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Genetic variation in *CYP11A1* and *StAR* in relation to endometrial cancer risk

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Abstract

Objective—Together, steroidogenic acute regulator (*StAR*) and the cholesterol side chain cleavage enzyme (P450_{scc}), which is encoded by *CYP11A1*, mediate the initial and rate-limiting step in steroidogenesis. Given the role of estrogens in endometrial carcinogenesis, we hypothesized that genetic variation in *StAR* and *CYP11A1* genes may influence endometrial cancer risk.

Methods—We genotyped four *CYP11A1* tagging single nucleotide polymorphisms (SNPs) and two *StAR* SNPs in endometrial cancer case-control studies nested within the Nurses' Health Study (553 cases, 1,339 controls) and the Women's Health Study (137 cases, 411 controls). We calculated odds ratios and 95% confidence intervals using conditional and unconditional logistic regression adjusted for endometrial cancer risk factors to examine the association between SNPs/haplotypes and endometrial cancer.

Results—We observed an increased risk for women carrying the variant allele for rs4555110 (odds ratio (OR)=1.3, 95% confidence interval (CI)= 1.1–1.7), rs3825944 (OR=1.4, 95% CI=1.1–1.8), and rs7173655 (OR=1.3, 95% CI=1.0–1.7) *CYP11A1* SNPs but no significant associations with *CYP11A1* haplotypes. *CYP11A1* SNPs were not predictive of plasma estradiol levels. We observed no associations between *StAR* SNPs and endometrial cancer risk.

Conclusions—Genetic variants in *CYP11A1* may influence endometrial cancer risk or may be markers for causal variants elsewhere. Polymorphisms in *StAR* are not associated with endometrial cancer risk but further research is needed.

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Keywords

endometrial cancer; polymorphism; *CYP11A1*; *StAR*

INTRODUCTION

Prolonged exposure to estrogens unopposed by progesterone plays an important role in the etiology of endometrial cancer [1,2]. Risk factors related to an increased exposure to estrogen such as early age at menarche, late age at menopause [3], use of unopposed postmenopausal estrogen [4], and obesity [5] are associated with increased endometrial cancer risk. Both the proliferative effects of estrogen and the oxidative damage of estrogen metabolites may contribute to endometrial cancer risk [6–10].

Inherited susceptibility to endometrial cancer includes the familial syndrome hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome, which is characterized by highly penetrant mutations in DNA mismatch repair genes (*MSH2*, *MLH1*, *PMS1*, and *PM2*) and a family history of colon cancer [11]. Population-based studies have demonstrated that endometrial cancer in a first-degree relative increases individual risk by 1.5–3 fold [12,13] while familial syndromes account for only 2–5% of endometrial cancer cases, suggesting that common genetic variation may be important to risk [14]. Genes in steroidogenic pathways have received attention as potential candidates in inherited susceptibility (reviewed in [14]). In this study we address the potential role of polymorphisms in two genes that regulate the initiation of steroid biosynthesis.

Together, steroidogenic acute regulator (*StAR*) and the cholesterol side chain cleavage enzyme (*P450scc*), which is encoded by *CYP11A1*, mediate the initial and rate-limiting step in steroidogenesis. *StAR* facilitates the movement of cholesterol into the inner mitochondrial membrane where *P450scc* cleaves the cholesterol side chain, converting cholesterol to pregnenolone, the precursor of androgens, estrogens, and progesterone. *P450scc* can only function in the inner mitochondrial membrane; thus the synergy between these enzymes is critical for successful steroidogenesis.

Mice lacking either of these genes are steroid deficient and accumulate excess lipids [15,16]. *StAR* knockout female mice have a sexually immature uterus and oviducts, suggesting impaired estrogen action. In addition, hormone assays in these mice revealed a marked decrease in the level of circulating progesterone, suggesting that decreased steroidogenesis in these mice impairs ovulation [15]. In humans, chronic anovulation is associated with increased endometrial cancer risk [17,18]. *CYP11A1*-null mice have a phenotype very similar to that of *StAR*-null mice [16]. Furthermore, in humans *StAR* or *P450scc* deficiency results in severely impaired adrenal and gonadal steroidogenesis as well as lipid deposits in these tissues [19–21]. The pentanucleotide repeat in the 5' regulatory region of *CYP11A1* has been shown to be associated with ovarian androgen excess and polycystic ovary syndrome (PCOS) [22–25], a common endocrine disorder that increases a woman's risk of endometrial cancer.

