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Endometrial cancer and genetic variation in *PTEN*, *PIK3CA*, *AKT1*, *MLH1*, and *MSH2* within a population-based case-control study

James V. Lacey Jr, Ph.D.^{a,1,*}, Hannah Yang, Ph.D.^a, Mia M. Gaudet, Ph.D.^{a,b}, Alison Dunning, Ph.D.^c, Jolanta Lissowska, Ph.D.^{a,d}, Mark E. Sherman, M.D.^a, Beata Peplonska, M.D.^{a,e}, Louise A. Brinton, Ph.D.^a, Catherine S. Healey, B.A.^c, Shahana Ahmed, B.A.^c, Paul Pharoah, M.D., Ph.D.^c, Douglas Easton, Ph.D.^f, Stephen Chanock, M.D.^{a,g,h}, and Montserrat Garcia-Closas, M.D., Dr.P.H.^a

^a Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA ^b Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, USA ^c Cancer Research UK Human Cancer Genetics Research Group, Department of Oncology, University of Cambridge, Cambridge, UK ^d Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland ^e Department of Occupational and Environmental Epidemiology, Nofer Institute of Occupational Medicine, Lodz, Poland ^f Cancer Research UK Genetic Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK ^g Core Genotyping Facility at the Advanced Technology Center, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA ^h Laboratory of Translational Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA

Abstract

Objective—We assessed whether common genetic variation in *PTEN*, *PIK3CA*, *AKT1*, *MLH1*, and *MSH2*—genes that reportedly are frequently altered in endometrial cancer—was associated with risk of endometrial cancer.

Methods—Using data from a population-based case-control study in Poland (PECS) of 417 cases and 407 matched controls, we genotyped 76 tagging single nucleotide polymorphisms (tagSNPs; located in or within 10kb upstream or 5kb downstream of the gene of interest, minor allele frequency $\geq 5\%$ among various ethnic groups, and not already represented by another tagSNP at a LD of $r^2 \geq 0.80$) on an Illumina Custom Infinium iSelect assay that included over 29,000 SNPs in 1316 genes. For individual SNPs, we used unconditional logistic regression models, adjusted for age and site, to generate odds ratios (ORs) and 95% confidence intervals (CIs). To replicate the

*Corresponding author. 1500 East Duarte Road, Duarte, CA 91010-3000, USA. Telephone: +1 626 256 4673 Fax: +1 626 471 7308. jlacey@coh.org (J. V. Lacey Jr.).

¹Present address: Division of Cancer Etiology, Department of Population Sciences, Beckman Research Institute, City of Hope, Duarte, CA, USA

Conflict of interest statement

The authors have no conflicts of interest to report.

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one statistically significant association in PECS, we independently genotyped that tagSNP among 1141 endometrial cancer cases and 2275 controls from the SEARCH study in the UK. We assessed haplotypes via extended haplotype blocks and the sequential haplotype scan method.

Results—The rs2677764 tagSNP in *PIK3CA* was statistically significantly associated with endometrial cancer in PECS (OR=1.42, 95% CI, 1.03–1.95; P=0.03) but not SEARCH (OR=0.98, 95% CI=0.82–1.17). Of the 25 haplotypes observed in at least 5% of cases and controls in PECS, only 1, in *PIK3CA*, was statistically significantly associated with endometrial cancer (OR=1.39, 95% CI, 1.00–1.93). All haplotype global p-values were null.

Conclusion—Common genetic variation in *PTEN*, *PIK3CA*, *AKT1*, *MLH1*, or *MSH2* was not statistically significantly associated with endometrial cancer.

