evaluating joint and independent effects of each polymorphism and the potential effect modifier relative to a common referent group. An example such a model for the joint effects of BMI and a gene variant relative to a common referent exposure group (wildtype polymorphism and BMI <25) is as follows:

$$\log [p(\text{fibroids})] = \beta_0 + \beta_1(\text{var}) + \beta_2(\text{BMI} < 25) + \beta_3(\text{BMI } 25\text{-}29.99) + \beta_4(\text{BMI} \geq 30) + \beta_5(\text{BMI} < 25 * \text{var}) + \beta_6 (\text{BMI } 25\text{-}29.99 * \text{var}) + \beta_7 (\text{BMI} > 30 * \text{var}),$$

where wt = wildtype polymorphism (two wildtype alleles) and var = variant polymorphism (either homozygous variant or heterozygous variant). Models assessing effect modification by smoking and alcohol use were similar. For multiplicative interaction, a likelihood ratio test determined the goodness of fit of the model with main effects terms only (for example, BMI 25-35, BMI >35, and the polymorphism), and the magnitude of risk (prevalence) ratio modification were assessed by comparing observed joint effect estimates with expected values assuming multiplicative risks. Interaction on the additive scale (prevalence difference modification) were evaluated by comparing observed versus expected joint effects of the polymorphism with BMI, smoking status, and alcohol assuming additive risks. In addition, Interaction Contrast Ratios and 95% CI were calculated to estimate departures from additive risks.

A priori assessments using a Directed Acyclic Graph revealed race as a potential confounder. Race is related to uterine leiomyoma, which occur more frequently in African Americans than in other races[1,9-12]. Race is also related to the genetic polymorphism *CYP1A1*3*, which is more prevalent in African Americans than in other races[4]. Population stratification may cause other factors to confound the relationship between the polymorphisms and uterine leiomyoma. Smoking, BMI, oral contraceptive use, and parity may be related to uterine fibroids; they are

related to the genetic polymorphism *CYP1A1*3*, via race. Since all analyses will be stratified by race, it is less likely that population stratification would pose a bias. In order to pose a bias, there would have to be subgroups that differ from other subgroups with regard to genotype and fibroid prevalence within the same racial category. All factors were included in the model (by race) one at a time. A factor is considered a confounder if it's inclusion into the model produces a change in effect >10%.

Prevalence risk estimates for *CYP1A1*3* will were estimated only for the African American strata due to the small numbers of African Americans with these alleles. The precision of all estimates would be limited by small numbers in the other races category; consequently, we did not conduct analyses separately for this group.

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IV. RESULTS

A. *Val153Met* Polymorphism of *Catechol-O-Methyltransferase* and Prevalence of Uterine Leiomyomata

1. Abstract

The *catechol-O-methyltransferase* (*COMT*) gene encodes enzymes that inactivate catechol estrogens and may have a protective role in estrogen induced tumorigenesis, such as uterine leiomyomata (fibroids). *Val158Met* is a common single nucleotide polymorphism (SNP) of the *COMT* gene (Ex4-12 G>A; rs4680) that results in a lower activity enzyme, possibly increasing susceptibility to tumorigenesis. However, a recent study reported an association between the high activity allele and fibroids. The purpose of this study was to evaluate the relation between the *COMT Val158Met* polymorphism and uterine fibroids. The data are from the National Institute of Environmental Health Science Uterine Fibroid Study, including 583 African American and 404 White women with genotype and ultrasound screening data for assessment of fibroid status. Log regression models were used to estimate prevalence ratios for fibroids prevalence. In addition, main effects were evaluated for effect measure modification by alcohol use, BMI, smoking, births after age 24, oral contraceptive use, and age. No associations between fibroids and *Val158Met* were observed for either ethnic group. This study suggests variation in this polymorphism alone does not affect fibroid prevalence.

2. Introduction

The catechol-*O*-methyltransferase (COMT) inactivates catechol estrogens by catalyzing the transfer of a methyl group from S-adenosylmethionine to catecholamines. A by-product of COMT, 2-methoxyestradiol, may have a protective role in estrogen induced tumorigenesis by inhibiting angiogenesis[1] and cell-proliferation[2-4]. *Val158Met* (Ex4-12 G>A; rs4680) is a common polymorphism that may result in a lower activity enzyme[5]. The low-activity *Met* allele has been positively associated with breast cancer in some, but not all studies[6-8]. The present study evaluated the relation between *COMT Val158Met* and uterine fibroids, which are common benign estrogen-responsive tumors in premenopausal women that were positively associated with the Val/Val genotype in a recent study [9].

3. Methods

Study Population

Data are from premenopausal participants in the National Institute of Environmental Health Sciences Uterine Fibroid Study (UFS). Participants were women randomly selected from a prepaid urban health plan between 35-49 years of age. Detailed information about the UFS is published elsewhere[10]. Women included in this analysis had available DNA and self-reported their race as African American or White (N=987).

Demographic and medical history data were obtained via telephone interview (including oral contraceptive use, smoking, height, and full-term births after age 24 which were associated with fibroids in previous analysis of this study population[11]) and a self-administered questionnaire (which included race, alcohol use.)

Outcome Assessment

Fibroid status was ascertained by screening with transabdominal and transvaginal ultrasound examinations (performed under the supervision of a radiologist by sonographers certified by the American Registry of Diagnostic Medical Sonographers) or was derived from clinical radiology records for women with a recent pelvic ultrasound exam at the study clinic. The presence or absence of fibroids and the diameter of the largest fibroid were determined for each participant.

Genotype Assessment

DNA was extracted from whole blood samples using a modified salt precipitation DNA extraction kit (GeneQuick). The *Val158Met* polymorphism was identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF)[12].

Statistical Analysis

Binomial log regression models were used to estimate race-specific prevalence ratios for any fibroids versus no fibroids, or for small (<2cm), medium (2-<4), or large (≥4cm) fibroids versus no fibroids (Statistical Analytic Systems, Version 8.0; SAS Institute, Inc., Cary, NC; 1999)[13,14]. Confounding was identified based on ≥10% change in prevalence ratios for *COMT* when potential confounders (age, body mass index (BMI), smoking status, oral contraceptive use, alcohol use, births after age 24) were added to the model. All genotype main effects were examined for effect measure modification by alcohol use, BMI, smoking, births after age 24, oral contraceptive use, and age based on likelihood ratio tests and interaction contrast ratios (for departures from multiplicative and additive prevalences, respectively).

4. Results

Among 583 African Americans, 421 (72%) had at least one fibroid detected, and 24% had large fibroids. Among 404 Whites, 201 (50%) had at least one fibroid, and 11% had large fibroids (Table 1).

COMT genotypes varied by race. Among African Americans, 47% were *Val/Val*, 43% were *Val/Met*, and 10% were homozygous variant *Met/Met*. For Whites, 24% were *Val/Val*, 50% were *Val/Met*, and 26% were *Met/Met*. Distributions of *COMT* genotypes among noncases within each racial group were consistent with Hardy Weinberg Equilibrium.

In general, estimated prevalence ratios for fibroids in association with one or two copies of the *Met* allele versus the *Val/Val* genotype were close to the null for both African American and White women (Table 2). Estimated prevalence ratios for small, medium, or large fibroids in association with the *Met* allele also were close to null, with the exception of an inverse association between the *Met* allele and small fibroids (versus no fibroids) among African Americans (PR=0.74; 95%CI:0.54,1.03). Adjusting for potential confounders did not alter estimates ≥10%; therefore, final models included genotype only. Interaction contrast ratios were all close to 0 (consistent with additive scale homogeneity)[14], and likelihood ratio test p-values were all above 0.28 (consistent with multiplicative scale homogeneity) for the potential effect measure modifiers examined.

5. Discussion

Fibroids disproportionately affect African Americans compared with Whites[10,15-18]. Our study included 583 African Americans, who were more likely than White participants to have fibroids and the wildtype *COMT Val/Val* genotype; however the *Val158Met* polymorphism was not significantly related to prevalent fibroids among African American or White women in this study.

Al Hendy et al. reported a positive association between fibroids and the *Val/Val* versus *Met/Met* genotype (odds ratio=2.5; 95%CI:1.02,6.15)[9] in a study that compared 186 cases (59 White, 81 African American, 46 Hispanic) that underwent hysterectomy for "symptomatic uterine fibroids" and had two or more histologically confirmed fibroids to 142 controls

undergoing hysterectomy for cervical dysplasia, benign ovarian masses, or dysfunctional uterine bleeding. In contrast, our study included 423 African American and 201 White women with ultrasound evidence of fibroids (both symptomatic and asymptomatic), and 162 African American and 203 White women with no fibroids detected who were representative of members of a large health plan, rather than women undergoing hysterectomy. In addition, we estimated prevalence ratios instead of prevalence odds ratios, which may exaggerate associations with common outcomes [13,14] such as fibroids. We stratified analyses by race and grouped *Val/Met* and *Met/Met* genotypes together to increase sample size, but also found that fibroids were not associated with the *Met/Met* versus *Val/Val* genotype in the total population (PR=0.87; 95%CI:0.75,1.02), or among African Americans (PR=1.00, 95%CI:0.85,1.18), or Whites (PR=1.02, 95%CI:0.77,1.35). We had >80% power to detect a statistically significant (α=0.05) PR$\geq$1.16 for fibroids versus no fibroids in association with *Val/Val* versus *Val/Met* or *Met/Met* genotypes in African Americans, and >80% power to detect a PR$\geq$1.43 in Whites.

Our findings do not rule out an association with fibroids treated by hysterectomy, but large fibroids, which are more likely to lead to hysterectomy[19], were not associated with *Val158Met* in our study. We cannot rule out associations between prevalent fibroids and other *COMT* variants; therefore future research should evaluate additional polymorphisms, as well as other genes in the estrogen metabolism pathway.

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Table 4.1. Characteristics of premenopausal study participants by race and fibroid status, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Fibroid Status									
	All Women		African American				Caucasian			
	No Fibroids	Fibroids present	No Fibroids	Small	Medium	Large	No Fibroids	Small	Medium	Large
Total	350	622	155	94	187	140	195	70	87	44
Age										
35-39	161 (46.0)	186 (29.9)	78(50.3)	37(39.4)	65(34.8)	36(25.7)	83(42.6)	19(27.1)	18(20.7)	11(25.0)
40-44	125 (35.7)	215 (34.6)	52(33.5)	33(35.1)	72(38.5)	47(33.6)	73(37.4)	17(24.3)	31(35.6)	15(34.1)
>=45	64 (18.3)	221 (35.5)	25(16.1)	24(25.5)	50(26.7)	57(40.7)	39(20.0)	34(48.6)	38(43.7)	18(40.9)
BMI										
Normal	168 (48.0)	208 (33.4)	52(33.5)	19(20.2)	40(21.4)	36(25.7)	116(59.5)	39(55.7)	55(63.2)	19(43.2)
Overweight	94 (26.9)	178 (28.6)	45(29.0)	29(30.9)	52(27.8)	51(36.4)	49(25.1)	14(20.0)	18(20.7)	14(31.8)
Obese	88 (25.1)	236 (37.9)	58(37.4)	46(48.9)	95(50.8)	53(37.9)	30(15.4)	17(24.3)	14(16.1)	11(25.0)
Smoking Status										
Never Smoked	194 (55.4)	310 (50.2)	79(51.0)	41(43.6)	80(42.8)	75(53.6)	115(59.0)	44(62.9)	46(52.9)	24(54.5)
Past Smoker	99 (28.3)	170 (27.5)	32(20.6)	19(20.2)	50(26.7)	32(22.9)	67(34.4)	22(31.4)	32(36.8)	15(34.1)
Current Smoker	57 (16.3)	138 (22.3)	44(28.4)	33(35.1)	56(29.9)	31(22.1)	13(6.7)	4(5.7)	9(10.3)	5(11.4)
Oral Contraceptive Use										
Never Used OC	51 (14.6)	64 (10.3)	19(12.3)	5(5.3)	12(6.4)	12(8.6)	32(16.4)	10(14.3)	16(18.4)	9(20.5)
Current/Past User Of OC	299 (85.4)	558 (89.7)	136(87.7)	89(94.7)	175(93.6)	128(91.4)	163(83.6)	60(85.7)	71(81.6)	35(79.5)

Characteristics	Fibroid Status									
	All Women		African American				Caucasian			
	No Fibroids	Fibroids present	No Fibroids	Small	Medium	Large	No Fibroids	Small	Medium	Large
Number of Fullterm Births after age 24										
None	182 (52.0)	359 (57.7)	70(45.2)	48(51.1)	98(52.4)	75(53.6)	112(57.4)	44(62.9)	57(65.5)	37(84.1)
1	85 (24.3)	151 (24.3)	52(33.5)	31(33.0)	52(27.8)	45(32.1)	33(16.9)	7(10.0)	14(16.1)	2(4.5)
2	66 (18.9)	98 (15.8)	24(15.5)	12(12.8)	30(16.0)	18(12.9)	42(21.5)	18(25.7)	16(18.4)	4(9.09)
3 Or More	17 (4.9)	14 (2.3)	9(5.8)	3(3.2)	7(3.7)	2(1.4)	8(4.1)	1 (1.43)	0 (0)	1 (2.27)
Number of Alcoholic Drinks per Week										
<0.5	111 (34.4)	246 (27.2)	76(49.0)	47(50.0)	98(52.4)	76(54.3)	35(17.9)	20(28.6)	22(25.3)	14(31.8)
0.5-<3	95 (29.4)	138 (23.7)	38(24.5)	22(23.4)	35(18.7)	25(17.9)	57(29.2)	21(30.0)	25(28.7)	9(20.5)
3-<7	60 (18.6)	103 (17.7)	16(10.3)	12(12.8)	18(9.6)	18(12.9)	44(22.6)	20(28.6)	21(24.1)	8(18.2)
7 Or More	57 (17.7)	95 (16.3)	11(7.1)	12(12.8)	22(11.8)	12(8.6)	46(23.6)	4(5.7)	9(10.3)	3(6.8)

Table 4.2. Prevalence ratios and 95% Confidence Intervals (CI) for the association between *COMT Val158Met* genotypes and uterine fibroids in African American and White premenopausal participants in the Uterine Fibroid Study

Fibroid Status	***African Americans***					***Caucasians***				
	Val/Val		**Val/Met & Met/Met**			**Val/Val**		**Val/Met & Met/Met**		
	N	**%**	**N**	**%**	**RR (95% CI)**	**N**	**%**	**N**	**%**	**RR (95% CI)**
No Fibroids	65	23.7	90	29.2	1.00	47	49.0	148	48.1	1.00
Any Fibroids	206	75.2	214	69.5	0.96 (0.92, 1.01)	47	49.0	154	50.0	1.01 (0.90, 1.13)
Small	51	18.6	43	14.0	0.86 (0.73, 1.01)	14	14.6	56	18.2	1.09 (0.85, 1.41)
Medium	86	31.4	100	32.5	0.96 (0.87, 1.06)	23	24.0	64	20.8	0.96 (0.79, 1.17)
Large	69	25.2	71	23.1	0.93 (0.82, 1.04)	10	10.4	34	11.0	1.03 (0.75, 1.42)

F. Polymorphisms and Haplotypes of Cytochrome P-450 1A1 and 1B1, and Prevalence of Uterine Leiomyomata

1. Abstract

Uterine leiomyomata (fibroids) are hormone-dependent tumors that disproportionately affect African Americans compared to Whites. Single nucleotide polymorphisms (SNPs) in *Cytochrome P-450 1A1 (CYP1A1)* and *1B1 (CYP1B1)* encode enzymes that could affect estrogen's biologic ability to influence fibroid development. This study examined the relation between fibroid prevalence and common SNPs and haplotypes of *CYP1A1* (*2A, *3, *2C, *4) and *CYP1B1* (CYP1B1AS, CYP1B1LV). Relations between polymorphisms and fibroid size (small, medium, or large versus none) were also determined. The study population included a cross-sectional sample of premenopausal African American (n=583) and White (n=404) women who participated in the National Institute of Environmental Health Science's Uterine Fibroid Study. Blood was collected from participants for DNA, and telephone interviews and questionnaires were completed to gather demographic and reproductive history. Prevalence ratios (PR) and prevalence differences (PD) were estimated using race-specific log-risk and linear-risk regression models. Effect measure modification by age, body mass index, oral contraception use, fullterm births, smoking and alcohol use was evaluated. Distributions of genotypes and fibroid prevalence varied by race. An association between fibroids and variants of *CYP1A1*3* (PR=1.14; 95%CI: 1.02, 1.28) was observed among African Americans. *CYP1A1*2C* variants tended to be associated with a reduction in the PR among Whites (0.79; 95%CI: 0.54, 1.18). The PRs for the association between *CYP1A1*4* variants and fibroids were elevated among Whites (PR=1.20; 95%CI: 0.90, 1.61) and African Americans (PR=1.16; 95%CI:

0.92, 1.48). Haplotypes containing the above variants showed similar results. No associations were observed between fibroids and *CYP1B1* variants. Adjusting for potential confounders did not alter effect estimates ≥10% and were therefore not included in final models. Analysis of effect measure modification did not show deviations from additive or multiplicative expectations. Our results reveal possible relations between fibroid prevalence and polymorphisms of *CYP1A1*3* and **4* in African Americans and *CYP1A1 *2C* and **4* in Whites; however, results should be interpreted with caution due to the potential for non-causal associations, and future studies should include a more comprehensive assessment of variation in *CYP1A1* and *CYP1B1*, in addition to other estrogen metabolism genes that may influence the pathogenesis of uterine fibroids.

2. Introduction

Uterine leiomyomata, or fibroids, are the most common reproductive tract tumors in women[1], and they affect a substantially higher proportion of African American women than White women[2-6]. Although these smooth muscle tumors are benign, they are associated with significant morbidity and are the leading indication for hysterectomy in the United States[7,8]. Fibroids also are associated with dysmenorrhea, menorrhagia, and possibly infertility[1]. The causes of fibroids are unknown; however, clinical evidence suggests that they are strongly influenced by sex hormones, notably estrogen[9,10].

Estrogen metabolism may affect uterine fibroids by influencing exposure to estrogen and metabolites with estrogenic activity, or by influencing the formation of reactive catechol estrogens that may cause oxidative damage. Cytochrome P450 enzymes catalyze Phase I hydrozylation of estradiol. More specifically, *CYP1A1* enzymes catalyze hydrozylation at the 2 carbon position (2-hydroxyestradiol) and *CYP1B1* enzymes catalyze hydrozylation at the 4 carbon position (4-hydroxyestradiol). Hydrozylation at either position generates catechol estrogens and metabolites that vary in their ability to stimulate estrogen receptors and/or induce oxidative damage[11]. 4-hydroxyestradiol metabolites are more estrogenic (with respect to estrogen receptor activation) and are more likely to result in mutagenic DNA adducts than 2-hydroxyestradiol metabolites[12,13].

