Gynecologic Oncology 121 (2011) 54–58

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Polymorphisms in genes hydroxysteroid-dehydrogenase-17b type 2 and type 4 and endometrial cancer risk

Stalo Karageorgi [a,b,*], Monica McGrath [c,d], I-Min Lee [c,e], Julie Buring [c,e], Peter Kraft [b,c], Immaculata De Vivo [b,c,d]

[a] Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA
[b] Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA
[c] Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA
[d] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
[e] Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 22 August 2010
Available online 3 December 2010

Keywords:
Endometrial carcinoma
Polymorphisms
HSD17b2
HSD17b4

ABSTRACT

Objective. Hydroxysteroid-dehydrogenase-17b (*HSD17b*) genes control the last step in estrogen biosynthesis. The isoenzymes HSD17b2 and HSD17b4 in the uterus preferentially catalyze the conversion of estradiol, the most potent and active form of estrogen, to estrone, the inactive form of estrogen. Endometrial adenocarcinoma is linked to excessive exposure to estrogens. We hypothesized that single nucleotide polymorphisms (SNPs) in genes *HSD17b2* and *HSD17b4* may alter the enzyme activity, estradiol levels and risk of disease.

Methods. Pairwise tag SNPs were selected from the HapMap Caucasian database to capture all known common (minor allele frequency >0.05) genetic variation with a correlation of at least 0.80. Forty-eight SNPs were genotyped in the case-control studies nested within the Nurses' Health Study (NHS) (cases = 544, controls = 1296) and the Women's Health Study (WHS) (cases = 130, controls = 389). The associations with endometrial cancer were examined using conditional logistic regression to estimate odds ratio and 95% confidence intervals adjusted for known risk factors. Results from the two studies were using fixed effects models. We additionally investigated whether SNPs are predictive of plasma estradiol and estrone levels in the NHS using linear regression.

Results. Four intronic SNPs were significantly associated with endometrial cancer risk (p-value<0.05). After adjustment for multiple testing, we did not observe any significant associations between SNPs and endometrial cancer risk or plasma hormone levels.

Conclusions. This is the first study to comprehensively evaluate variation in *HSD17b2* and *HSD17b4* in relation to endometrial cancer risk. Our findings suggest that variation in *HSD17b2* and *HSD17b4* does not substantially influence the risk of endometrial cancer in Caucasians.

Published by Elsevier Inc.

Introduction

Hydroxysteroid dehydrogenase 17b (*HSD17b*) genes are involved in the synthesis and metabolism of sex steroid hormones. There are at least eleven human HSD17b isoenzymes expressed in a variety of tissues such as the ovary, placenta, uterus, liver, adipose tissue, prostate and testis [1]. *HSD17b* Type 2 (*HSD17b2*) and *HSD17b* Type 4 (*HSD17b4*) are expressed in the uterus [2,3] and control the last step in the formation of estrogens. These enzymes preferentially catalyze the conversion of estradiol (E2), the most potent and active form of estrogen, to estrone (E1), the inactive form of estrogen [4]. *HSD17b2* and *HSD17b4* are located on two different chromosomes, chromosome 16 and 5, respectively, span 63 kb and 90 kb each, and are composed of 5 and 24 exons, respectively. HSD17b2 and HSD17b4 function as intracrine regulators modulating local estrogen levels [5].

Endometrial cancer is a disease linked to prolonged exposure to estrogen unopposed by progesterone. This mechanism is supported by studies demonstrating an association of reproductive factors and exogenous hormone use (oral contraceptives, postmenopausal hormones) with endometrial cancer [6]. Family history of endometrial cancer has also been associated with sporadic endometrial cancer [7], suggesting genetic variability may play a role in the development of endometrial cancer. We hypothesized that genetic variation in the form of single nucleotide polymorphisms (SNPs) in estrogen metabolism genes *HSD17b2* and *HSD17b4* may influence enzyme activity, estradiol levels and risk of disease. A number of genes along the estrogen biosynthesis pathway have been previously examined in relation to endometrial cancer, however, no previous studies have

* Corresponding author. Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA. Fax: +1 617 432 1722.
E-mail address: skarageo@hsph.harvard.edu (S. Karageorgi).

0090-8258/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.ygyno.2010.11.014

examined *HSD17b2* and *HSD17b4* [8]. In addition, only a small number of SNPs in these two genes have been investigated in relation to other health outcomes [9–15]. We used the Nurses' Health Study (NHS) and Women's Health Study (WHS) nested case-control studies to comprehensively evaluate SNP variation in genes *HSD17b2* and *HSD17b4* and their association with endometrial cancer risk and circulating hormone levels.

Methods

Study populations

The NHS case-control study of endometrial cancer is nested within the NHS prospective cohort study established in 1976 when 121,700 married female nurses aged between 30 and 55 years and residing in 11 US states, agreed to participate in the study. The nurses were followed every two years by completing a self-administered mailed questionnaire with detailed information on lifestyle factors and disease status [6]. In 1989–1990, 32,826 women completed a blood questionnaire and provided a blood sample. In 2000–2002, 33,040 women who had not provided a blood sample completed a buccal cell questionnaire and provided a buccal cell sample. Cases in this study consisted of women who provided a blood or buccal cell sample were diagnosed with invasive endometrial cancer between 1976 through June 1, 2004 and were confirmed by medical records. Controls were randomly selected participants from the cohort who provided a blood or buccal cell sample, and had no previous report of hysterectomy and cancer through the questionnaire cycle in which the case was diagnosed. Controls were matched to cases, using a 2:1 or 3:1 ratio, on age, menopausal status at specimen collection and prior to diagnosis, postmenopausal hormone use at specimen collection, date of specimen collection, type of biospecimen, and fasting status at blood draw.