Keywords

SNPs; uterine cancer; epidemiology; haplotypes

Introduction

Endometrial adenocarcinoma is the predominant form of uterine cancer, which is the most frequently diagnosed gynecologic cancer among U.S. women [1]. Estrogen clearly drives endometrial carcinogenesis, as shown by strong epidemiologic evidence linking hormone-related factors (e.g., unopposed estrogen therapy, parity, and circulating endogenous hormone levels) with endometrial cancer risk [2], but other mechanisms are also important. Among these are alterations in the *PTEN* (phosphatase and tensin homolog detected on chromosome 10) gene. *PTEN*-knockout mice that are exposed to estrogen almost universally develop endometrial carcinoma and its precursors [3–4], and endometrial cancer is frequently found among patients with hereditary syndromes characterized by *PTEN* abnormalities [5–6]. The *PTEN* tumor suppressor gene regulates the oncogenic phosphatidylinositol 3-kinase (*PI3K*) signaling pathway that is involved in carcinogenesis [7]. Downstream of those two pathways is *AKT*, a serine-threonine kinase that is regulated by *PI3K* and influences apoptosis and cell proliferation [8]. Alterations in the *PTEN-PI3K-AKT* pathways have been reported for hormone-related tumors among women, including breast and ovarian [9–11], endometrial [12–13], and colorectal [14–15] cancers. Two other important mechanisms for endometrial cancer, DNA mismatch repair [16] and microsatellite instability [17], are influenced by *MLH1* and *MSH2*, which code for DNA repair enzymes.

Investigation of common genetic variation has revealed some single nucleotide polymorphisms (SNPs) in hormone-related pathways that are associated with endometrial cancer [18] [19]. We explored whether common genetic variation in these other genes—*PTEN*, *PIK3CA*, *AKT1*, *MLH1* and *MSH2*—was associated with endometrial cancer in a population-based case-control study from Poland.

Materials and methods

The Polish Endometrial Cancer Study (PECS) has been previously described [20–21]. Women from Warsaw and Lodz who were 20–74 years old when diagnosed with incident, pathology-confirmed, invasive endometrial cancer, identified via participating hospitals and local cancer registries, between June 1, 2001 and December 31, 2003 were eligible as cases. The PECS used the same procedures as a concurrent breast cancer case-control study in the same two cities from January 1, 2000 through September 30, 2003.

We used the Polish Electronic System (PESEL), a demographic database of all Polish residents, to identify controls. We randomly selected frequency-matched [both on 5-year age

groups and study site to the breast cancer cases (Jan. 2000 – Jun. 2001) or endometrial cancer cases (Jun. 2001 – Jun. 2004)] controls, excluding potential controls who had a personal history of breast cancer, endometrial cancer, or hysterectomy. In-person interviews ascertained risk-factor data. All participants gave written informed consent. Institutional review boards at all participating institutions, including the NCI, approved this study.

Of 695 eligible endometrial cancer cases and 2843 eligible endometrial cancer controls, 551 (79%) and 1925 (68%), respectively, completed the interview. Trained nurses collected venous blood samples during the interview from 486 (88%) participating cases and 1814 (94%) participating controls. The overall control:case ratio in PECS was 3.5:1. To reach a 1:1 control:case ratio for genotyping studies, we included all 458 eligible cases with a sufficient sample amount and then randomly selected 465 controls from the frequency-matching age and site strata. We isolated genomic DNA from buffy coats using the Autopure LS DNA purification system (Gentra Systems, Inc., Minneapolis, MN). Eleven cases and 26 controls had samples with insufficient DNA or that did not meet plating requirements, leaving 447 cases and 439 controls.

SNP selection

We included candidate SNPs based on published literature and chose tagging SNPs (tagSNPs) based on linkage disequilibrium (LD) in the HapMap. The tagSNPs, selected in October, 2007, a) were located in or within the regions 10kb upstream or 5kb downstream of the gene, b) had a minor allele frequency (MAF) $\geq 5\%$ among various ethnic groups (CEU, YRI, JPT, and CHB), and c) were not already represented by another tagSNP at a LD of $r^2 \geq 0.80$. Fourteen *PTEN*, 11 *PIK3CA*, 5 *AKT1*, 17 *MLH1*, and 29 *MSH2* SNPs (76 total) met these criteria. Twenty-four other SNPs did not, primarily because of $MAF < 5\%$ in our study population.