Polymorphisms that influence estrogen metabolism enzymes might influence fibroid tumorigenesis by influencing estrogen hydrozylation and the production of estrogenic by-products or DNA adducts. Several common polymorphisms have been identified in *CYP1A1* and *CYP1B1*[14,15]. *CYP1A1*2C* (A>G; rs1048943) and **4* (C>A; rs1799814) in the heme-binding region cause amino acid substitutions of isoleucine to valine, and threonine to asparagine, respectively. *CYP1A1*2C* has been associated with other estrogen mediated tumors, such as

breast cancer[16], although not all breast cancer studies have found an association[17,18]. The functional significance of *CYP1A1*2A* (T>C) and **3* (T>C) variants in the 3' non-coding region[19,20] is unknown, however *CYP1A1*2A* has been reported to be in linkage disequilibrium with the non-synonymous **2C* variant[14,15,21-23], and the **3* variant is very common among African Americans[18] who are at increased risk of fibroids[2-6]. Some[24,25] but not all[18,26,27] studies have associated *CYP1A1*2A* with breast cancer, which is also believed to be an estrogen dependent tumor[28,29].

CYP1B1 Leu432Val (G>C; rs1056836) and *Ala119Ser* (G>T; rs1056827) are located in exons 3 and 2 respectively. The Leu432Val polymorphism results in a leucine to valine amino acid substitution that increased 2 and 4-hydroxylation of estradiol by at least three-fold in cells isolated from *Escherichia coli*[30]. *Ala119Ser* results in an amino acid change of alanine to serine, but has not been shown to alter enzyme function[31]. Previous studies have found associations between breast cancer and *CYP1B1 Ala119Ser*[32]and *Leu432Val*[33]; however, associations with *Leu432Val* have been inconsistent[32,34].

The present study evaluated the relation between several common polymorphisms (*CYP1A1*1, CYP1A1*2, CYP1A1*3, CYP1A1*4, CYP1B1 Ala119Ser*, and *CYP1B1 Leu432Val*) and the prevalence of uterine fibroids in a population-based cross-sectional study of premenopausal women. In addition, we examined associations with estimated *CYP1A1* and *CYP1B1* haplotyes and evaluated effect measure modification by factors that may influence fibroids or estrogen metabolism.

3. Methods

Study Population

Data for this study are from the National Institute of Environmental Health Sciences Uterine Fibroid Study (UFS), which was conducted in collaboration with the George Washington

University Medical Center. UFS participants were randomly selected women from a prepaid urban health plan, chosen because of its substantial black membership and broad socioeconomic base. To be eligible, participants had to be aged 35-49 years, English speaking, and a member of the health plan's Washington, DC site. Eighty percent of eligible women participated (N=1430)[3]. This analysis was restricted to premenopausal UFS participants (n=1245) (defined as women who, at enrollment, had reported a menstrual period, pregnancy, or breastfeeding during the previous twelve months) that had available DNA for genotyping (N=1064) and self-reported either African American or White race, giving a final sample of 987.

Outcome Assessment

Fibroids 0.5cm in diameter or larger were identified by transabdominal and transvaginal ultrasounds performed by sonographers certified by the American Registry of Diagnostic Medical Sonographers, under the supervision of a radiologist trained in ultrasonography. Radiology records were used to assess fibroid status for women with a recent pelvic ultrasound examination done at the study site for clinical purposes. The size of the largest fibroid among women who had fibroids was categorized as small (diameter 2 cm or less), medium (diameter >2 to < 4 cm), or large (diameter 4 cm or larger).

Assessment of Genotype and Covariates

Fasting whole blood samples were collected from participants during a clinic visit (74% of the parent study population) and sent to an outside laboratory (BioServe Biotechnologies, Laurel, MD) for genotyping. DNA was extracted from whole blood using a modified salt precipitation DNA extraction kit, GeneQuick (manufactured by BioServe Biotechnologies, Laurel, MD). Polymorphisms of *CYP1A1* and *CYP1B1* were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectometry (MALDI-TOF MS), a high throughput, accurate technique that simultaneously performs multiple genotyping analyses on very small quantities of DNA[35]. Assays failed for less than 2% of all participants for each SNP. Repeat

samples were run for 30 randomly selected participants to assess genotyping error. Of 180 paired repeats, 5 failed to amplify on one of the two assays; all other pairs were concordant.

Data were collected on other factors known or suspected to be related to fibroids. Participant characteristics, such as age, race, and alcohol use were collected by a self-administered questionnaire. Trained staff conducted telephone interviews to collect data on oral contraceptive use, smoking, and reproductive history and height. Weight was measured at a clinic visit. The number of births after age 24, which was found to be inversely associated with fibroids in a previous analysis from this study[36], was determined from responses to the interview.

Data Analysis

Distributions of *CYP1A1* and *CYP1B1* polymorphisms were evaluated for consistency with Hardy Weinberg Equilibrium among noncases. Pairwise linkage disequilbrium (r^2) was estimated for pairs of *CYP1A1* single nucleotide polymorphisms (SNPs) and *CYP1B1* SNPs[37,38]. Haplotypes, defined by the measured SNPs (4 in *CYP1A1* and 2 in *CYP1B1*), were inferred using the PHASE program (version 2.0.2)[39,40], which estimates the most probable haplotype pair (or diplotype) for each observation and the posterior probability of each inference. Each allele at each of the 4 loci 5' to 3' for *CYP1A1,* and the 2 loci 5' to 3' for *CYP1B1* compose the haplotype for each gene. To reduce error due to random variation, haplotypes with posterior probabilities less than 90% were excluded from haplotype analyses (n=111 African Americans missing data for *CYP1B1* haplotypes). Women missing more than one SNP for *CYP1A1* (2 African American and 1 White woman), and women without complete SNP data for *CYP1B1* (6 African American and 2 White women) also were excluded. The analysis groups for were similar in study characteristics to the total study populations (581 African Americans and 403 Whites for *CYP1A1* haplotype analysis, 466 African Americans and 402 for *CYP1B1* haplotype analysis).

All parameter estimates were obtained using SAS 8.0 (Statistical Analytic Systems, Version 8.0; SAS Institute, Inc., Cary, NC; 1999). Race-specific log-risk and linear-risk regression

models were used to determine prevalence ratios (PR) and prevalence differences (PD)[41,42] for fibroids in association with variant versus wild type genotypes among African American and White women. Separate regression models were used to determine associations between fibroids and haplotypes, with the most frequent haplotype used as the reference group. Haplotypes and diplotypes that were inferred in five or fewer women in either racial group were not included in regression analyses. Separate log-risk models were used to estimate prevalence ratios for associations between genotypes and the size of the largest fibroid (small fibroids, medium fibroids, and large fibroids relative to no fibroids, respectively). All genotype main effects were examined for effect measure modification by alcohol use (number of drinks per week <0.5, 0.5-<3, 3-<7, 7 or more), body mass index (<25, 25-29.99, or $\geq$30 kg/m^2) smoking (never, past, current), fullterm births after age 24 (0, 1, 2, 3 or more), oral contraceptive use (never, current/past use), and age (35-39, 40-44, 45 or older). Effect modification was evaluated by comparing joint effect estimates for each main predictor (e.g. polymorphism or haplotype) and the potential modifier based on likelihood ratio tests (for departures from multiplicative prevalences, $\alpha = 0.20$) and interaction contrast ratios (for departures from additive prevalences). Confounding (by alcohol use, body mass index, smoking, fullterm births after age 24, oral contraceptive use, and age) was evaluated based on a 10% or greater change in the estimated prevalence ratio when the potential confounder was included in the model. None of the potential confounders met this criterion; consequently results are reported for race-specific models that included genotypes only.

4. Results

A total of 583 African American and 404 White participants were included in this analysis. The mean age was 42.2 (sd. 4.07) for cases and 40.6 (sd. 3.89) for noncases. Approximately 72% of African American and 50% of White participants had fibroids detected by ultrasound

(Table 4.3). White and African American women were equally likely to have small fibroids (17% vs. 16%) but Whites were less likely to have medium (22% vs. 32%) or large fibroids (11% vs. 24%) than African American women.

The distributions of variant alleles in African American and White participants (respectively) were: 39% and 24% for *CYP1A1*2A*, 14% and 0.5% for *CYP1A1*3*, 2% and 10% for *CYP1A1*2C*, 2% and 9% for *CYP1A1*4*, 73% and 49% for *CYP1B1AS* and 44% and 83% for *CYP1B1LV*. We did not find evidence of linkage disequilibrium among the four *CYP1A1* or two *CYP1B1* polymorphisms among African American women, but, consistent with previous studies[14,15,21-23], *CYP1A1*2A* and *CYP1A1*2C* were linked among White women (r^2=0.34).

The prevalence of fibroids was increased among African Americans with one or more variant CYP1A1*3 allele (Table 4.2, PR=1.14; 95%CI: 1.02, 1.28; PD=0.10; 95%CI: 0.01, 0.19), but the association between CYP1A1*3 and fibroids among Whites was not estimated due to small numbers. The *CYP1A1*4 CA* or *AA* genotype was positively associated with prevalent fibroids among both Whites (PR=1.20; 95%CI: 0.90, 1.61; PD=0.10; 95%CI: -0.07, 0.27) and African Americans (PR=1.16; 95%CI: 0.92, 1.48; PD=0.12; 95%CI: -0.08, 0.32). The CYP1A1*2C variant tended to be inversely associated with fibroids among White women (PR=0.79; 95%CI: 0.54, 1.18; PD=-0.11; 95%CI: -0.27, 0.06) but not among African American women. Associations were not evident between fibroids and the *CYP1A1*2A* variant or the two *CYP1B1* variants among women in either racial group.

Analyses of effect measure modification by BMI, alcohol use, births after age 24, smoking, oral contraceptive use, and age were consistent with expectations for additive and multiplicative joint effects with gene variants (Table A.35 - A.43). Prevalence ratio estimates for individual SNPs in association with fibroids categorized according to the size of the largest fibroid (small, medium or large compared with no fibroids) were consistent with associations estimated for each

SNP and fibroids as a whole, with little evidence of variation beyond what might be accounted for by chance (Table 4.5).

Associations between fibroids and estimated *CYP1A1* and *CYP1B1* haplotypes and diplotypes were consistent with findings for individual SNPs (Table 4.6). Specifically, haplotypes and diplotypes that included the *CYP1A1*4 A* allele were positively associated with fibroids in African Americans (*TTAA* PR=1.17; 95%CI: 0.93, 1.49; PD=0.13; 95%CI: -0.07, 0.32; *TTAC/TTAA* PR=1.25; 95%CI=1.01, 1.56; PD=0.18; 95%CI: -0.01, 0.37) and Whites (*TTAA* PR=1.18; 95%CI=0.87, 1.56; PD=0.08; 95%CI: -0.09, 0.25; *TTAC/TTAA* PR=1.14; 95%CI: 0.83, 1.56; PD=0.07; 95%CI: -0.11, 0.25). Similarly, haplotypes and diplotypes that included the *CYP1A1*3 C* allele were positively associated with fibroids in African Americans (*TCAC* PR=1.13; 95%CI: 1.01, 1.26; PD=0.09; 95%CI: 0.01, 0.18). Haplotypes and diplotypes that included the *CYP1A1*2C G* allele were inversely associated with fibroids in Whites (*CTGC* PR=0.84; 95%CI: 0.58, 1.22; PD=-0.08; 95%CI: -0.24, 0.08; *TTAC/CTGC* PR=0.71; 0.45, 1.15; PD=0.14; 95%CI: -0.32, 0.03) and to a lesser extent in African Americans (*CTGC* PR=0.92; 95%CI: 0.58, 1.47; PD=-0.05; 95%CI: -0.36, 0.26; *TTAC/CTGC* PR=0.87; 0.51, 1.49; PD=-0.09; 95%CI: -0.43, 0.25). There was a weak inverse association between the *CYP1B1* TC/TC diplotype and fibroids among both African Americans (PR=0.86; 95%CI: 0.60, 1.23; PD=-0.11; 95%CI: -0.35, 0.13) and Whites (PR=0.91; 95%CI: 0.91; 0.58, 1.43; PD=-0.05; 95%CI: -0.26, 0.17); otherwise, estimated associations for *CYP1B1* haplotypes and diplotypes were close to the null.

5. Discussion

Uterine fibroids are only diagnosed after menarche[9] and diminish in size or disappear after menopause[1,10]. In addition, estrogen receptor levels in fibroid tumor cells are significantly elevated compared with normal myometrial cells during the follicular phase[43], and 4-

hydroxyestradiol levels are increased in leiomyoma compared with normal surrounding tissue[44]. We therefore hypothesized that polymorphisms in estrogen metabolism genes might influence the prevalence of uterine fibroids by influencing levels of estrogenic (and potentially mutagenic) byproducts of estrogen metabolism. Gene variants that differ by race might also contribute to differences in the prevalence of uterine fibroids between African Americans and Whites.

Our results support an association between fibroids and the *CYP1A1*3* variant, which is found almost exclusively in African American women (14% of our African American study population) (PR=1.14; 95%CI: 1.02, 1.28; PD=0.10 95%CI: 0.01, 0.19). However, this polymorphism is located in a non-coding region of the gene, suggesting an association due to linkage with a causal variant, or a non-causal association due to chance or error. The *CYP1A1*2C* variant, which was less common among African Americans than Whites in our population (2% vs 10%), was inversely associated with fibroids only among White women when evaluated as a single SNP (PR=0.79; 95%CI: 0.54, 1.18 and PD=-0.11; 95%CI=-0.27, 0.06 in Whites; PR=0.96; 95%CI: 0.64, 1.44 and PD=-0.03; 95%CI: -0.32, 0.26 in African Americans), though there was a weak inverse association with fibroids among both White and African American women for haplotypes and diplotypes that included the variant SNP (*CTGC* and *TTAC/CTGC*). This variant has been positively associated with breast cancer in some previous studies[16,27], but associations have been null in others[17,18]. Thus, the direction of the association observed with fibroids was unexpected. We noted positive associations between the *CYP1A1*4* variant and fibroids among both African American (PR=1.16; 95%CI: 0.92, 1.48; PD=0.12; 95%CI: -0.08, 0.32) and White women (PR=1.20; 95%CI: 0.90, 1.61; PD=0.10; 95%CI: -0.07, 0.27), with positive associations also noted for haplotypes and diplotypes that included this variant (*TTAA* and *TTAC/TTAA*). This variant is associated with an amino acid change, but estimated associations with other hormonally-mediated cancers (such as breast[17,18] and endometrial[45]) have been null. It was somewhat less common in African American than Whites

(2% vs. 9%), thus it would not help explain the increased prevalence of fibroids in African American women.

We did not find evidence of associations between prevalent fibroids and two common SNPs in *CYP1B1*, both of which are associated with amino acid changes. During estrogen metabolism, hydroxylation at the 4-carbon position, which is catalyzed primarily by *CYP1B1*, produces metabolites that are more estrogenic and potentially mutagenic than 2-hydroxyestradiol metabolites that are more likely to be byproducts of *CYP1A1* metabolism; consequently, evidence of associations with *CYP1A1* but not *CYP1B1* variants are contrary to our prior expectations. However, we evaluated only two SNPs in *CYP1B1*, and an association with other variants cannot be ruled out.

We did not find evidence to support associations between the genotypes evaluated and the size of the largest fibroid detected. Evidence of stronger associations with larger fibroids would have been consistent with an effect of gene variants on fibroid growth, while a lack of associations with fibroid size was consistent with an effect early in pathogenesis. However, we were unable to evaluate associations with the number of fibroids or the average size of fibroids among women with multiple fibroids, and estimates were imprecise due to small numbers of observations within each size category.

Our study included a large number of African American women, who are disproportionately affected by fibroids[2-6] compared with Whites. Nonetheless, we had limited power to estimate race-specific prevalences for uncommon SNPs. We inferred haplotypes based on the Markov-Chain Monte Carlo algorithm, which assigns a posterior probability for the accuracy of the inference based on the measured SNPs, but gene coverage was sparse, and we had limited power to examine rare haplotypes and diplotypes. In addition, a large proportion of African American women (n=111) were excluded from *CYP1B1* haplotype analyses because posterior probabilities were less than 90%.

Since most fibroids are asymptomatic, determination of fibroid status has proven to be difficult in research[46]. The UFS study used ultrasound screening to identify fibroids, which improved upon the diagnostic methods of previous studies. Ultrasound examination, the standard non-invasive clinical method for diagnosis, has a reported sensitivity of 67-99% and specificity of 89-94% when operative hysteroscopy and histological classification is used as the gold standard[47-49].

In summary, we noted positive associations between fibroids and *CYP1A1*3* and **4* variants among African Americans, and between fibroids and *CYP1A1*2C* and **4* variants in Whites. Although the magnitudes of the estimated prevalence ratios for the association between fibroids and these gene variants were modest, prevalence difference estimates show the potential for a substantial impact for these variants on the prevalence of fibroids among both African American and White women. These apparent inconsistencies highlight the importance of estimating absolute differences when the prevalence of an outcome is high, as was the case in our cross-sectional population of premenopausal women. Nonetheless, these associations must be interpreted with caution given the potential of non-causal relations due to chance or bias. Clear associations were not seen between *CYP1B1* SNPs and prevalent fibroids; however, we evaluated only two polymorphisms within this gene. To our knowledge, our study was the first to evaluate associations between *CYP1A1* and *CYP1B1* polymorphisms and fibroids, and our findings must therefore be replicated in other populations. In addition, future research should consider a more comprehensive evaluation of variants throughout the*CYP1A1* and *CYP1B1* genes, in addition to evaluating other genes in the estrogen metabolism pathway that may influence the pathogenesis of fibroids, which are a major cause of morbidity among all women.