Hyperandrogenism can contribute to and alter the concentrations of unopposed estrogens. Both *StAR* and *CYP11A1* are expressed in endometrial cancer cells [26], suggesting that local steroid hormone production could be influenced by *StAR* or *CYP11A1* alterations. Together, *StAR*/*CYP11A1* expression and activities affect the rate of steroidogenesis, as they are the initial and rate-limiting steps. Therefore, common genetic differences in *StAR* and *CYP11A1* between individuals may contribute to the overall balance of bioavailable estradiol and hence endometrial cancer risk.

Among Chinese, the *CYP11A1* pentanucleotide repeat has been associated with breast cancer [27–29], a disease in which high estradiol levels are associated with risk [2]. However, the association between the *CYP11A1* repeat and breast cancer was not observed in other ethnic groups [30]. To date, one study has evaluated the pentanucleotide repeat in relation to endometrial cancer risk in 417 cases and 402 controls, but no association was observed [31]. Using a haplotype approach, Setiawan and colleagues evaluated common genetic variation across the *CYP11A1* gene in relation to breast cancer risk. They observed global differences in haplotype frequencies between 1,615 cases and 1,962 controls from the Multiethnic Cohort Study [30]. In our study, we have used the same approach to evaluate *CYP11A1* haplotypes and endometrial cancer risk.

To date, genetic variation in the *StAR* gene has not been evaluated in relation to endometrial cancer risk. Data from a recent genome-wide association study suggests an association between *StAR* SNP rs6474491 and age at menopause ($p=0.0075$) [32], a risk factor for endometrial cancer. We evaluated common genetic variation in these *CYP11A1* and *StAR* genes in 690 endometrial cancer cases and 1,750 matched controls from the Nurses' Health Study (NHS) and Women's Health Study (WHS).

METHODS

Nurses' Health Study

Study population—The Nurses' Health Study (NHS) began in 1976 when 121,701 female registered nurses between the ages of 30 and 55 completed a self-administered questionnaire. Information regarding endometrial cancer risk factors was obtained from questionnaires completed every two years and at the time of blood collection. During 1989 and 1990, blood samples were collected from 32,826 women. Postmenopausal hormone (PMH) use was assessed by questionnaire at blood draw. Between 2001 and 2004, cheek cell samples were collected by the “swish-and-spit” method [33] from 29,684 women who had not provided a blood sample.

Case-control study—We included cases from the NHS blood cohort with pathologically confirmed invasive endometrial cancer and no previously diagnosed cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with blood or cheek cell samples and had neither a hysterectomy nor diagnosed cancer (except nonmelanoma skin cancer). Cases diagnosed before June 1, 1998 who gave blood were matched to three controls, while cases diagnosed after June 1, 1998 who gave blood and all cases who gave cheek cells were matched to two controls. Matching factors included year of birth, menopausal status at specimen collection and at the cycle prior to diagnosis, and postmenopausal hormone use at time of specimen collection (current vs. not current users). For cases who gave blood, controls were also matched by time of day of blood collection, month of blood return, and fasting status at blood draw. For cases who gave cheek cells, controls were also matched on month and year of cheek cell collection. This case-control study consists of 553 endometrial cancer cases and 1,339 matched controls, through June 1, 2004. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

Controls for plasma analysis—In a previous study of breast cancer risk, estradiol, estrone, estrone sulfate, and progesterone were measured in 643 postmenopausal women with no history of cancer (except non-melanoma skin cancer) and no prior PMH use in the last three months [34]. Women were considered postmenopausal at the time of natural menopause, bilateral oophorectomy, or ages 56 (if non-smoker) and 54 (if smoker) for women who reported having a hysterectomy with either one or both ovaries remaining. For our analysis we restricted

to women at risk for endometrial cancer (intact uterus) and genotype data for the *CYP11A1* SNPs (n=471).