Genotyping

The Core Genotyping Facility (CGF) at the NCI's Division of Cancer Epidemiology and Genetics (DCEG) performed the genotyping using the iSelect bead chip (Illumina Custom Infinium, <http://www.illumina.com/pages.ilmn?ID=158>) as part of a large-scale effort that included over 29,000 SNPs covering over 1300 genes for almost 22,000 total DNA samples from 15 studies of 13 different tumor types. Assays and methods are described at <http://cgf.nci.nih.gov/operations/multiplex-genotyping.html>.

Concordance between quality control samples exceeded 98%. After removing 30 cases and 32 controls that failed lab validation, completion percentages for cases and controls were 95% or higher for all assays. Analysis included 417 cases (91% of the 458 eligible cases who donated blood) and 407 controls (88% of the 465 eligible controls who were selected for genotyping).

Statistical analysis

We used Chi-square tests to compare case-control frequencies of individual SNPs and unconditional logistic regression to generated odds ratios (ORs) with 95% confidence intervals (CIs), with adjustment for age and site. The most common homozygous genotype was the reference for genotypes, with separate indicator variables for heterozygous and minor homozygous genotypes. We also considered the number of minor alleles as an ordinal variable (0, 1, or 2) to evaluate trends under an assumed log-additive mode of inheritance. For SNP-based analyses, we used STATA version 9.0. To assess LD between SNPs in the same gene, we evaluated pairwise D' and r^2 values among controls using Haploview (<http://www.broad.mit.edu/mpg/haploview/>).

To evaluate extended haplotype blocks, Haplo.score version 1.3.8 (<http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm>) generated age- and site-adjusted global score statistics. To compare overall and individual haplotype frequencies among cases and controls and generate ORs and 95% CIs, we used an E-M algorithm to simultaneously reconstruct haplotypes and estimate ORs, which we implemented through the program language R (<http://www.r-project.org>). We also used the sequential haplotype scan method [22] to conduct a multi-locus analysis to identify any statistically significant haplotype associations.

To detect associations between genes and endometrial cancer, we used the adaptive rank truncated product (ARTP) method [23]. We first calculated a standardized summary test statistic and then conducted 10,000 permutations to obtain an empirical distribution of the test statistic under the null hypothesis of no association between the gene and endometrial cancer [23]. A previous report provides additional details of these analytic methods [24].

When our analysis produced a SNP-cancer association with a p-value below 0.05 (See Results, below), we attempted to replicate the finding in SEARCH, an ongoing study in the UK that, since 2001, has ascertained prevalent and incident cases, ages 18–69 years at diagnosis, through the Eastern Cancer Registration and Information Centre. Of 1555 prevalent cases diagnosed before Jan. 8, 2001, 693 (45%) provided a blood sample and 684 (44%) completed a questionnaire. Of 836 incident cases diagnosed since Jan. 8, 2001, 453 (54%) provided a blood sample and 445 (53%) completed a questionnaire. Controls in SEARCH are randomly selected from the Norfolk component of the European Prospective Investigation of Cancer (EPIC), a European-wide study of diet and cancer. The Norfolk component includes approximately 25,000 residents of Norfolk, East Anglia, the same region from which cases were recruited. Controls were not matched to cases but were generally similar to cases in age at blood draw. After 41% of invited controls returned a blood sample, DNA was available for 1146 cases and 2304 controls.

The Centre for Cancer Genetic Epidemiology in Cambridge, UK, genotyped rs2677764 using a 5' nuclease assay (Taqman, Applied Biosystems, Carlsbad, CA). Five cases and 29 controls who failed lab validation were excluded from this analysis. To generate ORs and 95% CIs, we used identical logistic regression models as those for the PECS data. To pool PECS and SEARCH data, we used both a fixed-effects and random-effects meta-analytic approach; both produced similar findings, so only fixed-effects results are shown here.

Results

Demographic, reproductive, and lifestyle-related risk-factor associations for the entire endometrial case-control population have been previously described [20] and are consistent with generally well-established associations for endometrial cancer. Similar associations emerged for the 417 cases and 407 controls in the genotyping analyses (Table 1). Most of the tumors were endometrioid adenocarcinomas (80%) or mixed histologic types (12%), well or moderately differentiated (70%), and confined to the uterus (62%) at diagnosis.