The WHS case-control study of endometrial cancer is nested within the completed WHS randomized clinical trial that examined the use of low-dose aspirin and vitamin E for the primary prevention of cancer and cardiovascular disease. The trial consists of 39,876 female health professionals across the US, aged 45 years or older when randomization began in April 1993 [16,17]. Upon randomization, every six months for the first year, and annually thereafter, participants completed a detailed questionnaire that provided information on known or potential risk factors for endometrial cancer [18]. From 1993–1995, 28,345 women in the WHS provided blood samples. The cases in this analysis included women who provided a blood sample and were diagnosed with endometrial cancer from blood collection through June 1, 2002. Only cases confirmed by a pathologist were considered. Controls were randomly selected from the rest of the participants who provided a blood sample, and had no previous report of cancer and hysterectomy. Controls were matched to cases, using a 3:1 ratio, on age, menopausal status and postmenopausal hormone use at specimen collection, date of specimen collection and fasting status at blood draw.

SNP selection

SNPs were selected using data from the HapMap population sample with European ancestry (CEU) (phase I + II release 24). SNP data were downloaded from a region including the gene and 20 kb upstream and 10 kb downstream of the gene, in order to capture 5′ and 3′ regulatory regions. We excluded SNPs with Minor Allele Frequency (MAF) <0.05. Following exclusions, a total of 71 SNPs were listed in HapMap in gene *HSD17b2* and a total of 87 SNPs in gene *HSD17b4*. All SNPs within the genes were in introns except 3 non-synonymous SNPs in *HSD17b4*. One SNP was in the 5′ untranslated region (UTR) of *HSD17b2*. Using the software Haploview and taking advantage of the linkage disequilibrium structure between SNPs, we randomly selected pairwise tag SNPs correlated with a minimum r^2 of 0.80 with all remaining SNPs in the regions of interest. We forced certain SNPs to be chosen as tag SNPs, for comparability of results between studies and because of possible functional significance. These included tag SNPs identified by the Breast and Prostate Cohort Consortium (personal communication), non-synonymous SNPs ($n = 3$), SNPs in regulatory regions ($n = 1$), and SNPs previously reported to be associated with other diseases ($n = 2$ of which one SNP overlapped with an exonic SNP) [14,15].

Power calculations were estimated using the program Quanto. The sample size of ~700 case-control pairs in this study provided more that 82% power to detect a minimum log-additive odds ratio of 1.3 for an allele with 20% or higher frequency at the 0.05 level.

Genotyping

DNA was extracted from leukocyte cell and buccal cell samples with the QIAGEN-QIAamp 96 DNA Blood Kit. The SNPs were genotyped using Taqman assay on Biotrove OpenArray® Real-Time qPCR system (Woburn, MA). Laboratory personnel were blinded to case status, and a random 5% of the samples were repeated for genotyping quality control. The concordance for the duplicate samples was 100%. Missing data was less than 10%. Both DNA extraction and genotyping were performed at the Harvard Partners Genotyping Facility at the Dana–Farber/Harvard Cancer Center High Throughput Genotyping Core.

Plasma hormone level measurements

In a previous study of breast cancer risk in the NHS, estradiol and estrone plasma levels were measured in 643 postmenopausal control women with no history of cancer (except non-melanoma skin cancer) and no prior PMH use in the last three months [19]. A subset of our tag SNPs ($n = 23/48$; RS2042429, RS4445895, RS2955163, RS996752, RS2955162 in *HSD17b2* and RS25640, RS32646, RS382719, RS442923, RS463513, RS2455466, RS2457221, RS2636961, RS2678070, RS3797371, RS3850201, RS6897978, RS10064000, RS10478424, RS11748477, RS11749784, RS12653702, RS17388769 in *HSD17b4*) was previously genotyped in these controls (unpublished data). For our analysis we restricted to women at risk for endometrial cancer (intact uterus) ($n = 471$) and who had genotype data available for *HSD17b2* and *HSD17b4* SNPs.

Statistical analysis

Genotype frequencies among controls were tested for Hardy–Weinberg equilibrium using a chi-square test. The success rate (SR) for each SNP was calculated as the percent of successfully genotyped samples for each SNP. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between *HSD17b2* and *HSD17b4* SNP genotypes and endometrial cancer risk. We used the additive model modeling the number of minor alleles as a continuous variable. Individuals homozygous for the more common allele were coded as having zero copies of the minor allele, individuals heterozygous as having one copy, and individuals homozygous for the rare allele as having two copies. We conducted analysis adjusted for the matching factors only and separate analysis additionally adjusted for age at menarche, parity and age at first birth, body mass index at diagnosis (BMI; kg/m^2), smoking status at diagnosis, postmenopausal hormone use at diagnosis, age at menopause at diagnosis, and ever oral contraceptive use. We used the fixed effects model to combine results from the two cohorts after testing for heterogeneity. We adjusted for multiple testing by controlling the false discovery rate [20]. All analyses were restricted to Caucasians (98%). We used linear regression adjusted for age and laboratory batch to evaluate the association between the number of