6. References

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Table 4.3. Characteristics of premenopausal African American and White participants in the cross-sectional National Institutes of Environmental Health Sciences Uterine Fibroid Study with genotype data[a]

Characteristics	*Fibroid Status*					
	African Americans			**Whites**		
	Fibroid Present	**Total**	**Fibroid Prevalence**	**Fibroid Present**	**Total**	**Fibroid Prevalence**
Total	421	583	0.72	201	404	0.50
Age (years)						
35-39	138	220	0.63	48	137	0.35
40-44	152	206	0.74	63	136	0.46
>=45	131	157	0.83	90	131	0.69
BMI (kg/m^2)						
Normal (<25)	95	148	0.64	113	237	0.48
Overweight (25-29.9)	132	179	0.74	46	95	0.48
Obese (≥30)	194	256	0.76	42	72	0.58
Smoking Status						
Never Smoked	124	277	0.45	114	234	0.49
Past Smoker	101	134	0.75	69	139	0.50
Current Smoker	120	168	0.71	18	31	0.58
Oral Contraceptive Use						
Never Used OC	29	48	0.60	35	69	0.51
Current/Past User Of OC	392	535	0.73	166	335	0.50
Fullterm Births after age 24						
None	221	296	0.75	138	257	0.54
1	128	181	0.71	23	57	0.40
2	61	85	0.72	38	80	0.48
3 Or More	12	21	0.57	2	10	0.20
Number of Alcoholic Drinks per Week						
<0.5	221	300	0.74	56	60	0.93
0.5-<3	82	122	0.67	55	115	0.48
3-<7	48	65	0.74	49	102	0.48
7 Or More	46	58	0.79	16	98	0.16

[a]Prevalent fibroids detected by ultrasound.

Table 4.4. Estimated prevalence ratio(PR), prevalence differences (PD) and 95% confidence intervals (95% CI) for selected estrog estrogen metabolism gene polymorphisms among premenopausal African American and White participants in the Uterine Fibroid Study

Genotype	***African Americans***					***Whites***				
	Fibroid Present	***Total***	**Prev**	**PR (95% CI)**	**PD (95% CI)**	**Fibroid Present**	**Total**	**Prev**	**PR (95% CI)**	**PD (95% CI)**
*CYP1A1*2A*										
TT	260	352	0.74	1.00	0	153	298	0.51	1.00	0
TC or *CC*	159	222	0.72	0.97 (0.87,1.08)	-0.02 (-0.10,0.05)	47	97	0.48	0.94 (0.75, 1.19)	-0.03 (-0.14, 0.09)
*CYP1A1*3*[a]										
TT	352	492	0.72	1.00	0	200	393	0.51	**	
TC or *CC*	67	82	0.82	1.14 (1.02,1.28)	0.10 (0.01, 0.19)	1	2	**	**	**
*CYP1A1*2C*										
AA	412	564	0.73	1.00	0	183	355	0.52	1.00	0
AG or *GG*	7	10	0.70	0.96 (0.64,1.44)	-0.03 (-0.32, 0.26)	16	39	0.41	0.79 (0.54, 1.18)	-0.11 (-0.27, 0.06)
*CYP1A1*4*										
CC	408	561	0.73	1.00	0	180	361	0.50	1.00	0
CA or *AA*	11	13	0.85	1.16 (0.92,1.48)	0.12 (-0.08, 0.32)	21	35	0.60	1.20 (0.90, 1.61)	0.10 (-0.07, 0.27)
CYP1B1AS										
GG	109	149	0.73	1.00	0	102	200	0.51	1.00	0
GT or *TT*	308	421	0.73	1.00 (0.90,1.12)	0.00 (-0.08, 0.08)	99	194	0.51	1.00 (0.83, 1.21)	0.00 (-0.10, 0.10)
CYP1B1LV										
GG	237	321	0.74	1.00	0	32	66	0.48	1.00	0
GC or *CC*	182	253	0.72	0.97 (0.88,1.08)	-0.02 (-0.09, 0.05)	169	330	0.51	1.06 (0.80, 1.38)	0.03 (-0.10, 0.16)

Table 4.5. Estimated prevalence ratios (PR*) and 95% confidence intervals (95% CI) for the association between selected estrogen metabolism gene polymorphisms and uterine fibroids classified according to the size of the largest fibroid among premenopausal African American and White participants in the Uterine Fibroid Study

Genotype	Fibroid Status													
	African Americans							**Whites**						
	No Fibroids	Small		Medium		Large		No Fibroids	Small		Medium		Large	
	N	N	PR (95% CI)	N	PR (95% CI)	N	PR (95% CI)	N	N	PR (95% CI)	N	PR (95% CI)	N	PR (95%CI)
Total	155	94		187		140		195	70		87		44	
*CYP1A1*2A*														
TT	92	61	1.00	115	1.00	84	1.00	145	52	1.00	66	1.00	35	1.00
TC or *CC*	63	32	0.84 (0.60,1.19)	71	0.95 (0.78,1.17)	56	0.99 (0.77, 1.26)	50	18	1.00 (0.63,1.59)	20	0.91 (0.60, 1.39)	9	0.78 (0.40, 1.54)
*CYP1A1*3*														
TT	140	79	1.00	157	1.00	116	1.00	193	70		87		43	
TC or *CC*	15	14	1.33 (0.88,2.03)	29	1.25 (0.98,1.58)	24	1.36 (1.02,1.80)	1	0	**	0	**	1	**
CYP1B1AS														
GG	40	24	1.00	46	1.00	39	1.00	98	38	1.00	43	1.00	21	1.00
GT or *TT*	113	70	1.02 (0.71,1.47)	138	1.03 (0.82,1.29)	100	0.95 (0.73,1.24)	95	32	0.90 (0.60,1.35)	44	1.04 (0.73,1.47)	23	1.10 (0.65,1.88)
CYP1B1LV														
GG	84	51	1.00	104	1.00	82	1.00	34	11	1.00	14	1.00	7	1.00
GC or *CC*	71	43	1.00 (0.73,1.38)	81	0.96 (0.79,1.17)	58	0.91 (0.71,1.16)	161	59	1.10 (0.63,1.92)	73	1.07 (0.66,1.73)	37	1.09 (0.53,2.28)

*PR compare the prevalence of fibroids versus no fibroids for each size category in association with variant alleles. Estimates are not shown for *CYP1A1*3* among Whites due to small numbers.

Table 4.6. Estimated prevalence ratios (PR), prevalence differences (PD) and 95% confidence intervals (95% CI) for haplotypes and diplotypes based on selected estrogen metabolism gene polymorphisms among premenopausal African American and White participants in the Uterine Fibroid Study.

Genotype	Fibroid Status									
	African Americans					Whites				
	Fibroid present	**Total**	**Prev**	**PR (95% CI)**	**PD (95% CI)**	**Fibroid present**	**Total**	**Prev**	**PR (95%CI)**	**PD (95%CI)**
CYP1A1 haplotypes[a]										
Total	838	1148	0.73			400	790	0.51		
TTAC	579	803	0.72	Ref	0	327	648	0.50	Ref	0
CTAC	171	236	0.72	1.00 (0.92,1.10)	0.00 (-0.06,0.07)	35	66	0.53	1.06 (0.83, 1.34)	0.03 (-0.10,0.15)
CTGC	6	9	0.67	0.92 (0.58,1.47)	-0.05(-0.36,0.26)	17	40	0.43	0.86 (0.58, 1.22)	-0.08(-0.24,0.08)
TCAC	70	86	0.81	1.13 (1.01,1.26)	0.09 (0.01, 0.18)	1	2	**	**	**
TTAA	11	13	0.85	1.17 (0.93,1.49)	0.13 (-0.07, 0.32)	20	34	0.59	1.18 (0.87, 1.56)	0.08 (-0.09,0.25)
CYP1A1 diplotypes[a]										
Total	400	574	0.70			201	396	0.51		
TTAC/TTAC	200	278	0.72	Ref		133	263	0.51	Ref	0
CTAC/CTAC	17	22	0.78	1.07 (0.85,1.36)	0.05 (-0.13, 0.24)	1	3	**	**	**
TTAA/CTAC	1	2	**	**	**	29	54	0.54	1.06 (0.81, 1.40)	0.03 (-0.11,0.18)
TTAC/CTAC	118	170	0.69	0.96 (0.85,1.09)	-0.03(-0.11,0.06)	0	0	0	**	
TTAC/CTGC	5	8	0.63	0.87 (0.51,1.49)	-0.09(-0.43,0.25)	12	33	0.36	0.71 (0.45, 1.15)	-0.14(-0.32,0.03)
TTAC/TCAC	46	58	0.79	1.10 (0.95,1.28)	0.07 (-0.04, 0.19)	1	2	**	**	**
TTAC/TTAA	9	10	0.90	1.25 (1.01, 1.56)	0.18 (-0.01, 0.37)	19	33	0.58	1.14 (0.83, 1.56)	0.07 (-0.11,0.25)

Genotype	Fibroid Status									
	African Americans					Whites				
	Fibroid present	**Total**	**Prev**	**PR (95% CI)**	**PD (95% CI)**	**Fibroid present**	**Total**	**Prev**	**PR (95%CI)**	**PD (95%CI)**
CYP1B1 haplotypes[b]										
Total	678	918	0.74			402	788	0.51		
GG	302	408	0.74	Ref	0	164	326	0.50	Ref	0
GC	49	65	0.75	1.04 (0.90,1.20)	0.01 (-0.10, 0.13)	126	241	0.52	1.04 (0.90, 1.20)	0.02 (-0.6,0.11)
TC	80	112	0.71	0.97 (0.87,1.12)	-0.03(-0.12,0.07)	110	216	0.51	1.02 (0.94, 1.11)	0.01 (-0.07,0.10)
TG	247	333	0.74	1.00 (0.94,1.11)	0.00 (-0.06, 0.07)	2	5	**	**	**
CYP1B1 diplotypes[b]										
Total	339	459	0.74			201	394	0.51		
GG/TG	121	160	0.76	Ref		1	4	**	**	**
GG/GC	31	40	0.78	1.02 (0.85,1.24)	0.02 (-0.13, 0.16)	54	106	0.51	Ref	
GC/GC	3	5	**	**	**	17	32	0.53	1.04 (0.72,1.52)	0.02 (-0.18,0.22)
GC/TC	12	15	0.80	1.06 (0.81,1.38)	0.04 (-0.17, 0.26)	38	71	0.54	1.05 (0.79,1.40)	0.03 (-0.12,0.18)
GG/GG	75	104	0.72	0.95 (0.82, 1.11)	-0.04(-0.14,0.07)	31	62	0.50	0.98 (0.72,1.34)	-0.01(-0.17,0.15)
GG/TC	0	0	**	**	**	47	92	0.51	1.00 (0.76, 1.32)	0.00 (-0.14,0.14)
TC/TC	11	17	0.65	0.86 (0.60,1.23)	-0.11(-0.35,0.13)	12	26	0.46	0.91 (0.58,1.43)	-0.05(-0.26,0.17)
TG/TC	46	63	0.73	0.97 (0.81,1.15)	-0.03(-0.15,0.10)	1	0	**	**	**
TG/TG	40	55	0.73	0.96 (0.80,1.16)	-0.03(-0.16,0.11)	0	0	**	**	**

[a]Order of SNPs: CYP1A1*2A, CYP1A1*3, CYP1A1*2C, CYP1A1*4
[b]Order of SNPs: CYP1B1 Ala119Ser, CYP1B1 Leu432Val
**Not enough participants with this haplotype (or diplotype) to estimate associations.

V. CONCLUSIONS

A. Overview of Study Purpose

Uterine leiomyomas, or fibroids, cause significant morbidity among premenopausal women[1]. They disproportionately affect African Americans compared with Whites. In addition, fibroids among African Americans are larger and present with more symptoms than Whites[2-4]. Although the causes of fibroids are unknown, clinical, molecular, and observational evidence suggests that they are hormonally dependent. One hormone in particular that could be related to fibroid development is estrogen.

The biologic effect of estrogen depends, in part, on how it is metabolized. Metabolism of estrogen primarily occurs in the liver, but can also occur in other tissues. Estrogen hydroxylation at the C-2 position is catalyzed primarily by CYP1A1 enzymes, yielding the catechol estrogens 2-hydroxyestrone and 2-hydroxyestradiol (2-OH); likewise, hydroxylation at the 4 carbon (C-4) position is catalyzed primarily by CYP1B1 enzymes, yielding the catechol estrogens 4-hydroxyestrone and 4-hydroxyestradiol (4-OH)[5,6]. In general, initial hydroxylation at the C-4 position leads to metabolites that are more estrogenic and potentially genotoxic than those produced by hydroxylation at the C-2 position[8,9]. Phase II conjugation reactions (methylation, glucuronidation, and sulfation) produce estrogen metabolites that may be excreted through the urine and feces, or be maintained in circulation. Phase II methylation of 2-OH and 4-OH to less reactive metabolites (2-methoxyestrone and 2-methoxyestradiol, and 4-methoxyestrone and 4-methoxyestradiol, respectively) by the COMT enzyme reduces the formation of highly reactive

quinones[5,6], which are tumor promoters [7]. Variation in genes that encode estrogen metabolism enzymes may influence the pathogenesis of estrogen-dependent tumors, including fibroids.

Our primary aim was to examine relations between fibroids and polymorphisms of *CYP1A1, CYP1B1*, and *COMT* genes that are involved in estrogen metabolism. In addition, we inferred haplotypes for *CYP1A1* and *CYP1B1* and evaluated relations between these haplotypes and fibroids. We hypothesized that genotypes with variant alleles that alter estrogen metabolism would be associated with the prevalence or size of uterine fibroids. Given that CYP1B1 is primarily responsible for catalyzing reactions at the C-4 position, resulting in metabolites that may be more estrogenic or mutagenic than 2-hydroxy metabolites produced by CYP1A1 metabolism[8,9], we hypothesized that CYP1B1 variants might be more strongly associated with fibroids than variants in CYP1A1. We also hypothesized that ethnic differences in the prevalence of estrogen metabolism gene polymorphisms might at least partly explain ethnic differences in the prevalence of uterine fibroids. As a secondary aim, we examined whether prevalence effect estimates were modified by smoking status, age, alcohol use or BMI; specifically, we hypothesized that women with one or more variant alleles that also were smokers, obese, of older age, or that consumed alcohol would be more likely to have uterine fibroids than women who were not jointly exposed to a variant allele and one of these factors. We evaluated these hypotheses using data from the National Environmental and Health Sciences Uterine Fibroid Study (UFS), a cross sectional sample of women from an urban health plan. Log-risk and linear-risk regression models were constructed to determine prevalence ratio and prevalence difference estimates of the associations between prevalent fibroids and the genotypes of interest.

B. Summary of Findings

There were a total of 583 African American and 404 White participants included in this study. The mean age was 42.2 (sd. 4.07) for cases and 40.6 (sd. 3.89) for noncases. Approximately 64% of women in the study had fibroids, which were detected disproportionately in African American (73%), compared with White (51%) women. Overall, the majority of women with detectable fibroids had fibroids that were 2-4 cm in diameter. Whites and African Americans were equally likely to have small fibroids (17% vs. 16%), but White participants less likely to have medium (22% vs. 32%) or large fibroids (11% vs. 24%) than African American participants.

The distibution s of *CYP1A1*, *CYP1B1*, and *COMT* polymorphisms were consistent with Hardy Weinberg Equilibrium among noncases. The distributions of all gene variants varied by race. Approximately 60% of African American and White women were homozygous wildtype for *CYP1A1*2A*, while only 4% of African Americans and 2% of Whites were homozygous variant. The overwhelming majority of African American and White women were homozygous wildtype for *CYP1A1*2C* (98% and 90% respectively), while only one white woman and no African American women were homozygous variant for *CYP1A1*2C*. Approximately 86% of African American participants were homozygous wildtype, 14% were heterozygous, and <1% were homozygous variant for *CYP1A1*3*. In contrast, over 99% of White participants were homozygous wildtype. The prevalence of variant alleles of *CYP1A1*4* was slightly higher among Whites than African Americans. More than 97% of African American were homozygous wildtype and less than 2% were heterozygous. For Whites, approximately 91% were homozygous wildtype and 9% were heterozygous.

About one quarter of African American participants were homozygous wildtype for the *CYP1B1 Ala119Ser* polymorphism, while 50% were heterozygous. In contrast, about half of

White participants were homozygous wildtype for *Ala119Ser*, and only a third were heterozygous. Half of the African American participants were homozygous wildtype for the *CYP1B1 Leu432Val* polymorphism, while 37% were heterozygous and 7% were homozygous variant. In contrast, only 17% of White participants were homozygous wildtype, while the majority had one or more variant alleles (51% heterozygous, 33% homozygous variant).

Almost half of the African American participants were homozygous wildtype genotype for the *COMT Val158Met* polymorphism, 43% were heterozygous and 9.62% were homozygous variant. Approximately one quarter of White participants were homozygous wildtype for this polymorphism, half were heterozygous, and one quarter were homozygous variant.

We found both positive and negative associations between polymorphisms and prevalent fibroids. *CYP1A1*3*, a polymorphism almost exclusively found in African Americans[11], was positively associated with fibroid prevalence (PD=0.10; 95%CI: 0.01, 0.19). *CYP1A1*2C* was inversely associated with fibroid prevalence in Whites (PD=-0.11; 95%CI: -0.27, 0.06), but was not clearly associated with fibroids in African Americans (PD=-0.03; 95%CI: -0.32, 0.26). *CYP1A1*4* variant genotypes were positively associated with prevalent fibroids among women in both racial groups (PD for African Americans = 0.12, 95%CI: -0.08, 0.32; PD for Whites = 0.10, 95%CI: -0.07, 0.27). Overall the *CYP1A1*2A* polymorphism did not appear to be associated with fibroids in African Americans or Whites. Main effects for both *CYP1B1* polymorphisms were close to the null value. Adjusting for additional covariates (age, BMI, oral contraceptive use, births after age 24, smoking status, and number of drinks per week) did not alter prevalence ratios by 10% or more, therefore these potential confounders were not included in final models. There was no clear evidence of effect measure modification by smoking status, age, alcohol use or BMI in combination with any of the genotypes examined. Observed joint effects did not deviate from expected effects on the additive nor multiplicative scales.

C. Strengths and Limitations

To our knowledge, this is the first study to examine relations between uterine fibroids and polymorphisms of *CYP1A1* and *CYP1B1*. One other study examined the relation between the *COMT Val158Met* polymorphism and fibroids and reported a positive association with the high activity *Val/Val* genotype (OR=2.5; 95% CI: 1.02, 6.15)[12]. Although we found a similar direction of effect as Al-Hendy's study, the magnitude of effect estimate was not as strong in our study population, which was larger in size. In addition, we used different methods to ascertain fibroid status (ultrasonography versus hysterectomy for symptomatic fibroids) and for estimating associations (prevalence ratios versus odds ratios).