Three (rs4608577 and rs7355698 in *MSH2* and rs1234123 in *PTEN*) of the 76 SNPs were inconsistent with Hardy-Weinberg Equilibrium (HWE) at the ≤ 0.01 level, but all three remained in our analyses [25].

Associations with endometrial cancer were statistically non-significant for 75 of the 76 SNPs in *PTEN* and *PIK3CA* (Table 2) and *AKT1*, *MLH1*, and *MSH2* (Supplementary Table 1). The rs2677764 tagSNP in *PIK3CA* had a p-value of 0.03: compared with CC, the ORs for CT and TT were 1.47 (95% CI, 1.03–2.10) and 1.56 (95% CI, 0.43–5.60), respectively.

The OR (95% CI) and p-value for the ordinal variable were 1.42 (1.03–1.95) and 0.03, respectively.

The rs2677764 genotype ORs for CT (0.93, 95% CI, 0.77–1.13) and TT (1.27, 95% CI, 0.68–2.39) revealed null associations (p-value, 0.81) with endometrial cancer in the UK's SEARCH study of 1141 cases and 2275 controls (Table 3). Combining the PECS and SEARCH data yielded study-adjusted ORs of 1.14 (95% CI, 0.73–1.79) for CT vs. CC, 1.32 (95% CI, 0.75–2.33) for TT, and 1.15 (95% CI, 0.80–1.66; p-value, 0.45) for the trend.

None of the 3 common haplotypes for *AKT1*, the 5 for *MLH1*, or the 5 for *MSH2* was associated with endometrial cancer (Table 4). Of four common haplotypes for *PIK3CA*, 1 generated an OR of 1.39, with a 95% CI of 1.00–1.93. Twelve percent of cases and 9% of controls had this haplotype. Endometrial cancer was not statistically significantly associated with any of the 8 common haplotypes for *PTEN*. Each haplotype was observed among at least 5% of cases and controls, but no haplotype was seen in more than 25% of either group. None of the combined groups of rare haplotypes (i.e., less than 1% of cases and controls) was associated with endometrial cancer. Global p-values for all 5 genes ranged from 0.53 to 0.97. The sequential scan and gene-based ARTP methods identified no statistically significant haplotype associations for *PTEN* or *PIK3CA* (Figure 1) or *AKT1*, *MLH1*, or *MSH2* (data not shown).

Discussion

This analysis revealed primarily null associations between endometrial cancer and common genetic variation in *PTEN*, *PIK3CA*, *AKT1*, *MLH1*, and *MSH2*, genes in cell-cycle and mismatch repair pathways that are linked to endometrial carcinogenesis. Our results showed that individual tagSNPs and constructed haplotypes were, overall, similarly distributed among these cases and their matched, population-based controls.

After *PIK3CA* rs2677764 emerged as statistically significantly associated with endometrial cancer in the PECS, we attempted to replicate that association in an independent dataset, SEARCH study, by directly genotyping that SNP among 1141 cases and 2275 controls. In SEARCH and the pooled analysis of PECS and SEARCH data, rs2677764 in *PIK3CA* was not statistically significantly associated with endometrial cancer, but the magnitude of the per-allele OR from the pooled analysis (1.15) is consistent with the types of associations that reached genome-wide significance in recent GWAS for other cancer types. One haplotype in *PIK3CA* was also statistically significantly associated with endometrial cancer in the PECS data, but chance alone would be expected to produce one significant association among the 25 haplotype comparisons we conducted. These two statistically significant associations in the PECS analysis therefore seem likely to be false-positive findings, but it may be premature to definitively rule out a valid association between endometrial cancer and rs2677764, other potentially causal markers in LD with rs2677764, or haplotypes that contain rs2677764 until definitive results from a GWAS for endometrial cancer have been published and replicated.