Women's Health Study

Study population—Begun in April 1993, the Women's Health Study (WHS) is a completed randomized, double-blind, placebo-controlled trial investigating the benefits and risks of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease among 39,876 female health professionals, aged 45 years or older without a history of cancer (except nonmelanoma skin cancer), coronary heart disease, or cerebrovascular disease [35,36]. Written informed consent was obtained. A detailed description of the participants has been published previously [37]. At the time of randomization, blood samples were obtained from 28,345 women (71%).

Upon enrollment, all participants completed a detailed questionnaire including known or potential risk factors for endometrial cancer. Every six months for the first year and annually thereafter, participants were sent follow-up questionnaires. Women were asked to report new diagnoses of major illnesses on their follow-up questionnaires; some women also provided this information through letters, or telephone calls. An Endpoints Committee of physicians blinded to treatment reviewed records to confirm diagnoses. The trial was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Case-control study—Eligible WHS incident cases were women with pathologically confirmed invasive endometrial cancer diagnosed after blood collection (1993–1995) and before June 1, 2002, with no previously diagnosed cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with a blood sample but with neither hysterectomy nor diagnosed cancer (except nonmelanoma skin cancer) at the time of blood draw. Controls were 3:1 matched to cases according to age at randomization, menopausal status, and postmenopausal hormone use at blood draw (current vs. not current users). Controls were also matched to cases by date of blood return and fasting status at blood draw. This case-control study consists of 137 incident endometrial cancer cases and 411 matched controls.

Genotyping

A parsimonious set of tagging SNPs in *CYP11A1* were identified by the Breast and Prostate Cohort Consortium; their methods have been described previously [30]. Briefly, SNPs spanning 67 kb of the *CYP11A1* gene were identified through public databases and resequencing of 95 advanced breast cancer cases. Sixty-three SNPs with greater than 5% frequency were genotyped in a multiethnic panel of 349 women from the Multiethnic Cohort Study, and haplotypes were estimated with the expectation-maximization algorithm [38] for each ethnicity. A parsimonious set of tagging SNPs were chosen for each ethnic group ($R^2_h > 0.7$). Four tagging SNPs (rs4555110, rs3825944, rs34578565, rs7173655) were identified in one haplotype block for Caucasians (<http://ccnt.hsc.usc.edu/Core/DocManager>).

Based on HapMap data, which contains the most complete SNP data on *StAR* to our knowledge, *StAR* SNPs are largely monomorphic in Caucasians except for rs6474491, rs7823144, and SNPs that are in complete linkage disequilibrium with these two SNPs (www.hapmap.org).

In the Nurses' Health Study and Women's Health Study, DNA was extracted from buffy coat and cheek cell samples with the QIAGEN QIAamp Blood Kit (QIAGEN, Inc., Chatsworth, CA). DNA extracted from NHS blood samples was whole-genome amplified with GE healthcare Genomiphi (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). We have previously demonstrated that this method is robust for SNP genotyping [39]. Allelic discrimination was determined on the ABI 7900HT. Primers and conditions are available upon

request. Replicate samples (5% of sample size) were included for quality control. Laboratory personnel were blinded to the location of quality-control replicates and case/control status. Concordance of quality-control replicates was 100%.

Plasma hormone measurements

Estradiol and estrone were assayed from blood plasma at Quest Diagnostic's Nichols Institute (San Juan Capistrano, CA). The first batch of estrone sulfate was assayed at University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory (Worcester) and the remaining batches were assayed at the Nichol's Institute along with progesterone. Details of the hormone assays have been described previously [40]. Briefly, samples were extracted with a mixture of hexane and ethyl acetate and applied to celite column, steroids were eluted from the column, and fractions were subjected to radioimmunoassay. To quantify estrone sulfate levels, estrone was first extracted from the plasma, and then the estrone sulfate bond was enzymatically cleaved to release estrone, which was then extracted from the plasma by an organic solvent and was subjected to chromatography and then radioimmunoassay. To assess laboratory precision, blinded replicates (10% of samples) were randomly included in the sample set. Within batch laboratory coefficients ranged from 6–15%.

Statistical analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by conditional logistic regression to assess the association of *CYP11A1* and *StAR* genotypes with endometrial cancer risk. We evaluated a co-dominant model, a dominant model, and a trend in risk. Since we observed significant differences in allele frequency by ethnicity, all analyses were restricted to Caucasians.