Null findings for the two *CYP1B1* variants and the *COMT* variant do not rule out a relation between these genes and uterine fibroids. In addition, associations that were observed between fibroids and *CYP1A1* variants may have resulted from linkage disequilibrium between the measured SNP and one or more unmeasured variants, rather than from a direct causal effect of the SNP that was evaluated; this is particularly likely for the association observed between fibroids in African American women and the CYP1A1*3 variant, which is located in an intronic region of the gene. More importantly, the potential for non-causal associations due to chance or bias cannot be ruled out, and positive findings for *CYP1A1* variants should be interpreted with caution given that this is the first study to evaluate these relations. We evaluated only a subset of variants in the genes examined, and effects of a single polymorphism, even if functional, might not be strong enough to influence the net effect of estrogen metabolism. We inferred haplotypes and evaluated their associations with fibroids to strengthen our ability to capture other unmeasured variants in the gene which may be important; however, the SNPs used to define these haplotypes covered the gene only sparsely, and we had limited power to evaluate associations with uncommon SNPs or haplotypes. We also had limited power to examine

modification of SNP main effects by smoking, age, BMI, number of births after age 24, alcohol and oral contraception use. Genotyping data were not complete, but the majority of eligible women in the parent study had DNA available (75%). There were no clear differences between the analysis group and the total study population with respect to all variables.

Our study design is cross-sectional. We therefore cannot distinguish between incident and prevalent fibroids. The examination of incident fibroids would help to ascertain temporality; however this is not an issue since we are evaluating the effects of genotypes. Moreover, since most fibroids are asymptomatic, it would be logistically difficult to detect incident fibroids. It would require multiple ultrasound examinations beginning at the age of menarche.

The Uterine Fibroid Study improved upon previous fibroid research (including the study of *COMT* by Al-Hendy et al.) by using ultrasound examinations to identify fibroids, thereby greatly increasing the sensitivity with which fibroids status was determined. Because fibroids are highly prevalent, we estimated prevalence ratios instead of odds ratios (which overestimate relative risks for common outcomes[13,14]) and used prevalence difference estimates to assess the potential public health impact of gene variants on fibroids. We also used MALDI-TOF, a high throughput and accurate technique that simultaneously performs multiple genotyping analysis on very small quantities of DNA, to genotype DNA[15]. MALDI-TOF assays used to identify SNPs were reliable on repeat assays (>90% concordance for all SNPs). PHASE was used to infer haplotypes, which has been found to be similar[16, 17] or slightly superior to other haplotying programs. This program allowed us to reduce random variation error by restricting analysis to haplotype probabilities ≥90%[18].

All estimates were stratified by race due to the strong associations between race and fibroids, and between race and the gene variants examined; however, we did not note evidence of substantial variation in associations between gene variants and fibroids between African Americans and Whites. The study included a large number of African Americans, which

strengthened our ability to evaluate genetic variation as a cause of racial disparities in fibroid prevalence and morbidity. However, race is a proxy measure of a variety of social and behavioral factors that may be relevant to fibroid pathogenesis, as well as a crude measure of biologic ancestry.

D. Future directions

This study provides evidence supporting the hypothesis that *CYP1A1*2C, CYP1A1*3,* and *CYP1A1*4* polymorphisms are related to fibroids, but these findings must be confirmed in other study populations. Future research should examine the functional significance of these polymorphisms with regard to estrogen metabolism and fibroid pathogenesis, in addition to evaluating other candidate genes. Null findings for *COMT* and *CYP1B1* variants do not rule out a role of these genes, and a larger more comprehensive study is needed to fully assess variability within *CYP1A1, CYP1B1* and *COMT* genes, with sufficient numbers of African Americans and Whites to explore determinants of racial differences in fibroids. In addition, several genes pertinent to estrogen metabolism's pathway should be considered simultaneously to determine the net effect of estrogen metabolism gene polymorphisms on fibroid pathogenesis.

Although our findings provide no immediate public health benefit, they do provide additional evidence for the importance of estrogen in fibroid development. The associations with CYP1A1 variants document a need for molecular research to determine the functional significance of each. This is especially warranted for CYP1A1*3 polymorphism, since African Americans are the predominant carriers of the variant allele and are disproportionately affected by uterine fibroids. If the functional significance of all of these SNPs are uncovered, we can do additional studies examining modifiable risk factors that increase the risk of prevalent fibroids in the presence of these genotypes.

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APPENDIX

Table A.1. Description of *CYP1A1, CYP1B1*, and *COMT* polymorphisms evaluated in the National Institute of Environmental Health Sciences' Uterine Fibroid Study

Polymorphism	Alternate Name	Location	Nucleotide Change	Amino Acid Change
CYP1A1				
CYP1A1*2A	CYP1A1m1	3' non-coding region	T—C	None
CYP1A1*2C	CYP1A1m2	exon 7	A—G	Isoleucine to Valine
CYP1A1*3	CYP1A1m3	3' non-coding region	T—C	None
CYP1A1*4	CYP1A1m4	exon 7	C—A	Threonine to Asparagine
CYP1B1				
Leu432Val	None	exon 3	G—C	Leucine to Valine
Ala119Ser	None	exon 2	G—T	Alanine to Serine
COMT				
Val158Met	None	exon 4	G—A	Valine to Methionine

Table A.2: Relevant association studies of *CYP1A1*, *CYP1B1*, and *COMT* variants

Author (Year)	Study Design	Population	Variant(s)	Findings
Ał Hendy et al 2006	Case-control	186 cases, 142 controls	COMT Val158Met	Increased risk of fibroids among women with Val/Val genotype (OR=2.5; 95%CI: 1.02, 6.15)
Ambrosone et al 1995	Case-control	265 female cases, 322 female controls; all Caucasian	CYP1A1*2C	Increased risk of breast cancer among women with CYP1A1*2C variant (OR=1.61; 95% CI 0.94-2.75). Risk highest among those who smoked up to 29 pack-years (OR=5.22; 95% CI 1.16-23.56)
Bailey et al (1998b)	Case-control	223 female cases; 221 female controls; 27% African American	CYP1A1*2A, CYP1A1*2C, CYP1A1*3, CYP1A1*4	No association of any CYP1A1 variants with breast cancer
Cascorbi et al. (1996)	Case-control	157 cases, 314 controls; Caucasian males and females	CYP1A1*4	No association of CYP1A1*4 with lung cancer
De Vivo et al. (2002)	Case-control	453 female cases, 456 female controls; 99% Caucasian	CYP1B1 Val432Leu	No association of Val432Leu with breast cancer
Drakoulis et al 1994	Case-control	142 cases, 171 controls; all German	CYP1A1*2A, CYP1A1*2C	No association of CYP1A1*2A with lung cancer; CYP1A1*2C associated with lung cancer (OR=2.16, 95% CI 0.96-5.11)
Fritsche et al. (1999)	Case-control	187 cases, 101 controls; all Germans	Leu432Val	Association of Leu432Val with colorectal cancer (OR=1.93; 95% CI 1.15-3.24)
Goodman et al. (2001)	Case-control	129 cases, 144 controls; 45% were Asian, 28% Caucasian, 27% Other	Leu432Val	Association of Leu432Val with ovarian cancer (OR=3.85; 95% CI 1.2-11.4)

Author (Year)	Study Design	Population	Variant(s)	Findings
Goth Goldstein et al. (2000)	Case-control	27 cases, 32 controls (race unknown)	CYP1A1*2A	No association of CYP1A1*2A with breast cancer
Hirvonen et al (1992)	Case-control	106 cases, 122 controls; Finnish population	CYP1A1*2A	No association of CYP1A1*2A with lung cancer
Huang et al (1999)	Case-control	150 cases, 150 controls	COMT Val158Met	Increase risk in breast cancer among women with 2 variant alleles compared to women with one or less variant alleles
Ishibe et al 1998	Case-control	466 female cases, 466 female controls; >97% Caucasian	CYP1A1*2A	Increase in breast cancer risk among women with CYP1A1*2A variant and who smoked before age 18 (RR=5.65; 95% CI, 1.11-11.7)
Laden et al. (2002)	Case-control	367 female cases, 367 female controls	CYP1A1*2C	Increased in breast cancer risk among women with CYP1A1*2C variant and who had PCB levels in the highest category
Lavigne et al. (1997)	Case-control	112 cases, 112 controls	COMT Val158Met	Increased risk in breast cancer among postmenopausal women with 2 variant alleles; inverse association among premenopausal women with 2 variant alleles
Mitrunen et al (2001)	Case-control	483 cases, 482 controls, Finnish	COMT Val158Met	Decrease risk in breast cancer among postmenopausal women with 2 alleles and low body mass index (OR=0.33; 95% CI 0.13-0.83); increase in risk for postmenopausal women with 2 alleles and long term estrogen use (OR=4.02; 95% CI 1.13-

Author (Year)	Study Design	Population	Variant(s)	Findings
				14.30), and one variant allele and early age at menarche (OR=8.59; 95% CI 1.85-39.80)
Nakachi et al 1991	Case-control		CYP1A1*2A	Increased risk of lung cancer among those with CYP1A1*2A (OR=7.31; 95% CI 2.13-25.12)
Rebbeck et al. (1994)	Case-control	96 female cases, 146 female controls; Caucasians	CYP1A1*2C	No association of CYP1A1*2C with breast cancer
Taioli et al 1995	Case-control	49 female cases, 256 female controls; 33% African American, 67% Caucasian	CYP1A1*2A, CYP1A1*3	Increased association with breast cancer among African American women with CYP1A1*2A variant (OR=9.7; 95% CI 2.0-47.9); No association found among Caucasians; No association found with the CYP1A1*3 variant
Tang et al. (2000)	Case-control	189 male cases, 147 male controls; all Caucasian	CYP1B1 Leu432Val	Leu432Val associated with increased prostate cancer risk (OR=3.3; 95% CI 1.9-9.0)
Tefre et al 1991	Case-control	221 cases, 212 controls; all Norwegian	CYP1A1*2A	No association found between CYP1A1*2A and lung cancer
Thompson et al (1998)	Cohort	570 women	COMT Val158Met	Increased risk of breast cancer in premenopausal women with 2 variant alleles; inverse relationship in postmenopausal women
Watanabe et al. (2000)	Case-control	339 female cases, 361 female controls; all Japanese	CYP1B1 Leu432Val, Ala119Ser	Leu432Val not associated with increased risk of breast cancer; Ala119Ser associated with both breast and lung cancers

Author (Year)	Study Design	Population	Variant(s)	Findings
Zheng et al. (2000)	Case-control	186 female cases, 200 female controls; all Chinese	CYP1B1 Leu432Val	Leu432Val associated with increased risk of breast cancer (OR=2.3; 95% CI 1.2–4.5)

Table A.3. Distribution of outcome and exposure variables by race among premenopausal participants in the National Institute of Environmental Health Sciences Uterine Fibroid Study

Variable	African Americans	Caucasians	Other
Total	583	404	77
Fibroid present (%)	421 (72.21)	201 (49.75)	43 (55.84)
Fibroid size (%)			
No fibroids	155 (26.59)	195 (49.24)	32 (42.67)
Fibroid < 2cm	94 (16.12)	70 (17.68)	13 (17.33)
Fibroid 2-3.999cm	187 (32.08)	87 (21.97)	19 (25.33)
Fibroid ≥ 4cm	140 (24.01)	44 (11.11)	11 (14.67)
Missing	7 (1.20)	8 (1.98)	2 (2.60)
BMI (%)			
Normal (<25)	148 (25.39)	237 (58.66)	35 (45.45)
Overweight (25 – 29.9)	179 (30.70)	95 (23.51)	19 (24.68)
Obese (≥30)	256 (43.91)	72 (17.82)	23 (29.87)
Age (%)			
35-39	220 (37.74)	137 (33.91)	29 (37.66)
40-44	206 (35.33)	136 (33.66)	25 (32.47)
45 and over	157 (26.93)	131 (32.43)	23 (29.87)
Mean number of alcoholic drinks/week (%)			
Less than 0.5	300 (51.46)	60 (14.85)	0 (0)
0.5 – 2	122 (20.93)	115 (28.47)	0 (0)
3 – 6	65 (11.15)	102 (25.25)	0 (0)
7 or more	58 (9.95)	98 (24.26)	0 (0)
Missing	38 (6.52)	29 (7.18)	77 (0)
Oral Contraception Use (%)	535 (91.77)	335 (82.92)	60 (77.92)
Births after age 24 (%)			
0	296 (50.77)	257 (63.61)	43 (55.84)
1	181 (31.05)	57 (14.11)	21 (27.27)
2	85 (14.58)	80 (19.80)	10 (12.99)
3 or more	21 (3.60)	10 (2.48)	3 (3.90)
Smoking Status (%)			
Never smoker	277 (47.51)	234 (57.92)	47 (61.04)
Former smoker	134 (22.98)	139 (34.41)	18 (23.38)
Smoker	168 (28.82)	31 (7.67)	12 (15.58)
Missing	4 (0.69)	0 (0)	0 (0)

Variable	African Americans	Caucasians	Other
CYP1A1*2A (%)			
Wildtype	354 (60.72)	305 (75.50)	38 (49.35)
Heterozygous	204 (34.99)	89 (22.03)	27 (35.06)
Homozygous Variant	23 (3.95)	9 (2.23)	12 (15.58)
Missing	2 (0.34)	1 (0.25)	0 (0)
CYP1A1*2C (%)			
Wildtype	571 (97.94)	363 (89.85)	52 (67.53)
Heterozygous	10 (1.72)	38 (9.41)	18 (23.38)
Homozygous Variant	0 (0)	1 (0.25)	7 (9.09)
Missing	2 (0.34)	2 (0.50)	0 (0)
CYP1A1*3 (%)			
Wildtype	498 (85.42)	401 (99.26)	73 (94.81)
Heterozygous	79 (13.55)	2 (0.50)	4 (5.19)
Homozygous Variant	4 (0.69)	0 (0)	0 (0)
Missing	2 (0.34)	1 (0.25)	0 (0)
CYP1A1*4 (%)			
Wildtype	568 (97.43)	368 (91.09)	74 (96.10)
Heterozygous	13 (2.23)	36 (8.91)	2 (2.60)
Homozygous Variant	0 (0)	0 (0)	1 (1.30)
Missing	2 (0.34)	0 (0)	0 (0)
Ala119Ser (%)			
Wildtype	152 (26.07)	204 (50.50)	38 (49.35)
Heterozygous	289 (49.57)	171 (42.33)	28 (36.36)
Homozygous Variant	136 (23.33)	27 (6.68)	11 (14.29)
Missing	6 (1.03)	2 (0.50)	0 (0)
Leu432Val (%)			
Wildtype	326 (55.92)	67 (16.58)	20 (25.97)
Heterozygous	216 (37.05)	205 (50.74)	31 (40.26)
Homozygous Variant	39 (6.69)	132 (32.67)	26 (33.77)
Missing	2 (0.34)	0 (0)	0 (0)
COMT (%)			
Wildtype	274 (47.08)	96 (23.76)	35 (45.45)
Heterozygous	252 (43.30)	202 (50.00)	29 (37.66)
Homozygous	56 (9.62)	106 (26.24)	13 (16.88)
Missing	56 (9.61)	0 (0)	0 (0)

Table A.4. Examination of Hardy Weinberg Equilibrium among noncases, National Institute of Environmental Health Sciences Uterine Fibroid Study

Genotype[a]	African Americans			Whites		
	Expected frequency	Observed frequency	χ^2	Expected frequency	Observed frequency	χ^2
CYP1A1*2A						
AA	94.46	92	1.33	144.74	145	0.02
AB	53.08	58		46.52	46	
BB	7.46	5		3.74	4	
CYP1A1*2C						
AA	152.04	152	0.01	172.68	172	0.77
AB	2.97	3		21.64	23	
BB	0.01	0		0.68	0	
CYP1A1*3						
AA	139.41	140	0.93	193	193	0.001
AB	15.17	14		1	1	
BB	0.41	1		0.0012	0	
CYP1A1*4						
AA	153.01	153	0.001	181.25	181	0.27
AB	1.99	2		13.50	14	
BB	0.01	0		0.25	0	
Ala119Ser						
AA	39.26	40	0.06	99.39	98	0.24
AB	76.49	75		78.22	81	
BB	37.26	38		15.39	14	
Leu432Val						
AA	83.85	84	0.004	35.33	34	0.15
AB	60.31	60		95.34	98	
BB	10.85	11		64.33	63	
COMT						
AA	47.2	47	0.01	69.00	65	2.10
AB	97.5	98		69.00	77	
BB	50.3	50		17.00	13	

[a] AA=Wildtype (p^2), AB=Heterzygous (2pq), BB=Homozygous Variant (q^2)

Table A.5. Primers used during MALDI-TOF procedure to genotype polymorphisms of *CYP1A1, CYP1B1*, and *COMT*

Polymorphism	Forward Primer	Reverse Primer	Mass Ext
CYP1A1			
CYP1A1*2A	ACGTTGGATGGGATAGCCAGGAAGAGAAAG	ACGTTGGATGTATCTTTGGCATGGGCAAGC	AGCGGAAGTC
CYP1A1*2C	ACGTTGGATGTATCTTTGGCATGGGCAAGC	ACGTTGGATGGGATAGCCAGGAAGAGAAAG	AGACCTCCCA
CYP1A1*3	ACGTTGGATGACTACTCAGAGGCTGAGGTG	ACGTTGGATGAGTGCACTGGTACCATTTTG	CACTGTAACC
CYP1A1*4	ACGTTGGATGATATGTGCACTCCCTGTGCG	ACGTTGGATGCTTCTGGCCTTGTAAGACCC	GTAAGACCCT
CYP1B1			
Ala119Ser	ACGTTGGATGTAGTGGTGCTGAATGGCGAG	ACGTTGGATGGACACCACACGGAAGGAGG	CACGGAAGGA
Leu432Val	ACGTTGGATGTCCAAGAATCGAGCTGGATC	ACGTTGGATGTTGTCAACCAGTGGTCTGTG	GGTCTGTGAA
COMT			
Val158Met	ACGTTGGATGACCCAGCGGATGGTGGATTT	ACGTTGGATGGCCCTTTTTCCAGGTCTGAC	GGCATGCACA

Table A.6: Estimated power to detect main effects odds ratios (OR) by exposure prevalence ($\alpha = .05$) for Whites

Gene Prevalence (Wildtype) (One or more variant alleles)	*OR* 1.5	2.0	3.0
CYP1A1*2A	0.404	0.824	0.994
0.7568			
0.2432			
CYP1A1*2C	0.216	0.502	0.846
0.9030			
0.0970			
CYP1A1*3	0.046	0.067	0.102
0.9950			
0.0050			
CYP1A1*4	0.207	0.480	0.825
0.9109			
0.0891			
CYP1B1 Ala119Ser	0.522	0.926	1.000
0.5075			
0.4925			
CYP1B1 Leu432Val	0.331	0.728	0.974
0.1658			
0.8342			
COMT Val158Met	0.411	0.831	0.994
0.2376			
0.7624			