Somatic *PTEN* mutations have been reported in endometrial cancers [26], especially for early-onset tumors or tumors among patients with Cowden's syndrome [27], but data on *PTEN* SNPs and endometrial cancer are limited. Seven of the 14 SNPs in our analysis were evaluated in breast and prostate cancer by Haiman et al. in a large, multi-ethnic cohort that showed no significant associations [28]. For *PIK3CA*, most of the existing data on genetic mutations focused on mutations in tumors [29], rather than common genetic variation in population-based studies. For *AKT*, a statistical methods paper that described an alternative approach for modeling interactions mentioned seven tagSNPs for *AKT1* that were

statistically significantly associated with endometrial cancer ($p=0.042$) after applying a partial least squares regression algorithm [30]. The individual SNPs and associations were not presented, and three other approaches for evaluating associations produced p -values above 0.05. Until additional information is available about the testing method and results, direct comparisons between our data and that study are difficult.

MSH2 and *MLH1* are integral to mismatch repair (MMR) and microsatellite instability (MSI), which have been implicated in endometrial carcinogenesis [17]. However, as with *PTEN*, few data on associations between endometrial cancer and common genetic variation in these two genes are available. One previous study [31] of 672 hospital-based cases collected over 40 years and 880 unmatched hospital-based controls reported statistically significant associations between endometrial cancer and two SNPs, rs2303428 (OR=1.4, 95% CI, 1.0–1.9) and rs2059520 (OR=1.3, 95% CI, 1.0–1.7), in *MSH2*, but both associations were confined to heterozygous genotypes; neither of the homozygous minor alleles was associated with endometrial cancer. These SNPs were not associated with endometrial cancer in our population-based study.

Strengths of the PECS include a relatively large sample size, high levels of participation, and reasonably extensive candidate-gene genotyping of tagSNPs in genes hypothesized to be related to endometrial carcinogenesis. Misclassification of genotypes was negligible. Our analysis was further strengthened by the ability to assess the rs2677764 findings in an independent dataset, the larger SEARCH study. The SEARCH-specific null results and the null pooled estimate from PECS and SEARCH provide good evidence for an overall null association between rs2677764 and endometrial cancer. To avoid false-positive subgroup associations that could emerge by chance alone [32], we did not look for any gene-environment interactions in our data. However, the extensive risk factor data in our study could be used for that purpose if future or consortial studies identify any confirmed susceptibility loci.

Our sample size was not large enough to completely rule out small-magnitude associations between tag SNPs—especially the uncommon variants—and endometrial cancer in the PECS. We used generally accepted methods to identify and analyze tagSNPs, but it is possible that other untested markers related to these genes are associated with endometrial cancer. Selection bias from differential non-participation is a concern for case-control studies, but relatively high participation rates in PECS and the low probability that genetic variation was associated with participation make it unlikely to have biased our findings. The SEARCH study provided a large sample size for replication but could have introduced other error or bias if genetic variation in rs2677764 was associated with participation rates, which were lower than PECS, or survival, because SEARCH included both prevalent and incident cases.