Haplotype frequencies were estimated in cases and controls together by the expectation-maximization (E-M) algorithm [38]. Posterior probabilities of the haplotypes given the observed genotypes were calculated for each individual as previously described by Zaykin and colleagues [41]. Haplotypes with less than 5% frequency were grouped together. Global tests of association between common haplotype patterns and endometrial cancer risk were assessed using a likelihood ratio test to compare a model with all haplotypes in a particular block to a model with covariates only. To evaluate haplotype-disease associations, odds ratios and 95% CIs were calculated by conditional logistic regression adjusted for the matching factors, assuming an additive model of haplotype inheritance.

Multivariate models were adjusted for age at menarche, parity and age at first birth, first-degree family history of colon cancer, smoking status at diagnosis, body mass index at diagnosis, age at menopause, and menopausal status at diagnosis. We used a test for heterogeneity to determine whether results from the two cohorts differed significantly. After observing no significant differences, we combined results from the two studies using the DerSimonian and Laird random effects model. All analyses were performed with SAS v 9.1 (SAS Institute, Cary, NC).

For plasma analyses, we used the extreme Studentized deviate Many-Outlier procedure[42] to identify and exclude outliers in each laboratory set. We used linear regression adjusted for age and laboratory batch to evaluate the association between *CYP11A1* genotypes and log-transformed hormone levels.

RESULTS

We genotyped four *CYP11A1* and two *StAR* SNPs in 553 cases and 1,339 controls from the NHS and 137 cases and 411 controls from WHS. Characteristics of these populations have

been described previously [43]. Briefly, matching factors were distributed similarly in cases and controls. In both studies, cases were more likely than controls to be younger at menarche, to be older at menopause, and to have taken postmenopausal hormones [43].

Analyses were restricted to Caucasians (547 cases and 1315 controls for NHS; 130 cases and 391 controls for WHS) due to significant differences in genotype frequency by ethnicity. Furthermore, we did not include genotype data from cheek cells in the analysis because rs6474491 and rs7823144 in *StAR* and rs34578565 in *CYP11A1* were significantly out of HWE ($p \leq 0.01$) for these samples.

Women carrying the variant allele (heterozygous or homozygous variant genotypes) for *CYP11A1* SNPs rs4555110, rs3825944, or rs7173655 had a 30–40% greater risk of endometrial cancer than women with two wild-type alleles for these SNPs (Table 1). Results remained unchanged when we restricted to endometrioid cases (data not shown). We observed no significant associations between *StAR* SNPs and endometrial cancer risk (Table 1).

We observed three common *CYP11A1* haplotypes, accounting for over 93% of the alleles in this population. Two haplotypes (AGCT and AACC) were associated with non-significant increases in endometrial cancer risk (Table 2).

Among a group of NHS controls ($n=471$), *CYP11A1* genotypes were not predictive of plasma estradiol, estrone, estrone sulfate, or progesterone levels.

DISCUSSION

This is the first study to evaluate genetic variation in *StAR* and tagging SNPs in *CYP11A1* in relation to endometrial cancer risk. In our study, three *CYP11A1* tagging SNPs were significantly associated with increased endometrial cancer risk. However, *CYP11A1* haplotypes, the linear combination of these SNPs, were not significantly associated with risk. The lack of an association for *CYP11A1* haplotypes and endometrial cancer risk is likely due to the increase variability introduced by the estimation of haplotypes.

For the *StAR* gene, we observed no associations with endometrial cancer risk. However, the *StAR* associations we observed were weak (ORs= 1.1 and 0.8), and we did not have adequate power to test for significance of these weak associations.

Olson and colleagues evaluated the *CYP11A1* pentanucleotide repeat in relation to endometrial cancer risk in the Estrogen, Diet, Genetics, and Endometrial Cancer study, a population-based case-control study with 819 participants from northern New Jersey who donated buccal specimens, and observed no association [31]. Several studies reported no association between the pentanucleotide repeat and plasma hormone levels (progesterones, androgens, and estrogens), suggesting that this polymorphism may not be functional at least with respect to changes in hormone levels [22,23,44–46]. However, genetic variation in other parts of the gene could play an important role in *CYP11A1* function.