Table A.7: Estimated power to detect main effects odds ratios (OR) by exposure prevalence ($\alpha = .05$) for African American

Gene Prevalence (Wildtype) (One or more variant alleles)	*OR*		
	1.5	2.0	3.0
CYP1A1*2A			
0.6093	0.501	0.902	0.999
0.3907			
CYP1A1*2C			
0.9828	0.056	0.090	0.149
0.0172			
CYP1A1*3			
0.8571	0.282	0.635	0.940
0.1429			
CYP1A1*4			
0.9776	0.066	0.114	0.204
0.0224			
CYP1B1 Ala119Ser			
0.2634	0.462	0.856	0.994
0.7366			
CYP1B1 Leu432Val			
0.5611	0.519	0.914	0.999
0.4389			
COMT Val158Met			
0.4708	0.504	0.899	0.998
0.5292			

Table A.8. Estimated pairwise linkage disequilibrium (LD) among White premenopausal women in Uterine Fibroid Study (using r^2)

	CYP1A1*2A	CYP1A1*3	CYP1A1*2C
CYP1A1*2A			
CYP1A1*3	<0.001		
CYP1A1*2C	0.341	<0.001	
CYP1A1*4	<0.001	<0.001	0.002
	Ala119Ser		
Leu432Val	0.239		

Table A.9. Estimated pairwise linkage disequilibrium (LD) among African American premenopausal women in Uterine Fibroid Study (using r^2)

	CYP1A1*2A	CYP1A1*3	CYP1A1*2C
CYP1A1*2A			
CYP1A1*3	0.021		
CYP1A1*2C	0.022	<0.001	
CYP1A1*4	0.003	<0.001	<0.001
	Ala119Ser		
Leu432Val	0.045		

Table A.10. Distribution of haplotype and diplotype frequencies among premenopausal African American and White participants in the Uterine Fibroid Study

Genotype	Fibroid Status									
	African American					Caucasian				
	No Fibroids	Fibroids present	Small	Medium	Large	No Fibroids	Fibroids present	Small	Medium	Large
CYP1A1 haplotypes[a]										
N	310	838	186	372	280	390	400	140	172	88
CTAC	65(21.0)	171(20.3)	36(19.1)	75(20.1)	60(21.4)	31(7.9)	35(8.7)	15(10.7)	14(8.0)	6(6.8)
CTGC	3(1.0)	6(0.7)	1(0.5)	2(0.5)	3(1.1)	23(5.9)	17(4.2)	3(2.1)	10(5.7)	4(4.5)
TCAC	16(5.2)	70(8.3)	14(7.4)	31(8.3)	25(8.9)	1(0.3)	1(0.2)	0(0.0)	0(0.0)	1(1.1)
TTAA	2(0.6)	11(1.3)	1(0.5)	3(0.8)	7(2.5)	14(3.6)	20(5.0)	6(4.3)	11(6.3)	3(3.4)
TTAC	224(72.3)	579(68.8)	134(71.3)	260(69.5)	185(66.1)	321(82.3)	327(81.3)	116(82.9)	137(78.7)	74(84.1)
TTGC	0(0.0)	1(0.1)	0(0.0)	1(0.3)	0(0.0)	0	0	0	0	0
CYP1A1 diplotypes[a]										
N	155	419	93	186	140	195	201	70	86	44
CTAC/CTAC	5(3.2)	17(4.0)	4(4.3)	6(3.2)	7(5.0)	2(1.0)	1(0.5)	0(0.0)	1(1.1)	0(0.0)
CTAC/CTGC	0(0.0)	1(0.2)	1(1.1)	0(0.0)	0(0.0)	2(1.0)	3(1.5)	0(0.0)	2(2.3)	1(2.3)
CTGC/CTGC	0	0	0	0	0	0(0.0)	1(0.5)	0(0.0)	1(1.1)	0(0.0)
TCAC/CTAC	2(1.3)	17(4.0)	5(5.3)	7(3.7)	5(3.6)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(2.3)
TCAC/TCAC	1(0.6)	3(0.7)	0(0.0)	2(1.1)	1(0.7)	0	0	0	0	0

Genotype	Fibroid Status									
	African American					Caucasian				
	No Fibroids	Fibroids present	Small	Medium	Large	No Fibroids	Fibroids present	Small	Medium	Large
TTAA/CTAC	1(0.6)	1(0.2)	0(0.0)	0(0.0)	1(0.7)	25(12.8)	29(14.4)	15(21.4)	10(11.5)	4(9.1)
TTAA/TCAC	0(0.0)	1(0.2)	0(0.0)	0(0.0)	1(0.7)	0	0	0	0	0
TTAC/CTAC	52(33.5)	118(28.0)	22(23.4)	56(29.9)	40(28.6)	0	0	0	0	0
TTAC/CTGC	3(1.9)	5(1.2)	0(0.0)	2(1.1)	3(2.1)	21(10.8)	12(6.0)	3(4.3)	6(6.9)	3(6.8)
TTAC/TCAC	12(7.7)	46(10.9)	9(9.6)	20(10.7)	17(12.1)	1(0.5)	1(0.5)	0(0.0)	0(0.0)	1(2.3)
TTAC/TTAA	1(0.6)	9(2.1)	1(1.1)	3(1.6)	5(3.6)	14(7.2)	19(9.5)	6(8.6)	11(12.6)	2(4.5)
TTAC/TTAC	78(50.3)	200(47.5)	51(54.3)	89(47.6)	60(42.9)	130(66.7)	133(66.2)	46(65.7)	55(63.2)	32(72.7)
TTAC/TTGC	0(0.0)	1(0.2)	0(0.0)	1(0.5)	0(0.0)	0	0	0	0	0
CYP1B1 haplotypes[b]										
N	240	678	146	302	230	386	402	140	174	88
GC	16(5.2)	49(5.8)	7(3.7)	22(5.9)	20(7.1)	115(29.5)	126(31.3)	47(33.6)	55(31.6)	24(27.3)
GG	106(34.2)	302(35.9)	66(35.1)	133(35.6)	103(36.8)	162(41.5)	164(40.8)	55(39.3)	70(40.2)	39(44.3)
TC	32(10.3)	80(9.5)	21(11.2)	33(8.8)	26(9.3)	106(27.2)	110(27.4)	38(27.1)	49(28.2)	23(26.1)
TG	86(27.7)	247(29.3)	52(27.7)	114(30.5)	81(28.9)	3(0.8)	2(0.5)	0(0.0)	0(0.0)	2(2.3)

Genotype	Fibroid Status									
	African American					Caucasian				
	No Fibroids	Fibroids present	Small	Medium	Large	No Fibroids	Fibroids present	Small	Medium	Large
CYP1B1 diplotypes[b]										
N	120	339	73	170	115	193	201	70	87	44
GC/GC	2(1.3)	3(0.7)	0(0.0)	0(0.0)	3(2.1)	15(7.7)	17(8.5)	7(10.0)	8(9.2)	2(4.5)
GC/TC	3(1.9)	12(2.9)	3(3.2)	4(2.1)	5(3.6)	33(16.9)	38(18.9)	13(18.6)	18(20.7)	7(15.9)
GG/GC	9(5.8)	31(7.4)	4(4.3)	18(9.6)	9(6.4)	52(26.7)	54(26.9)	20(28.6)	21(24.1)	13(29.5)
GG/GG	29(18.7)	75(17.8)	20(21.3)	28(15.0)	27(19.3)	31(15.9)	31(15.4)	11(15.7)	14(16.1)	6(13.6)
GG/TC	0	0	0	0	0	45(23.1)	47(23.4)	13(18.6)	21(24.1)	13(29.5)
GG/TG	39(25.2)	121(28.7)	22(23.4)	59(31.6)	40(28.6)	3(1.5)	1(0.5)	0(0.0)	0(0.0)	1(2.3)
TC/TC	6(3.9)	11(2.6)	3(3.2)	4(2.1)	4(2.9)	14(7.2)	12(6.0)	6(8.6)	5(5.7)	1(2.3)
TG/TC	17(11.0)	46(10.9)	12(12.8)	21(11.2)	13(9.3)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	1(2.3)
TG/TG	15(9.7)	40(9.5)	9(9.6)	17(9.1)	14(10.0)	0	0	0	0	0

[a]Order of SNPs: CYP1A1*2A, CYP1A1*3, CYP1A1*2C, CYP1A1*4
[b]Order of SNPs: CYP1B1 Ala119Ser, CYP1B1 Leu432Val

Table A.11. Assessment of confounding for the main effects of COMT allele and presence of fibroids among premenopausal African American and White women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
COMT	0.92	(0.84, 1.02)
COMT, Age	0.91	(0.83, 1.00)
COMT, BMI	0.93	(0.85, 1.03)
COMT, Smoking	0.92	(0.83, 1.02)
COMT, Oral conceptive use	0.93	(0.84, 1.03)
COMT, Full term births after age 24	0.93	(0.84, 1.03)
COMT, Alcohol use	0.94	(0.85, 1.04)
Full Model[a]	0.97	(0.88, 1.07)
Whites		
COMT	1.02	(0.81, 1.28)
COMT, Age	1.02	(0.83, 1.27)
COMT, BMI	1.02	(0.81, 1.28)
COMT, Smoking	1.01	(0.80, 1.28)
COMT, Oral conceptive use	1.02	(0.81, 1.28)
COMT, Full term births after age 24	1.01	(0.81, 1.27)
COMT, Full term (full25pc)	1.01	(0.80, 1.27)
COMT, Alcohol use	1.03	(0.81, 1.31)
Full Model[a]	1.03	(0.82, 1.28)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.12. Assessment of confounding for the main effects of COMT allele and presence of largest fibroids ≤ 2cm among African American and White premenopausal women in the the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
COMT	0.74	(0.54, 1.03)
COMT, Age	0.72	(0.53, 0.99)
COMT, BMI	0.77	(0.56, 1.05)
COMT, Smoking	0.73	(0.53, 1.00)
COMT, Oral conceptive use	0.76	(0.55, 1.05)
COMT, Full term births after age 24	0.75	(0.54, 1.03)
COMT, Alcohol use	0.78	(0.57, 1.08)
Full Model[a]	0.80	(0.59, 1.09)
Whites		
COMT	1.17	(0.72, 1.99)
COMT, Age	1.16	(0.71, 1.89)
COMT, BMI	1.19	(0.72, 1.97)
COMT, Smoking	1.21	(0.72, 2.01)
COMT, Oral conceptive use	1.21	(0.72, 2.02)
COMT, Full term births after age 24	1.19	(0.72, 1.99)
COMT, Alcohol use	1.13	(0.68, 1.88)
Full Model[b]	1.12	(1.65, 2.02)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

[b] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.13 Assessment of confounding for the main effects of COMT allele and presence of largest fibroids >2cm ≤4 among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
COMT	0.92	(0.76, 1.12)
COMT, Age	0.90	(0.75, 1.09)
COMT, BMI	0.94	(0.78, 1.13)
COMT, Smoking	0.91	(0.75, 1.10)
COMT, Oral conceptive use	0.93	(0.77, 1.13)
COMT, Full term	0.92	(0.76, 1.11)
COMT, Full term births after age 24	0.93	(0.77, 1.13)
COMT, Alcohol use	0.97	(0.79, 1.19)
Full Model [a]	0.99	(0.82, 1.20)
Whites		
COMT	0.92	(0.62, 1.36)
COMT, Age	0.95	(0.65, 1.38)
COMT, BMI	0.92	(0.62, 1.36)
COMT, Smoking	0.90	(0.61, 1.34)
COMT, Oral conceptive use	0.91	(0.61, 1.35)
COMT, Full term	0.91	(0.62, 1.35)
COMT, Full term births after age 24	0.94	(0.63, 1.40)
COMT, Alcohol use	0.95	(0.62, 1.44)
Full Model [b]	0.95	(0.64, 1.42)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

[b] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.14 Assessment of confounding for the main effects of COMT allele and presence of largest fibroids >4 among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
COMT	0.84	(0.66, 1.07)
COMT, Age	0.82	(0.66, 1.01)
COMT, BMI	0.85	(0.67, 1.08)
COMT, Smoking	0.85	(0.67, 1.09)
COMT, Oral conceptive use	0.85	(0.67, 1.08)
COMT, Births after age 24	0.85	(0.67, 1.09)
COMT, Alcohol use	0.86	(0.67, 1.11)
Full Model[a]	0.89	(0.71, 1.12)
Whites		
COMT	1.06	(0.56, 2.02)
COMT, Age	1.08	(0.58, 2.03)
COMT, BMI	1.06	(0.56, 2.00)
COMT, Smoking	1.04	(0.55, 1.98)
COMT, Oral conceptive use	1.05	(0.55, 1.99)
COMT, Full term births after age 24	1.02	(0.55, 1.92)
COMT, Alcohol use	1.10	(0.56, 2.16)
Full Model[b]	1.07	(0.54, 2.01)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

[b] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.15 Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of COMT genotype and participant characteristics among premenopausal African Americans, Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	PR (95% CI)	N (%)	PR (95% CI)
Total	274(.)		308(.)	
Ag e				
35-39	106(38.7)	1.0	114(37.0)	0.87 (0.71, 1.06)
40-44	97(35.4)	1.07 (0.90, 1.28)	109(35.4)	1.18 (0.91, 1.52)
>=45	71(25.9)	1.31 (1.13, 1.53)	85(27.6)	1.01 (0.79, 1.28)
BMI				
Normal	63(23.0)	1.0	84(27.3)	0.90 (0.71, 1.14)
Overweight	85(31.0)	1.13 (0.92, 1.39)	94(30.5)	1.03 (0.77, 1.39)
Obese	126(46.0)	1.16 (0.96, 1.40)	130(42.2)	1.05 (0.80, 1.39)
Smoking Status				
Never Smoked	132(48.2)	1.0	145(47.1)	0.99 (0.85, 1.15)
Past Smoker	59(21.5)	1.09 (0.91, 1.29)	74(24.0)	0.96 (0.75, 1.23)
Current Smoker	82(29.9)	1.13 (0.97, 1.32)	86(27.9)	0.82 (0.64, 1.04)
Oral Contraceptive Use				
Never Used OC	16(5.8)	1.0	32(10.4)	0.95 (0.59, 1.53)
Current/Past User Of OC	258(94.2)	1.23 (0.84, 1.81)	276(89.6)	0.98 (0.60, 1.60)
Number Of Full term Births				
None	144(52.6)	1.0	152(49.4)	0.91 (0.80, 1.04)
1	83(30.3)	0.94 (0.81, 1.09)	97(31.5)	0.99 (0.79, 1.24)
2	40(14.6)	0.85 (0.67, 1.07)	45(14.6)	1.21 (0.90, 1.65)
3 Or More	7(2.6)	0.90 (0.56, 1.45)	14(4.5)	0.77 (0.37, 1.56)
Number Of Alcoholic Drinks per week				
Less Than 0.5	151(55.1)	1.0	148(48.1)	0.96 (0.84, 1.10)
0.5-2	51(18.6)	0.92 (0.75, 1.13)	71(23.1)	1.00 (0.76, 1.32)
3-6	22(8.0)	1.14 (0.94, 1.38)	43(14.0)	0.83 (0.62, 1.12)
7 Or More	32(11.7)	1.03 (0.84, 1.26)	26(8.4)	1.12 (0.84, 1.49)

Table A.16 Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of COMT genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	WT/Wt		Wt/Var & Var/Var	
	N (%)	**PR (95% CI)**	**N (%)**	**PR (95% CI)**
Total	96(.)		308(.)	
Age				
35-39	29(30.2)	1.0	108(35.1)	0.82 (0.49, 1.35)
40-44	36(37.5)	0.91 (0.50, 1.64)	100(32.5)	1.54 (0.78, 3.04)
>=45	31(32.3)	1.63 (1.00, 2.66)	100(32.5)	1.22 (0.69, 2.16)
BMI				
Normal	56(58.3)	1.0	181(58.8)	1.03 (0.75, 1.41)
Overweight	22(22.9)	0.94 (0.55, 1.61)	73(23.7)	1.05 (0.58, 1.92)
Obese	18(18.8)	1.27 (0.80, 2.01)	54(17.5)	0.91 (0.53, 1.56)
Smoking Status				
Never Smoked	61(63.5)	1.0	173(56.2)	1.02 (0.75, 1.37)
Past Smoker	29(30.2)	0.98 (0.62, 1.55)	110(35.7)	1.05 (0.63, 1.75)
Current Smoker	6(6.3)	1.36 (0.73, 2.53)	25(8.1)	0.83 (0.40, 1.71)
Oral Contraceptive Use				
Never Used OC	12(12.5)	1.0	57(18.5)	0.87 (0.51, 1.50)
Current/Past User Of OC	84(87.5)	0.84 (0.49, 1.42)	251(81.5)	1.20 (0.66, 2.18)
Number Of Full term Births				
None	59(61.5)	1.0	198(64.3)	0.94 (0.74, 1.20)
1	19(19.8)	0.55 (0.73, 1.10)	38(12.3)	1.55 (0.70, 3.42)
2 or more	18(18.7)	0.77 (0.44, 1.35)	72 (23.4)	1.06 (0.57, 2.00)
Number Of Alcoholic Drinks per week				
Less Than 0.5	16(16.7)	1.0	44(14.3)	0.77 (0.42, 1.43)
0.5-2	23(24.0)	0.96 (0.50, 1.83)	92(29.9)	1.35 (0.62, 2.94)
3-6	26(27.1)	0.80 (0.40, 1.59)	76(24.7)	1.97 (0.88, 4.39)
7 Or More	24(25.0)	1.22 (0.68, 2.19)	74(24.0)	1.03 (0.49, 2.16)