Common genetic variation in *PTEN*, *PIK3CA*, *AKT1*, *MLH1*, and *MSH2* was not associated with endometrial cancer in our study, but continued exploration of processes these genes affect, including proliferation, apoptosis, mismatch repair, and microsatellite instability, may provide additional insight into endometrial carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin.* 2008; 58:71–96. [PubMed: 18287387]
2. Purdie DM, Green AC. Epidemiology of endometrial cancer. *Best Pract Res Clin Obstet Gynaecol.* 2001; 15:341–54. [PubMed: 11476557]
3. Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in *pten*^{+/-} mice. *Cancer Res.* 2000; 60:3605–11. [PubMed: 10910075]
4. Wang H, Douglas W, Lia M, Edelmann W, Kucherlapati R, Podsypanina K, Parsons R, Ellenson LH. DNA mismatch repair deficiency accelerates endometrial tumorigenesis in *Pten* heterozygous mice. *Am J Pathol.* 2002; 160:1481–6. [PubMed: 11943731]
5. Marsh DJ, Dahia PL, Caron S, Kum JB, Frayling IM, Tomlinson IP, Hughes KS, Eeles RA, Hodgson SV, Murday VA, Houlston R, Eng C. Germline PTEN mutations in Cowden syndrome-like families. *J Med Genet.* 1998; 35:881–5. [PubMed: 9832031]
6. Zhou XP, Marsh DJ, Morrison CD, Chaudhury AR, Maxwell M, Reifemberger G, Eng C. Germline inactivation of PTEN and dysregulation of the phosphoinositol-3-kinase/Akt pathway cause human Lhermitte-Duclos disease in adults. *Am J Hum Genet.* 2003; 73:1191–8. [PubMed: 14566704]
7. Saal LH, Johansson P, Holm K, Gruvberger-Saal SK, She QB, Maurer M, Koujak S, Ferrando AA, Malmstrom P, Memeo L, Isola J, Bendahl PO, Rosen N, Hibshoosh H, Ringner M, Borg A, Parsons R. Poor prognosis in carcinoma is associated with a gene expression signature of aberrant PTEN tumor suppressor pathway activity. *Proc Natl Acad Sci U S A.* 2007; 104:7564–9. [PubMed: 17452630]
8. Blanco-Aparicio C, Renner O, Leal JF, Carnero A. PTEN, more than the AKT pathway. *Carcinogenesis.* 2007; 28:1379–86. [PubMed: 17341655]
9. Campbell IG, Russell SE, Choong DY, Montgomery KG, Ciavarella ML, Hooi CS, Cristiano BE, Pearson RB, Phillips WA. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res.* 2004; 64:7678–81. [PubMed: 15520168]
10. Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res.* 2005; 11:2875–8. [PubMed: 15837735]
11. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, Neve RM, Kuo WL, Davies M, Carey M, Hu Z, Guan Y, Sahin A, Symmans WF, Pusztai L, Nolden LK, Horlings H, Berns K, Hung MC, van de Vijver MJ, Valero V, Gray JW, Bernards R, Mills GB, Hennessy BT. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res.* 2008; 68:6084–91. [PubMed: 18676830]
12. Hayes MP, Wang H, Espinal-Witter R, Douglas W, Solomon GJ, Baker SJ, Ellenson LH. PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. *Clin Cancer Res.* 2006; 12:5932–5. [PubMed: 17062663]
13. Oda K, Stokoe D, Taketani Y, McCormick F. High frequency of coexistent mutations of PIK3CA and PTEN genes in endometrial carcinoma. *Cancer Res.* 2005; 65:10669–73. [PubMed: 16322209]
14. Velculescu VE. Defining the blueprint of the cancer genome. *Carcinogenesis.* 2008; 29:1087–91. [PubMed: 18495658]
15. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. High frequency of mutations of the PIK3CA gene in human cancers. *Science.* 2004; 304:554. [PubMed: 15016963]

16. Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, White KG, Luthra R, Gershenson DM, Broaddus RR. Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. *J Clin Oncol*. 2007; 25:5158–64. [PubMed: 17925543]
17. Imai K, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis*. 2008; 29:673–80. [PubMed: 17942460]
18. Olson SH, Bandera EV, Orlov I. Variants in estrogen biosynthesis genes, sex steroid hormone levels, and endometrial cancer: a HuGE review. *Am J Epidemiol*. 2007; 165:235–45. [PubMed: 17110639]
19. Setiawan VW, Doherty JA, Shu XO, Akbari MR, Chen C, De Vivo I, Demichele A, Garcia-Closas M, Goodman MT, Haiman CA, Hankinson SE, Henderson BE, Horn-Ross PL, Lacey JV Jr, Le Marchand L, Levine DA, Liang X, Lissowska J, Lurie G, McGrath M, Narod SA, Rebbeck TR, Ursin G, Weiss NS, Xiang YB, Yang HP, Zheng W, Olson SH. Two estrogen-related variants in CYP19A1 and endometrial cancer risk: a pooled analysis in the Epidemiology of Endometrial Cancer Consortium. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:242–7. [PubMed: 19124504]
20. Brinton LA, Sakoda LC, Lissowska J, Sherman ME, Chatterjee N, Peplonska B, Szeszenia-Dabrowska N, Zatonski W, Garcia-Closas M. Reproductive risk factors for endometrial cancer among Polish women. *Br J Cancer*. 2007; 96:1450–6. [PubMed: 17426703]
21. Gaudet MM, Lacey JV Jr, Lissowska J, Peplonska B, Brinton LA, Chanock S, Garcia-Closas M. Genetic variation in CYP17 and endometrial cancer risk. *Hum Genet*. 2008; 123:155–62. [PubMed: 18172694]
22. Yu Z, Schaid DJ. Sequential haplotype scan methods for association analysis. *Genet Epidemiol*. 2007; 31:553–64. [PubMed: 17487883]
23. Yu K, Li Q, Bergen AW, Pfeiffer RM, Rosenberg PS, Caporaso N, Kraft P, Chatterjee N. Pathway analysis by adaptive combination of P-values. *Genet Epidemiol*. 2009; 33:700–9. [PubMed: 19333968]
24. Yang HP, Gonzalez Bosquet J, Li Q, Platz EA, Brinton LA, Sherman ME, Lacey JV Jr, Gaudet MM, Burdette LA, Figueroa JD, Ciampa JG, Lissowska J, Peplonska B, Chanock SJ, Garcia-Closas M. Common genetic variation in the sex hormone metabolic pathway and endometrial cancer risk: pathway-based evaluation of candidate genes. *Carcinogenesis*. 2010; 31:827–33. [PubMed: 20053928]
25. Balding DJ. A tutorial on statistical methods for population association studies. *Nat Rev Genet*. 2006; 7:781–91. [PubMed: 16983374]
26. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. *Cancer Res*. 1997; 57:4736–8. [PubMed: 9354433]
27. Eng C. PTEN: one gene, many syndromes. *Hum Mutat*. 2003; 22:183–98. [PubMed: 12938083]
28. Haiman CA, Stram DO, Cheng I, Giorgi EE, Pooler L, Penney K, Le Marchand L, Henderson BE, Freedman ML. Common genetic variation at PTEN and risk of sporadic breast and prostate cancer. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:1021–5. [PubMed: 16702386]
29. Ollikainen M, Gylling A, Puputti M, Nupponen NN, Abdel-Rahman WM, Butzow R, Peltomaki P. Patterns of PIK3CA alterations in familial colorectal and endometrial carcinoma. *Int J Cancer*. 2007; 121:915–20. [PubMed: 17471559]
30. Wang T, Ho G, Ye K, Strickler H, Elston RC. A partial least-square approach for modeling gene-gene and gene-environment interactions when multiple markers are genotyped. *Genet Epidemiol*. 2008
31. Beiner ME, Rosen B, Fyles A, Harley I, Pal T, Siminovitch K, Zhang S, Sun P, Narod SA. Endometrial cancer risk is associated with variants of the mismatch repair genes MLH1 and MSH2. *Cancer Epidemiol Biomarkers Prev*. 2006; 15:1636–40. [PubMed: 16985024]
32. Weiss NS. Subgroup-specific associations in the face of overall null results: should we rush in or fear to tread? *Cancer Epidemiol Biomarkers Prev*. 2008; 17:1297–9. [PubMed: 18559544]

Figure 1.

Table 1

Demographic characteristics and endometrial cancer risk and protective factors for cases and controls* in a population-based endometrial cancer case-control study in Poland

	Cases (n=417)		Controls (n=407)		P-value [†]
	N	%	N	%	
Age at Recruitment*					
<49	43	10.3%	41	10.1%	
50–59	132	31.7%	124	30.5%	
60–69	171	41.0%	171	42.0%	
>70	71	17.0%	71	17.4%	0.98
Recruitment Site*					
Lodz	299	71.7%	288	15.0%	
Warsaw	118	28.3%	119	6.2%	0.77
Education (grade)					
<12th	151	36.5%	153	38.2%	
12th	147	35.5%	144	35.9%	
>12th	116	28.0%	104	25.9%	0.78
Age at Menarche (years)					
<12	115	27.8%	75	18.7%	
13	102	24.6%	92	22.9%	
14	106	25.6%	110	27.4%	
>15	91	22.0%	125	31.1%	<0.01
Number of Pregnancies					
None	50	12.0%	23	5.7%	
1	78	18.7%	61	15.0%	
2	116	27.8%	134	32.9%	
3	90	21.6%	79	19.4%	
4+	83	19.9%	110	27.0%	<0.01
Age at First Birth (years old)					
Nulliparous	76	18.2%	35	8.6%	
<20	47	11.3%	46	11.3%	