Setiawan and colleagues took a comprehensive approach by evaluating genetic variation across the *CYP11A1* gene in relation to breast cancer risk in a multiethnic population [30]. They genotyped 12 tagging SNPs across three haplotype blocks to capture the genetic variation in *CYP11A1* in multiethnic populations. They reported a significantly increased risk of breast cancer with a haplotype in block 2, which is not found in Caucasians and therefore not assessed in our study. In addition, they observed an increased risk with a haplotype in block 1 that is found in less than 5% of our population. Although we did not test this block-1 haplotype independently due to limited power, we did observe an increased risk of endometrial cancer

for all rare haplotypes combined. Additional research with a larger sample size will be needed to determine whether this haplotype is relevant to endometrial cancer risk.

CYP11A1 is responsible for initiating biosynthesis of steroid hormones such as estrogen, and endometrial cancer is an estrogen-related disease [2]. Therefore, genetic variation in *CYP11A1* could plausibly influence endometrial cancer risk. We observed an increased risk with the variant allele of three *CYP11A1* SNPs (rs4555110, rs3825944, and rs7173655), suggesting that *CYP11A1* may play a role in endometrial cancer risk. These associations need to be validated in other Caucasian populations. Although these SNPs may directly influence *CYP11A1* function, they may simply be markers for the true causal variant.

Our *a priori* hypothesis was that these variants may influence endometrial cancer risk by altering estrogen biosynthesis, yet we observed no association between these SNPs and plasma estrogen levels in women without cancer. Consequently, these variants may influence endometrial cancer risk through another pathway. Alternatively, a single plasma estrogen measurement may not be a sensitive enough measure of lifetime estrogen exposure to capture relative differences between women with and without these variants.

Interestingly, the *StAR* gene has little genetic variation, suggesting that it has been evolutionarily conserved, perhaps due to its importance in the hormone biosynthesis pathway. We observed no association between *StAR* SNPs and endometrial cancer risk, although we had insufficient power to test the significance of these associations.

Study strengths include our homogeneous population (which minimizes confounding by race), nested case-control design, prospectively collected data on potential confounders, and comprehensive disease follow-up. Additional studies in non-Caucasians are needed to evaluate the role of *CYP11A1* and *StAR* in endometrial cancer risk for minority populations.

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Table 1

Association between *CYP11A1* and *StAR* polymorphisms and endometrial cancer risk, Nurses' Health Study and Women's Health Study

Gene	SNP	Minor Allele	Minor Allele Frequency	Pooled OR (95% CI) ^a
<i>CYP11A1</i>	rs4555110	A	0.13	1.33 (1.05, 1.68)
<i>CYP11A1</i>	rs3825944 ^b	A	0.14	1.39 (1.10, 1.75)
<i>CYP11A1</i>	rs34578565	C	0.37	1.17 (0.89, 1.54)
<i>CYP11A1</i>	rs7173655	C	0.30	1.31 (1.02, 1.70)
<i>StAR</i>	rs6474491 ^b	A	0.25	1.10 (0.71, 1.72)
<i>StAR</i>	rs7823144 ^b	A	0.07	0.82 (0.34, 1.96)

^aOdds ratio and 95% confidence interval for carrying at least one copy of the variant allele adjusted for matching factors plus age at menarche, parity and age at first birth, family history of colon cancer, body mass index at diagnosis, smoking status at diagnosis, postmenopausal hormone use at diagnosis, age at menopause at diagnosis, oral contraceptive use at blood draw

^bNHS cheek samples were excluded for this SNP because genotyping results were out of Hardy-Weinberg Equilibrium

Table 2*CYP11A1* haplotypes and endometrial cancer risk, Nurses' Health Study and Women's Health Study

<i>CYP11A1</i> Haplotypes	Pooled OR (95% CI) ^a
<i>GGGT</i>	1.00
<i>AGCT</i>	1.24 (0.57, 2.72)
<i>AACC</i>	1.67 (0.74, 3.79)

^a Adjusted for matching factors plus age at menarche, parity and age at first birth, family history of colon cancer, body mass index at diagnosis, smoking status at diagnosis, postmenopausal hormone use at diagnosis, age at menopause at diagnosis, oral contraceptive use at blood draw