Table A.17 Assessment of confounding for the main effects of COMT allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*2A	0.97	(0.87, 1.08)
CYP1A1*2A, Age	0.98	(0.89, 1.08)
CYP1A1*2A, BMI	0.99	(0.89, 1.10)
CYP1A1*2A, Smoking	0.97	(0.88, 1.08)
CYP1A1*2A, Oral conceptive use	0.98	(0.88, 1.08)
CYP1A1*2A, Full term births after age 24	0.98	(0.88, 1.09)
CYP1A1*2A, Alcohol use	0.96	(0.86, 1.07)
Full Model[a]	1.01	(0.91, 1.12)
Whites		
CYP1A1*2A	0.94	(0.75, 1.19)
CYP1A1*2A, Age	0.98	(0.79, 1.21)
CYP1A1*2A, BMI	0.93	(0.74, 1.18)
CYP1A1*2A, Smoking	0.93	(0.74, 1.18)
CYP1A1*2A, Oral conceptive use	0.94	(0.75, 1.19)
CYP1A1*2A, Full term births after age 24	0.95	(0.76, 1.20)
CYP1A1*2A, Alcohol use	0.92	(0.71, 1.18)
Full Model[a]	0.93	(0.74, 1.17)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.18 Assessment of confounding for the main effects of CYP1A1*3 allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*3	1.15	(1.02, 1.29)
CYP1A1*3, Age	1.16	(1.06, 1.28)
CYP1A1*3, BMI	1.13	(1.00, 1.26)
CYP1A1*3, Smoking	1.14	(1.01, 1.28)
CYP1A1*3, Oral conceptive use	1.13	(1.01, 1.27)
CYP1A1*3, Full term	1.16	(1.03, 1.30)
CYP1A1*3, Full term births after age 24	1.15	(1.02, 1.29)
CYP1A1*3, Alcohol use	1.15	(1.02, 1.29)
Full Model[a]	1.08	(0.97, 1.20)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.19 Assessment of confounding for the main effects of CYP1A1*2C allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*2C	0.96	(0.64, 1.45)
CYP1A1*2C, Age	0.95	(0.65, 1.39)
CYP1A1*2C, BMI	0.91	(0.60, 1.37)
CYP1A1*2C, Smoking	0.97	(0.65, 1.47)
CYP1A1*2C, Oral conceptive use	0.99	(0.67, 1.48)
CYP1A1*2C, Full term 24	0.97	(0.65, 1.46)
CYP1A1*2C, Full term births after age	0.96	(0.64, 1.45)
CYP1A1*2C, Alcohol use	0.96	(0.64, 1.44)
Full Model[a]	0.97	(0.61, 1.54)
Whites		
CYP1A1*2C	0.80	(0.54, 1.17)
CYP1A1*2C, Age	0.82	(0.57, 1.18)
CYP1A1*2C, BMI	0.79	(0.53, 1.16)
CYP1A1*2C, Smoking	0.79	(0.53, 1.16)
CYP1A1*2C, Oral conceptive use	0.80	(0.54, 1.18)
CYP1A1*2C, Full term births after age 24	0.82	(0.56, 1.21)
CYP1A1*2C, Alcohol use	0.69	(0.43, 1.10)
Full Model[a]	0.73	(0.46, 1.13)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.20 Assessment of confounding for the main effects of CYP1A1*4 allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*4	1.16	(0.92, 1.48)
CYP1A1*4, Age	1.05	(0.84, 1.33)
CYP1A1*4, BMI	1.12	(0.88, 1.42)
CYP1A1*4, Smoking	1.18	(0.93, 1.50)
CYP1A1*4, Oral conceptive use	1.15	(0.91, 1.46)
CYP1A1*4, Full term births after age 24	1.15	(0.91, 1.46)
CYP1A1*4, Alcohol use	1.26	(1.04, 1.54)
Full Model[a]	1.08	(0.89, 1.32)
Whites		
CYP1A1*4	1.20	(0.90, 1.61)
CYP1A1*4, Age	1.20	(0.94, 1.53)
CYP1A1*4, BMI	1.18	(0.89, 1.58)
CYP1A1*4, Smoking	1.22	(0.91, 1.64)
CYP1A1*4, Oral conceptive use	1.20	(0.90, 1.61)
CYP1A1*4, Full term births after age 24	1.25	(0.94, 1.66)
CYP1A1*4, Alcohol use	1.26	(0.95, 1.68)
Full Model (full term)	1.26	(0.92, 1.72)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.21 Assessment of confounding for the main effects of *CYP1B1AS* allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1AS	1.00	(0.90, 1.12)
CYP1B1AS, Age	1.01	(0.91, 1.13)
CYP1B1AS, BMI	1.02	(0.91, 1.14)
CYP1B1AS, Smoking	1.00	(0.89, 1.12)
CYP1B1AS, Oral conceptive use	1.01	(0.90, 1.13)
CYP1B1AS, Full term births after age 24	1.02	(0.91, 1.14)
CYP1B1AS, Alcohol use	1.02	(0.91, 1.15)
Full Model[a]	1.07	(0.95, 1.20)
Whites		
CYP1B1AS	1.00	(0.83, 1.21)
CYP1B1AS, Age	1.01	(0.84, 1.20)
CYP1B1AS, BMI	0.97	(0.80, 1.18)
CYP1B1AS, Smoking	1.00	(0.83, 1.22)
CYP1B1AS, Oral conceptive use	1.00	(0.82, 1.21)
CYP1B1AS, Full term births after age 24	1.01	(0.84, 1.23)
CYP1B1AS, Alcohol use	0.98	(0.80, 1.20)
Full Model[b]	0.98	(0.80, 1.20)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.22. Assessment of confounding for the main effects of *CYP1B1LV* allele and presence of fibroids among African American and White premenopausal women in the Uterine Fibroid Study

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1LV	0.97	(0.88, 1.08)
CYP1B1LV, Age	1.00	(0.91, 1.10)
CYP1B1LV, BMI	0.98	(0.89, 1.09)
CYP1B1LV, Smoking	0.97	(0.87, 1.07)
CYP1B1LV, Oral conceptive use	0.98	(0.88, 1.08)
CYP1B1LV, Full term births after age 24	0.98	(0.89, 1.08)
CYP1B1LV, Alcohol use	0.98	(0.88, 1.09)
Full Model[a]	0.99	(0.90, 1.09)
Whites		
CYP1B1LV	1.06	(0.81, 1.38)
CYP1B1LV, Age	1.08	(0.84, 1.39)
CYP1B1LV, BMI	1.05	(0.80, 1.37)
CYP1B1LV, Smoking	1.06	(0.81, 1.39)
CYP1B1LV, Oral conceptive use	1.06	(0.81, 1.39)
CYP1B1LV, Full term births after age 24	1.05	(0.80, 1.37)
CYP1B1LV, Alcohol use	1.01	(0.77, 1.33)
Full Model (full term)	1.03	(0.80, 1.33)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.23. Assessment of confounding for the main effects of *CYP1A1*2A* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was <2cm (small) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*2A	0.85	(0.60, 1.19)
CYP1A1*2A, Age	0.87	(0.62, 1.22)
CYP1A1*2A, BMI	0.91	(0.64, 1.28)
CYP1A1*2A, Smoking	0.86	(0.61, 1.21)
CYP1A1*2A, Oral conceptive use	0.87	(0.62, 1.22)
CYP1A1*2A, Full term births after age 24	0.86	(0.61, 1.22)
CYP1A1*2A, Alcohol use	0.83	(0.59, 1.17)
Full Model[a]	0.95	(0.68, 1.33)
Whites		
CYP1A1*2A	1.00	(0.63, 1.59)
CYP1A1*2A, Age	1.04	(0.67, 1.59)
CYP1A1*2A, BMI	0.97	(0.61, 1.53)
CYP1A1*2A, Smoking	1.02	(0.64, 1.62)
CYP1A1*2A, Oral conceptive use	1.01	(0.63, 1.59)
CYP1A1*2A, Full term births after age 24	1.01	(0.64, 1.59)
CYP1A1*2A, Alcohol use	1.10	(0.69, 1.74)
Full Model (full term)	1.07	(0.70, 1.64)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.24. Assessment of confounding for the main effects of *CYP1A1*3* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was <2cm (small) compared to no fibroids

Variables in model	Prevalence Ratio	PR 95% CI
African Americans		
CYP1A1*3	1.35	(0.89, 2.05)
CYP1A1*3, Age	1.44	(0.96, 2.16)
CYP1A1*3, BMI	1.29	(0.86, 1.94)
CYP1A1*3, Smoking	1.36	(0.90, 2.07)
CYP1A1*3, Oral conceptive use	1.31	(0.87, 1.99)
CYP1A1*3, Full term	1.39	(0.91, 2.10)
CYP1A1*3, Full term births after age 24	1.37	(0.90, 2.09)
CYP1A1*3, Alcohol use	1.38	(0.92, 2.05)
Full Model[a]	1.36	(0.93, 2.00)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.25. Assessment of confounding for the main effects of *CYP1B1AS* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was <2cm (small) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1AS	1.05	(0.72, 1.52)
CYP1B1AS, Age	1.06	(0.74, 1.54)
CYP1B1AS, BMI	1.10	(0.76, 1.60)
CYP1B1AS, Smoking	1.02	(0.70, 1.48)
CYP1B1AS, Oral conceptive use	1.07	(0.74, 1.55)
CYP1B1AS, Full term	1.08	(0.74, 1.58)
CYP1B1AS, Full term births after age 24	1.08	(0.74, 1.57)
CYP1B1AS, Alcohol use	1.09	(0.75, 1.58)
Full Model[a]	1.09	(0.84, 1.80)
Whites		
CYP1B1AS	0.90	(0.60, 1.35)
CYP1B1AS, Age	0.91	(0.62, 1.32)
CYP1B1AS, BMI	0.84	(0.55, 1.26)
CYP1B1AS, Smoking	0.90	(0.60, 1.35)
CYP1B1AS, Oral conceptive use	0.91	(0.61, 1.38)
CYP1B1AS, Full term births after age 24	0.90	(0.60, 1.35)
CYP1B1AS, Alcohol use	0.92	(0.61, 1.38)
Full Model[a]	0.91	(0.61, 1.35)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.26. Assessment of confounding for the main effects of *CYP1B1LV* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was <2cm (small) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1LV	0.98	(0.71, 1.36)
CYP1B1LV, Age	1.02	(0.74, 1.41)
CYP1B1LV, BMI	1.02	(0.74, 1.40)
CYP1B1LV, Smoking	0.96	(0.69, 1.33)
CYP1B1LV, Oral conceptive use	0.99	(0.72, 1.36)
CYP1B1LV, Full term births after age 24	1.00	(0.72, 1.38)
CYP1B1LV, Alcohol use	1.01	(0.74, 1.40)
Full Model[a]	1.03	(0.76, 1.39)
Whites		
CYP1B1LV	1.10	(0.63, 1.92)
CYP1B1LV, Age	1.14	(0.67, 1.93)
CYP1B1LV, BMI	1.07	(0.61, 1.86)
CYP1B1LV, Smoking	1.08	(0.61, 1.89)
CYP1B1LV, Oral conceptive use	1.09	(0.62, 1.90)
CYP1B1LV, Full term births after age 24	1.09	(0.63, 1.91)
CYP1B1LV, Alcohol use	1.00	(0.58, 1.74)
Full Model[a]	1.03	(0.62, 1.70)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.27. Assessment of confounding for the main effects of *CYP1A1*2A* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was >2cm and ≤4cm (medium) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*2A	0.95	(0.78, 1.17)
CYP1A1*2A, Age	0.98	(0.80, 1.19)
CYP1A1*2A, BMI	1.00	(0.82, 1.22)
CYP1A1*2A, Smoking	0.97	(0.79, 1.18)
CYP1A1*2A, Oral conceptive use	0.97	(0.79, 1.18)
CYP1A1*2A, Full term	0.97	(0.79, 1.18)
CYP1A1*2A, Full term births after age 24	0.98	(0.80, 1.19)
CYP1A1*2A, Alcohol use	0.95	(0.77, 1.16)
Full Model[a]	1.03	(0.85, 1.26)
Whites		
CYP1A1*2A	0.91	(0.60, 1.39)
CYP1A1*2A, Age	0.97	(0.65, 1.45)
CYP1A1*2A, BMI	0.91	(0.60, 1.39)
CYP1A1*2A, Smoking	0.89	(0.58, 1.36)
CYP1A1*2A, Oral conceptive use	0.91	(0.60, 1.39)
CYP1A1*2A, Full term births after age 24	0.93	(0.61, 1.41)
CYP1A1*2A, Alcohol use	0.89	(0.48, 1.28)
Full Model[a]	0.82	(0.52, 1.31)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, and alcohol use

Table A.28. Assessment of confounding for the main effects of *CYP1A1*3* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was >2cm and ≤4cm (medium) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*3	1.25	(0.99, 1.59)
CYP1A1*3, Age	1.30	(1.05, 1.63)
CYP1A1*3, BMI	1.20	(0.95, 1.52)
CYP1A1*3, Smoking	1.23	(0.97, 1.56)
CYP1A1*3, Oral conceptive use	1.23	(0.97, 1.56)
CYP1A1*3, Full term	1.27	(1.00, 1.61)
CYP1A1*3, Full term births after age 24	1.25	(0.98, 1.59)
CYP1A1*3, Alcohol use	1.29	(1.02, 1.63)
Full Model[a]	1.22	(0.96, 1.55)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral contraceptive use, fullterm births after age 24, and alcohol use

Table A.29. Assessment of confounding for the main effects of *CYP1B1AS* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was >2cm and ≤4cm (medium) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1AS	1.03	(0.82, 1.29)
CYP1B1AS, Age	1.05	(0.84, 1.31)
CYP1B1AS, BMI	1.08	(0.86, 1.35)
CYP1B1AS, Smoking	1.02	(0.81, 1.29)
CYP1B1AS, Oral conceptive use	1.05	(0.84, 1.32)
CYP1B1AS, Full term	1.06	(0.84, 1.34)
CYP1B1AS, Full term births after age 24	1.06	(0.84, 1.33)
CYP1B1AS, Alcohol use	1.05	(0.83, 1.34)
Full Model[a]	1.04	(0.92, 1.48)
Whites		
CYP1B1AS	1.04	(0.73, 1.47)
CYP1B1AS, Age	1.05	(0.76, 1.46)
CYP1B1AS, BMI	1.05	(0.73, 1.50)
CYP1B1AS, Smoking	1.04	(0.73, 1.47)
CYP1B1AS, Oral conceptive use	1.03	(0.72, 1.46)
CYP1B1AS, Full term	1.05	(0.74, 1.49)
CYP1B1AS, Full term births after age 24	1.02	(0.72, 1.45)
CYP1B1AS, Alcohol use	1.00	(0.69, 1.45)
Full Model[a]	1.03	(0.74, 1.53)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, oral and contraceptive use

Table A.30. Assessment of confounding for the main effects of *CYP1B1LV* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was >2cm and ≤4cm (medium) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1LV	0.96	(0.79, 1.17)
CYP1B1LV, Age	0.99	(0.81, 1.20)
CYP1B1LV, BMI	0.98	(0.81, 1.19)
CYP1B1LV, Smoking	0.93	(0.77, 1.14)
CYP1B1LV, Oral conceptive use	0.96	(0.79, 1.17)
CYP1B1LV, Full term births after age 24	0.97	(0.79, 1.18)
CYP1B1LV, Alcohol use	0.98	(0.80, 1.20)
Full Model[a]	1.00	(0.83, 1.20)
Whites		
CYP1B1LV	1.07	(0.66, 1.73)
CYP1B1LV, Age	1.11	(0.70, 1.76)
CYP1B1LV, BMI	1.06	(0.66, 1.71)
CYP1B1LV, Smoking	1.09	(0.67, 1.77)
CYP1B1LV, Oral conceptive use	1.08	(0.67, 1.75)
CYP1B1LV, Full term	1.06	(0.66, 1.71)
CYP1B1LV, Full term births after age 24	1.00	(0.63, 1.61)
CYP1B1LV, Alcohol use	0.96	(0.59, 1.55)
Full Model [a]	1.01	(0.64, 1.62)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, oral contraceptive use, and alcohol use

Table A.31 Assessment of confounding for the main effects of *CYP1A1*2A* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was ≥4cm (large) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*2A	0.98	(0.77, 1.26)
CYP1A1*2A, Age	0.99	(0.80, 1.24)
CYP1A1*2A, BMI	1.00	(0.78, 1.28)
CYP1A1*2A, Smoking	0.98	(0.77, 1.26)
CYP1A1*2A, Oral conceptive use	0.99	(0.77, 1.27)
CYP1A1*2A, Full term births after age 24	1.00	(0.78, 1.28)
CYP1A1*2A, Alcohol use	0.95	(0.74, 1.23)
Full Model[a]	0.98	(0.85, 1.38)
Whites		
CYP1A1*2A	0.78	(0.40, 1.53)
CYP1A1*2A, Age	0.83	(0.43, 1.61)
CYP1A1*2A, BMI	0.77	(0.39, 1.49)
CYP1A1*2A, Smoking	0.75	(0.38, 1.46)
CYP1A1*2A, Oral conceptive use	0.78	(0.40, 1.53)
CYP1A1*2A, Full term births after age 24	0.83	(0.43, 1.60)
CYP1A1*2A, Alcohol use	0.73	(0.36, 1.48)
Full Model[a]	0.80	(0.41, 1.57)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, and oral contraceptive use

Table A.32 Assessment of confounding for the main effects of *CYP1A1*3* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was ≥4cm (large) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1A1*3	1.37	(1.03, 1.82)
CYP1A1*3, Age	1.39	(1.12, 1.71)
CYP1A1*3, BMI	1.35	(1.02, 1.79)
CYP1A1*3, Smoking	1.35	(1.02, 1.80)
CYP1A1*3, Oral conceptive use	1.36	(1.02, 1.80)
CYP1A1*3, Full term	1.41	(1.07, 1.85)
CYP1A1*3, Full term births after age 24	1.38	(1.04, 1.82)
CYP1A1*3, Alcohol use	1.35	(1.00, 1.82)
Full Model[a]	1.24	(0.94, 1.63)

[a] Full model includes the following variables: COMT genotype, age, births after age 25, smoking, BMI, and oral contraceptive use

Table A.33 Assessment of confounding for the main effects of *CYP1B1AS* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was ≥4cm (large) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1AS	0.95	(0.72, 1.24)
CYP1B1AS, Age	0.98	(0.78, 1.25)
CYP1B1AS, BMI	0.95	(0.73, 1.24)
CYP1B1AS, Smoking	0.96	(0.73, 1.25)
CYP1B1AS, Oral conceptive use	0.95	(0.73, 1.24)
CYP1B1AS, Full term births after age 24	0.97	(0.74, 1.27)
CYP1B1AS, Alcohol use	1.00	(0.75, 1.33)
Full Model[a]	0.98	(0.74, 1.35)
Whites		
CYP1B1AS	1.10	(0.65, 1.88)
CYP1B1AS, Age	1.11	(0.66, 1.87)
CYP1B1AS, BMI	0.98	(0.57, 1.69)
CYP1B1AS, Smoking	1.11	(0.65, 1.89)
CYP1B1AS, Oral conceptive use	1.08	(0.62, 1.85)
CYP1B1AS, Full term births after age 24	1.16	(0.69, 1.95)
CYP1B1AS, Alcohol use	1.05	(0.60, 1.82)
Full Model[a]	1.12	(0.57, 1.93)

[a] Full model includes the following variables: COMT genotype, age, smoking, BMI, and oral contraceptive use

Table A.34 Assessment of confounding for the main effects of *CYP1B1LV* allele and fibroid prevalence among African American and White premenopausal women in the Uterine Fibroid Study whose largest fibroid recorded was ≥4cm (large) compared to no fibroids

Variables in model	Prevalence Ratio(PR)	PR 95% CI
African Americans		
CYP1B1LV	0.92	(0.72, 1.18)
CYP1B1LV, Age	1.00	(0.80, 1.24)
CYP1B1LV, BMI	0.93	(0.72, 1.18)
CYP1B1LV, Smoking	0.93	(0.72, 1.19)
CYP1B1LV, Oral conceptive use	0.92	(0.72, 1.18)
CYP1B1LV, Full term births after age 24	0.94	(0.74, 1.20)
CYP1B1LV, Alcohol use	0.91	(0.71, 1.18)
Full Model	0.98	(0.79, 1.22)
Whites		
CYP1B1LV	1.09	(0.53, 2.28)
CYP1B1LV, Age	1.15	(0.56, 2.35)
CYP1B1LV, BMI	1.08	(0.52, 2.25)
CYP1B1LV, Smoking	1.10	(0.53, 2.31)
CYP1B1LV, Oral conceptive use	1.12	(0.54, 2.34)
CYP1B1LV, Full term births after age 24	1.07	(0.52, 2.19)
CYP1B1LV, Alcohol use	1.24	(0.56, 2.75)
Full Model (full25pc)	1.02	(0.50, 1.35)

Table A.35. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1A1*2A genotype and participant characteristics among African Americans, National Institute of Environmental Health Science Uterine Fibroid Study

	WT/Wt		Wt/Var & Var/Var	
Characteristics	**N (%)**	**PR (95% CI)**	**N (%)**	**PR (95% CI)**
Total	354(.)		227(.)	
Age				
35-39	130(36.72)		89(39.21)	0.87 (0.70, 1.08)
40-44	125(35.31)	1.06 (0.90, 1.25)	81(35.68)	1.28 (0.98, 1.67)
>=45	99(27.97)	1.28 (1.11, 1.48)	57(25.11)	1.07 (0.82, 1.39)
BMI				
Normal	82(23.16)	1.0	65(28.63)	0.87 (0.68, 1.12)
Overweight	112(31.64)	1.11 (0.92, 1.33)	67(29.52)	1.11 (0.81, 1.51)
Obese	160(45.20)	1.11 (0.93, 1.31)	95(41.85)	1.21 (0.91, 1.61)
Smoking Status				
Never Smoked	159(44.92)	1.0	117(51.54)	0.98 (0.84, 1.15)
Past Smoker	80(22.60)	1.04 (0.89, 1.22)	53(23.35)	1.05 (0.82, 1.35)
Current Smoker	112(31.64)	1.06 (0.91, 1.22)	56(24.67)	0.91 (0.70, 1.19)
Oral Contraceptive Use				
Never Used OC	28(7.91)	1.0	20(8.81)	0.86 (0.53, 1.39)
Current/Past User Of OC	326(92.09)	1.16 (0.88, 1.54)	207(91.19)	1.15 (0.70, 1.88)
Number Of Full term Births				
None	182(51.41)	1.0	113(49.78)	1.05 (0.92, 1.19)
1	115(32.49)	1.02 (0.90, 1.17)	65(28.63)	0.77 (0.60, 0.99)
2	45(12.71)	0.92 (0.75, 1.14)	40(17.62)	1.03 (0.77, 1.39)
3 Or More	12(3.39)	0.78 (0.48, 1.27)	9(3.96)	0.91 (0.42, 1.96)
Number Of Alcoholic Drinks per week				
Less Than 0.5	179(50.56)	1.0	119(52.42)	0.96 (0.83, 1.10)
0.5-2	77(21.75)	0.94 (0.80, 1.11)	45(19.82)	0.93 (0.69, 1.26)
3-6	38(10.73)	0.90 (0.71, 1.14)	27(11.89)	1.31 (0.97, 1.78)
7 Or More	34(9.60)	1.17 (1.01, 1.36)	24(10.57)	0.82 (0.59, 1.14)

Table A.36. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1A1*2A genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

	Wt/Wt		Wt/Var & Var/Var	
Characteristics	**N (%)**	**PR (95%CI)**	**N (%)**	**PR (95%CI)**
Total	305(.)		98(.)	
Age				
35-39	103(33.77)	1.0	34(34.69)	0.66 (0.36, 1.21)
40-44	105(34.43)	1.14 (0.83, 1.57)	31(31.63)	1.61 (0.77, 3.37)
>=45	97(31.80)	1.71 (1.30, 2.26)	33(33.67)	1.59 (0.82, 3.08)
BMI				
Normal	182(59.67)	1.0	55(56.12)	0.92 (0.67, 1.27)
Overweight	71(23.28)	0.92 (0.69, 1.24)	24(24.49)	1.27 (0.73, 2.19)
Obese	52(17.05)	1.22 (0.94, 1.59)	19(19.39)	0.84 (0.45, 1.54)
Smoking Status				
Never Smoked	180(59.02)	1.0	54(55.10)	1.06 (0.78, 1.42)
Past Smoker	106(34.75)	1.08 (0.85, 1.36)	33(33.67)	0.78 (0.46, 1.33)
Current Smoker	19(6.23)	1.29 (0.88, 1.87)	11(11.22)	0.68 (0.31, 1.50)
Oral Contraceptive Use				
Never Used OC	51(16.72)	1.0	18(18.37)	0.94 (0.55, 1.60)
Current/Past User Of OC	254(83.28)	0.96 (0.72, 1.28)	80(81.63)	1.00 (0.55, 1.81)
Number Of Full term Births				
None	201(65.90)	1.0	55(56.12)	0.93 (0.70, 1.23)
1	36(11.80)	0.66 (0.42, 1.04)	21(21.43)	1.38 (0.70, 2.74)
2 or more	68(22.30)	0.82 (0.61, 1.09)	22(19.39)	0.97 (0.51, 1.82)
Number Of Alcoholic Drinks per week				
Less Than 0.5	50(16.39)	1.0	10(10.20)	0.68 (0.25, 1.85)
0.5-2	76(24.92)	1.12 (0.76, 1.65)	38(38.78)	1.45 (0.49, 4.24)
3-6	79(25.90)	1.38 (0.96, 1.97)	23(23.47)	0.95 (0.30, 2.95)
7 Or More	80(26.23)	1.12 (0.76, 1.65)	18(18.37)	1.82 (0.61, 5.38)

Table A.37. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1A1*3 genotype and participant characteristics among African Americans, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	**PR (95% CI)**	**N (%)**	**PR (95% CI)**
Total	498(.)		83(.)	
Age				
35-39	193(38.76)	1.0	26(31.33)	0.96 (0.70, 1.33)
40-44	179(35.94)	1.14 (0.99, 1.31)	27(32.53)	1.21 (0.83, 1.75)
>=45	126(25.30)	1.26 (1.10, 1.45)	30(36.14)	1.24 (0.89, 1.75)
BMI				
Normal	127(25.50)	1.0	20(24.10)	1.29 (1.00, 1.67)
Overweight	150(30.12)	1.17 (0.99, 1.39)	29(34.94)	0.92 (0.67, 1.25)
Obese	221(44.38)	1.24 (1.06, 1.44)	34(40.96)	0.80 (0.58, 1.10)
Smoking Status				
Never Smoked	234(46.99)	1.0	42(50.60)	1.21 (1.03, 1.42)
Past Smoker	116(23.29)	1.11 (0.97, 1.27)	17(20.48)	0.76 (0.53, 1.10)
Current Smoker	144(28.92)	1.03 (0.90, 1.18)	24(28.92)	1.01 (0.79, 1.30)
Oral Contraceptive Use				
Never Used OC	43(8.63)	1.0	5(6.02)	1.38 (0.83, 2.28)
Current/Past User Of OC	455(91.37)	1.25 (0.97, 1.62)	78(93.98)	0.82 (0.49, 1.37)
Number Of Full term Births:				
None	256(51.41)	1.0	39(46.99)	1.17 (1.01, 1.35)
1	154(30.92)	0.93 (0.82, 1.06)	26(31.33)	1.00 (0.77, 1.29)
2 or more	88(17.68)	0.91 (0.78, 1.07)	18(21.69)	0.91 (0.64, 1.29)
Number Of Alcoholic Drink per week:				
Less Than 0.5	248(49.80)	1.0	50(60.24)	1.16 (1.00, 1.34)
0.5-2	105(21.08)	0.90 (0.77, 1.06)	17(20.48)	1.17 (0.90, 1.53)
3-6	60(12.05)	1.05 (0.90, 1.24)	5(6.02)	0.68 (0.32, 1.43)
7 Or More	53(10.64)	1.12 (0.96, 1.30)	5(6.02)	0.86 (0.53, 1.38)

Table A.38. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1A1*2C genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	PR (95% CI)	N (%)	PR (95% CI)
Total	363(.)		39(.)	
Age				
35-39	121(33.3)	1.0	15(38.5)	0.71 (0.30, 1.71)
40-44	121(33.3)	1.22 (0.89, 1.65)	15(38.5)	1.65 (0.60, 4.52)
>=45	121(33.3)	1.91 (1.47, 2.48)	9(23.1)	0.87 (0.28, 2.74)
BMI				
Normal	216(59.5)	1.0	21(53.8)	0.75 (0.43, 1.32)
Overweight	84(23.1)	0.94 (0.73, 1.23)	10(25.6)	1.39 (0.59, 3.31)
Obese	63(17.4)	1.19 (0.94, 1.52)	8(20.5)	0.82 (0.28, 2.41)
Smoking Status				
Never Smoked	212(58.4)	1.0	22(56.4)	0.90 (0.56, 1.46)
Past Smoker	127(35.0)	1.04 (0.84, 1.29)	12(30.8)	0.70 (0.27, 1.81)
Current Smoker	24(6.6)	1.16 (0.81, 1.67)	5(12.8)	0.76 (0.22, 2.57)
Oral Contraceptive Use				
Never Used OC	63(17.4)	1.0	6(15.4)	0.95 (0.41, 2.20)
Current/Past User Of OC	300(82.6)	0.98 (0.75, 1.27)	33(84.6)	0.80 (0.31, 2.07)
Number Of Full term Births:				
None	238(65.6)	1.0	17(43.6)	0.74 (0.41, 1.32)
1	47(12.9)	0.70 (0.48, 1.02)	10(25.6)	1.73 (0.69, 4.36)
2 or more	78(21.5)	0.86 (0.66, 1.12)	12(30.8)	1.26 (0.48, 3.30)
Number Of Alcoholic Drinks per week				
Less Than 0.5	55(15.2)	1.0	5(12.8)	0.46 (0.08, 2.71)
0.5-2	100(27.5)	1.15 (0.80, 1.64)	14(35.9)	1.87 (0.28, 12.37)
3-6	92(25.3)	1.34 (0.95, 1.90)	10(25.6)	1.12 (0.15, 8.47)
7 Or More	92(25.3)	1.18 (0.83, 1.70)	5(12.8)	1.69 (0.21, 13.61)

Table A.39. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1A1*4 genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	PR (95% CI)	N (%)	PR (95% CI)
Total	368(.)		36(.)	
Age				
35-39	125(34.0)	1.0	12(33.3)	1.42 (0.77, 2.62)
40-44	124(33.7)	1.30 (0.96, 1.77)	12(33.3)	0.77 (0.33, 1.81)
>=45	119(32.3)	1.94 (1.48, 2.55)	12(33.3)	0.84 (0.42, 1.67)
BMI				
Normal	217(59.0)	1.0	20(55.6)	1.07 (0.69, 1.68)
Overweight	86(23.4)	0.92 (0.71, 1.21)	9(25.0)	1.60 (0.87, 2.95)
Obese	65(17.7)	1.19 (0.93, 1.53)	7(19.4)	0.91 (0.41, 2.05)
Smoking Status				
Never Smoked	215(58.4)	1.0	19(52.8)	1.06 (0.68, 1.66)
Past/Current Smoker	153(41.6)	1.02 (0.82, 1.25)	17(47.3)	1.29 (0.72, 2.29)
Oral Contraceptive Use				
Never Used OC	64(17.4)	1.0	5(13.9)	0.75 (0.25, 2.25)
Current/Past User Of OC	304(82.6)	0.92 (0.71, 1.20)	31(86.1)	1.71 (0.55, 5.35)
Number Of Full term Births:				
None	236(64.1)	1.0	21(58.3)	1.40 (1.06, 1.86)
1	50(13.6)	0.76 (0.53, 1.09)	7(19.4)	0.75 (0.29, 1.96)
2 or More	82(22.3)	0.84 (0.65, 1.09)	8(22.3)	0.59 (0.23, 1.96)
Number Of Alcoholic Drinks per week:				
Less Than 0.5	56(15.2)	1.0	4(11.1)	0.58 (0.10, 3.27)
0.5-2	103(28.0)	1.10 (0.76, 1.58)	12(33.3)	2.65 (0.45, 15.61)
3-6	93(25.3)	1.24 (0.87, 1.78)	9(25.0)	2.50 (0.43, 14.67)
7 Or More	90(24.5)	1.21 (0.84, 1.74)	8(22.2)	1.66 (0.26, 10.74)

Table A.40. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1B1AS genotype and participant characteristics among African Americans, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	**PR (95% CI)**	**N (%)**	**PR (95% CI)**
Total	152(.)		425(.)	
Age				
35-39	63(41.4)	1.0	155(36.5)	0.92 (0.75, 1.14)
40-44	50(32.9)	1.11 (0.87, 1.40)	153(36.0)	1.08 (0.81, 1.43)
>=45	39(25.7)	1.17 (0.93, 1.48)	117(27.5)	1.16 (0.88, 1.53)
BMI				
Normal	35(23.0)	1.0	110(25.9)	0.87 (0.68, 1.13)
Overweight	48(31.6)	1.07 (0.82, 1.39)	130(30.6)	1.10 (0.80, 1.51)
Obese	69(45.4)	1.00 (0.77, 1.30)	185(43.5)	1.27 (0.93, 1.72)
Smoking Status				
Never Smoked	76(50.0)	1.0	199(46.8)	1.06 (0.89, 1.26)
Past Smoker	31(20.4)	1.08 (0.84, 1.40)	101(23.8)	0.97 (0.72, 1.29)
Current Smoker	43(28.3)	1.17 (0.94, 1.45)	123(28.9)	0.84 (0.65, 1.09)
Oral Contraceptive Use				
Never Used OC	10(6.6)	1.0	37(8.7)	0.85 (0.52, 1.38)
Current/Past User Of OC	142(93.4)	1.05 (0.69, 1.59)	388(91.3)	1.19 (0.73, 1.97)
Number Of Full term Births:				
None	83(54.6)	1.0	212(49.9)	1.11 (0.95, 1.30)
1	48(31.6)	1.10 (0.89, 1.35)	128(30.1)	0.81 (0.63, 1.04)
2 or More	18(11.8)	1.07 (0.80, 1.42)	67(15.8)	0.81 (0.63, 1.04)
Number Of Alcoholic Drinks per week				
Less Than 0.5	66(43.4)	1.0	230(54.1)	0.99 (0.84, 1.16)
0.5-2	32(21.1)	0.83 (0.62, 1.13)	88(20.7)	1.15 (0.82, 1.61)
3-6	22(14.5)	1.09 (0.86, 1.39)	43(10.1)	0.88 (0.64, 1.21)
7 Or More	16(10.5)	0.98 (0.70, 1.37)	42(9.9)	1.15 (0.79, 1.66)

Table A.41 Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1B1AS genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

	Wt/Wt		Wt/Var & Var/Var	
Characteristics	**N (%)**	**PR (95%CI)**	**N (%)**	**PR (95%CI)**
Total	204(.)		198(.)	
Age				
35-39	66(32.4)	1.0	70(35.4)	0.91 (0.58, 1.43)
40-44	72(35.3)	1.11 (0.74, 1.68)	64(32.3)	1.27 (0.72, 2.27)
>=45	66(32.4)	1.84 (1.30, 2.61)	64(32.3)	1.07 (0.65, 1.77)
BMI				
Normal	130(63.7)	1.0	106(53.5)	1.13 (0.87, 1.47)
Overweight	43(21.1)	0.99 (0.69, 1.44)	51(25.8)	0.97 (0.59, 1.59)
Obese	31(15.2)	1.58 (1.20, 2.09)	41(20.7)	0.55 (0.35, 0.88)
Smoking Status				
Never Smoked	117(57.4)	1.0	116(58.6)	1.02 (0.79, 1.32)
Past Smoker	70(34.3)	1.04 (0.77, 1.40)	68(34.3)	0.97 (0.64, 1.47)
Current Smoker	17(8.3)	1.19 (0.77, 1.84)	14(7.1)	0.95 (0.49, 1.84)
Oral Contraceptive Use				
Never Used OC	27(13.2)	1.0	42(21.2)	0.79 (0.51, 1.25)
Current/Past User Of OC	177(86.8)	0.83 (0.58, 1.18)	156(78.8)	1.32 (0.80, 2.17)
Number Of Full term Births:				
None	130(63.7)	1.0	126(63.6)	1.10 (0.88, 1.38)
1	30(14.7)	0.98 (0.66, 1.45)	27(13.6)	0.52 (0.25, 1.06)
2 or More	44(21.5)	0.86 (0.60, 1.24)	45(22.7)	0.89 (0.53, 1.48)
Number Of Alcoholic Drinks per week:				
Less Than 0.5	36(17.6)	1.0	24(12.1)	1.18 (0.65, 2.14)
0.5-2	53(26.0)	1.48 (0.93, 2.38)	61(30.8)	0.64 (0.32, 1.29)
3-6	46(22.5)	1.31 (0.80, 2.17)	56(28.3)	0.98 (0.49, 1.98)
7 Or More	53(26.0)	1.36 (0.84, 2.21)	44(22.2)	0.82 (0.40, 1.67)

Table A.42. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1B1LV genotype and participant characteristics among African Americans, National Institute of Environmental Health Science Uterine Fibroid Study

Characteristics	Wt/Wt		Wt/Var & Var/Var	
	N (%)	PR (95% CI)	N (%)	PR (95% CI)
Total	326(.)		255(.)	
Age				
35-39	124(38.0)	1.0	96(37.6)	0.87 (0.71, 1.07)
40-44	120(36.8)	1.12 (0.95, 1.31)	85(33.3)	1.10 (0.85, 1.44)
>=45	82(25.2)	1.18 (1.01, 1.39)	74(29.0)	1.26 (0.98, 1.61)
BMI				
Normal	87(26.7)	1.0	60(23.5)	0.81 (0.63, 1.05)
Overweight	91(27.9)	1.07 (0.89, 1.28)	88(34.5)	1.24 (0.90, 1.69)
Obese	148(45.4)	1.09 (0.92, 1.28)	107(42.0)	1.27 (0.95, 1.71)
Smoking Status				
Never Smoked	163(50.0)	1.0	114(44.7)	1.05 (0.91, 1.22)
Past Smoker	72(22.1)	1.15 (0.99, 1.34)	61(23.9)	0.83 (0.65, 1.07)
Current Smoker	89(27.3)	1.08 (0.93, 1.27)	78(30.6)	0.88 (0.69, 1.12)
Oral Contraceptive Use				
Never Used OC	20(6.1)	1.0	28(11.0)	1.36 (0.82, 2.25)
Current/Past User Of OC	306(93.9)	1.51 (0.97, 2.35)	227(89.0)	0.71 (0.42, 1.19)
Number Of Full term Births:				
None	160(49.1)	1.0	136(53.3)	1.06 (0.93, 1.21)
1	108(33.1)	1.01 (0.88, 1.17)	71(27.8)	0.82 (0.64, 1.04)
2	48(14.7)	1.01 (0.83, 1.22)	37(14.5)	0.86 (0.63, 1.16)
3 Or More	10(3.1)	0.81 (0.49, 1.36)	11(4.3)	0.86 (0.40, 1.82)
Number Of Alcoholic Drinks per Week				
Less Than 0.5	159(48.8)	1.0	140(54.9)	0.96 (0.84, 1.10)
0.5-2	73(22.4)	0.93 (0.79, 1.11)	48(18.8)	0.94 (0.70, 1.26)
3-6	38(11.7)	1.00 (0.82, 1.22)	27(10.6)	1.02 (0.74, 1.40)
7 Or More	33(10.1)	0.99 (0.80, 1.23)	25(9.8)	1.22 (0.92, 1.62)

Table A.43. Prevalence Ratio (PR) and 95% Confidence interval (CI) for the association between fibroid presence and interactions of CYP1B1LV genotype and participant characteristics among Whites, National Institute of Environmental Health Science Uterine Fibroid Study

	Wt/Wt		Wt/Var & Var/Var	
Characteristics	**N (%)**	**PR (95% CI)**	**N (%)**	**PR (95% CI)**
Total	67(.)		337(.)	
Age				
35-39	20(29.9)	1.0	117(34.7)	1.19 (0.59, 2.40)
40-44	23(34.3)	1.38 (0.61, 3.09)	113(33.5)	0.91 (0.38, 2.16)
>=45	24(35.8)	2.11 (1.03, 4.34)	107(31.8)	0.89 (0.41, 1.92)
BMI				
Normal	41(61.2)	1.0	196(58.2)	0.98 (0.70, 1.39)
Overweight	16(23.9)	0.75 (0.37, 1.52)	79(23.4)	1.37 (0.65, 2.91)
Obese	10(14.9)	1.20 (0.66, 2.17)	62(18.4)	0.98 (0.52, 1.88)
Smoking Status				
Never Smoked	31(46.3)	1.0	203(60.2)	0.96 (0.66, 1.39)
Past Smoker	28(41.8)	0.72 (0.39, 1.31)	111(32.9)	1.52 (0.80, 2.89)
Current Smoker	8(11.9)	1.45 (0.86, 2.46)	23(6.8)	0.73 (0.37, 1.42)
Oral Contraceptive Use				
Never Used OC	15(22.4)	1.0	54(16.0)	1.15 (0.64, 2.10)
Current/Past User Of OC	52(77.6)	1.05 (0.57, 1.93)	283(84.0)	0.90 (0.46, 1.75)
Number Of Full term Births:				
None	41(61.2)	1.0	216(64.1)	1.06 (0.77, 1.46)
1	9(13.4)	0.85 (0.39, 1.86)	48(14.2)	0.86 (0.36, 2.04)
2	12(17.9)	0.95 (0.50, 1.80)	68(20.2)	0.89 (0.44, 1.78)
3 Or More	5(7.5)	0.38 (0.06, 2.25)	5(1.5)	0.94 (0.08, 11.47)
Number Of Alcoholic Drinks per week:				
Less Than 0.5	14(20.9)	1.0	46(13.6)	1.22 (0.56, 2.65)
0.5-2	18(26.9)	1.24 (0.52, 2.98)	97(28.8)	0.93 (0.36, 2.42)
3-6	16(23.9)	1.75 (0.79, 3.89)	86(25.5)	0.71 (0.29, 1.73)
7 Or More	16(23.9)	1.49 (0.64, 3.48)	82(24.3)	0.79 (0.31, 2.01)

Figure A.1. Catechol Estrogen Metabolism

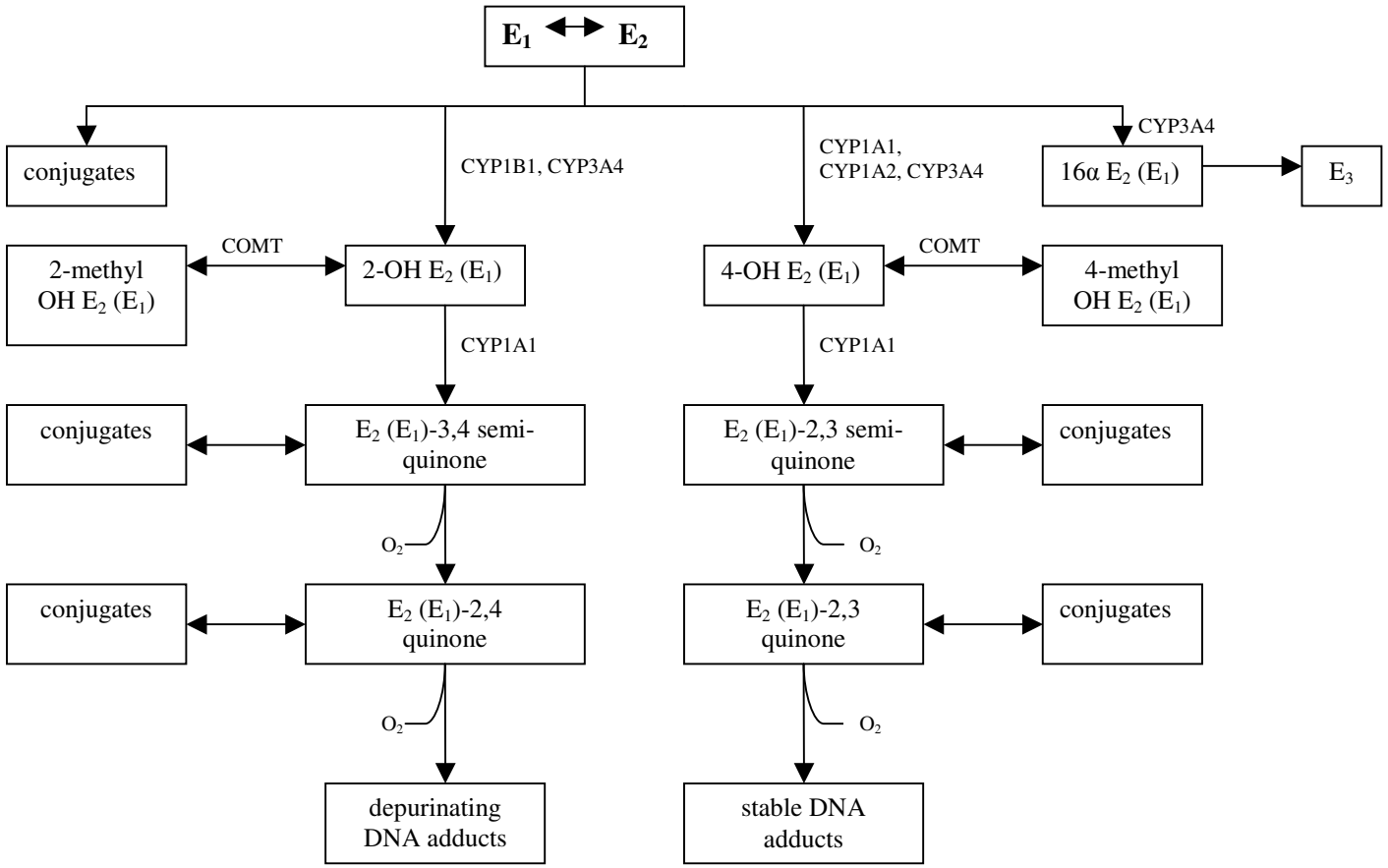

Figure A.2. Conceptual Model

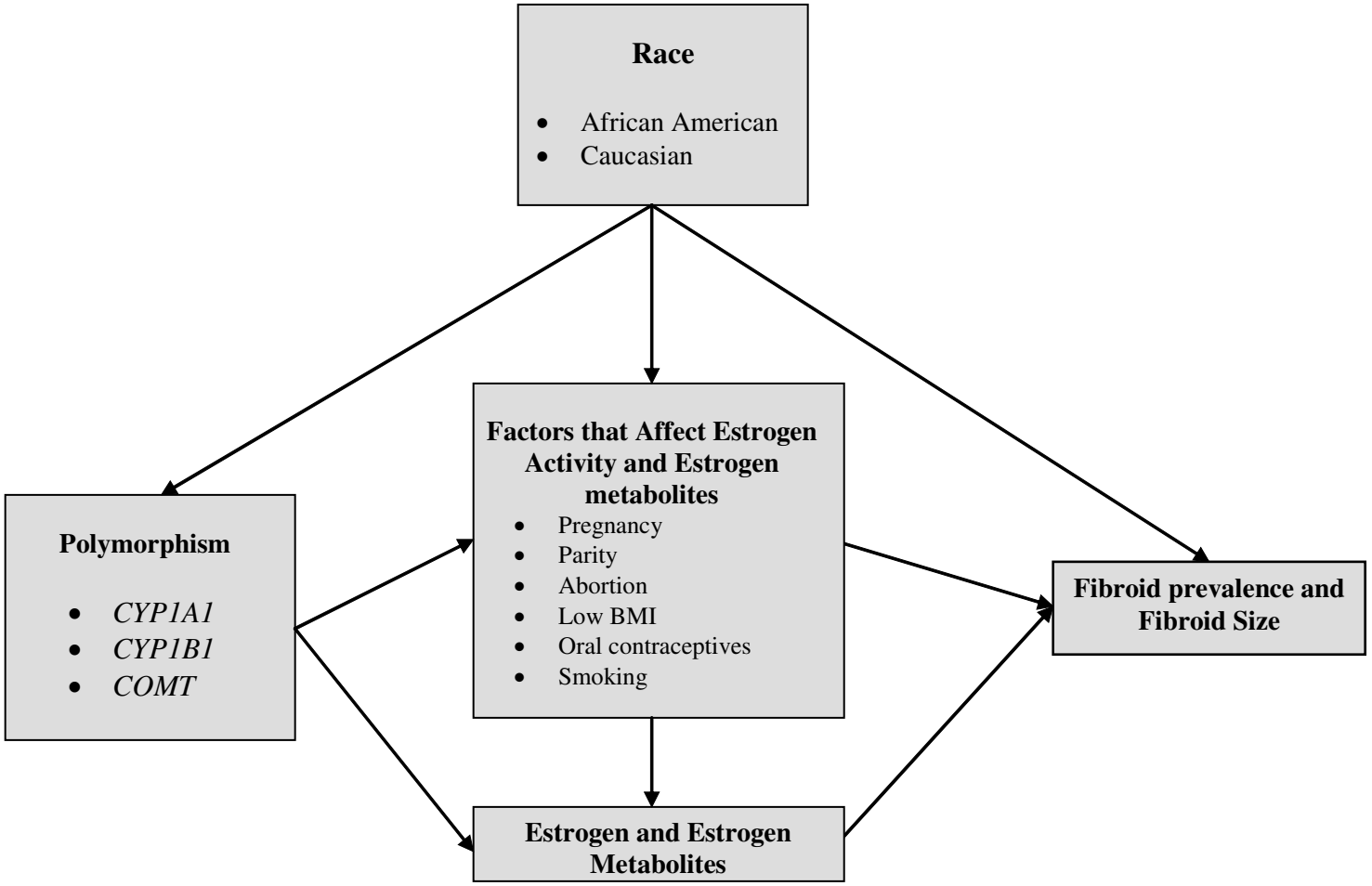

Figure A.3 . Location of *Val158Met* polymorphism on the membrame bound form of the *COMT* gene. The shaded boxes represent the exons.

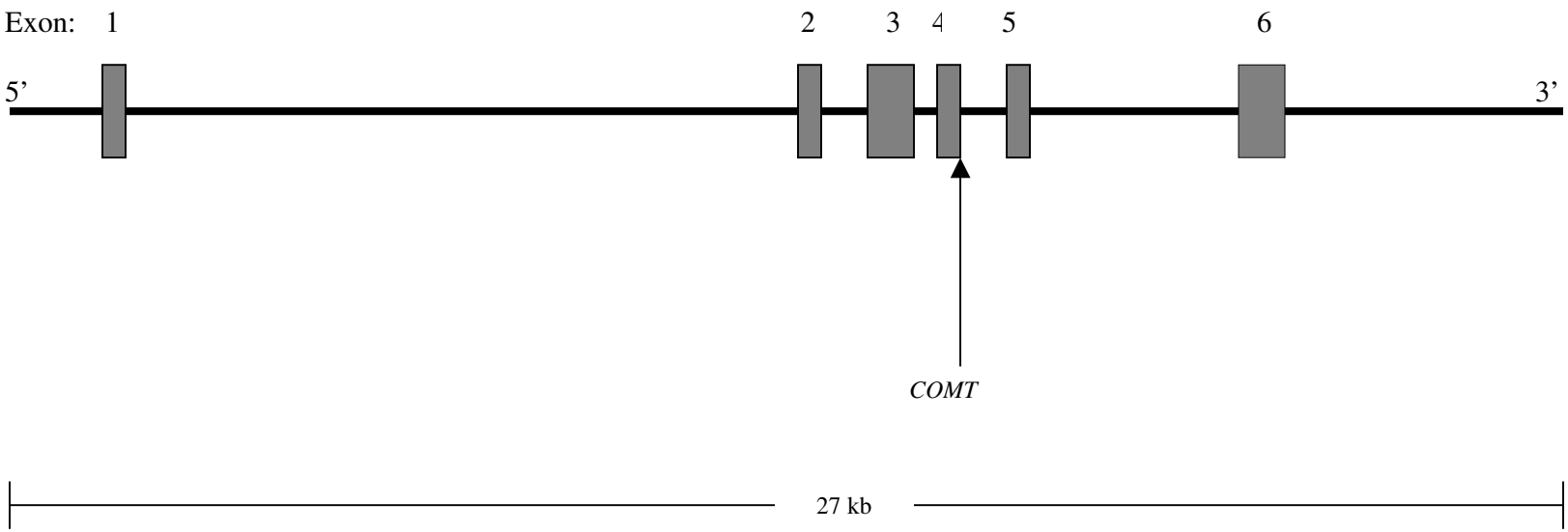

Figure A.4. Location of *CYP1A1*2A, CYP1A1*2C, CYP1A1*3*, and *CYP1A1*4* on the *CYP1A1* gene. The shaded boxes represent the exons.

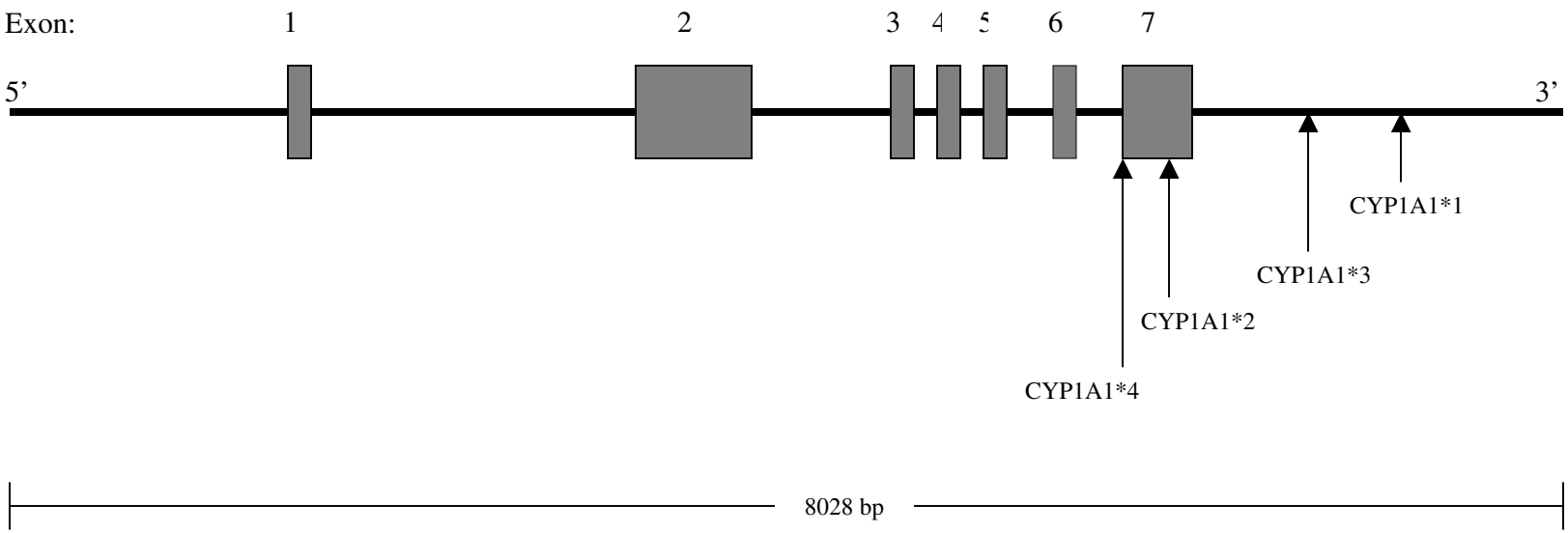

Figure A.5. Location of *CYP1B1AS* and *CYP1B1LV* on the *CYP1B1* gene. The shaded boxes represent the exons.