	Cases (n=417)		Controls (n=407)		P-value [†]
	N	%	N	%	
20–24	184	44.1%	185	45.5%	
25–29	84	20.1%	105	25.8%	
>30	26	6.2%	36	8.8%	<0.01
Body Mass Index at Interview (kg/m ²)					
<25	112	26.9%	157	38.6%	
25–29	157	37.6%	158	38.8%	
30–40	127	30.5%	79	19.4%	
40+	21	5.0%	13	3.2%	<0.01
Menopausal Status					
Premenopausal	94	22.7%	56	14.7%	
Postmenopausal	321	77.3%	324	85.3%	<0.01
Oral Contraceptive Use					
Never	395	95.4%	371	92.1%	
Ever	19	4.6%	32	7.9%	0.048
Oral Menopausal Hormone Use					
Never	309	63.3%	312	67.0%	
Ever	103	25.0%	87	21.8%	0.28
Estrogen only	7		8		
Estrogen plus progestin or progestin only	69		59		

Note: Numbers may not add up to case and control totals because of missing values

* Cases and controls frequency-matched on age ($\pm$ 5 year age categories) and site (Lodz, Warsaw)

[†] χ^2 test

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between tagSNPs* in *PIK3CA* and *PTEN* and endometrial cancer in a population-based case-control in Poland

Gene	hSNP	MAF	Cases (N=417)		Controls (N=407)		OR [†]	95% CI	P-value [‡]	Gene-based P-value [§]
			N	%	N	%				
<i>PIK3CA</i>	rs2699905	CC	205	49%	219	54%	1.00	Ref		0.23
		CT	172	41%	154	38%	1.18	(0.88–1.58)		
		TT	40	10%	34	8%	1.27	(0.77–2.09)		
		per allele					1.15	(0.93–1.42)	0.21	
rs7641889	CC	6%	368	88%	365	90%	1.00	Ref		
	CT		48	12%	38	9%	1.21	(0.77–1.91)		
	TT		1		4	1%	0.25	(0.03–2.29)		
	per allele						1.03	(0.69–1.55)	0.88	
rs7641983	CC	14%	306	73%	302	74%	1.00	Ref		
	CT		97	23%	97	24%	0.97	(0.70–1.35)		
	TT		14	3%	8	2%	1.72	(0.71–4.16)		
	per allele						1.08	(0.82–1.41)	0.59	
rs7651265	AA	9%	334	80%	335	83%	1.00	Ref		
	AG		78	19%	65	16%	1.18	(0.82–1.71)		
	GG		5	1%	6	1%	0.79	(0.24–2.65)		
	per allele						1.10	(0.80–1.51)	0.56	
rs6443624	CC	16%	292	70%	286	70%	1.00	Ref		
	AC		110	26%	113	28%	0.94	(0.69–1.28)		
	AA		15	4%	8	2%	1.83	(0.76–4.38)		
	per allele						1.06	(0.82–1.38)	0.66	
rs7640662	CC	18%	282	68%	270	66%	1.00	Ref		
	CG		121	29%	127	31%	0.91	(0.67–1.23)		
	GG		14	3%	10	2%	1.35	(0.58–3.13)		
	per allele						0.98	(0.76–1.27)	0.90	
rs2677760	CC	47%	108	26%	118	29%	1.00	Ref		
	CT		193	47%	194	48%	1.08	(0.77–1.50)		

Gene	htSNP	MAF	Cases (N=417)		Controls (N=407)		OR [†]	95% CI	P-value [‡]	Gene-based P-value [§]
	TT		N	%	N	%				
rs2677764	per allele		110	27%	92	23%	1.29	(0.88–1.89)		
	CC	9%	320	77%	337	83%	1.13	(0.94–1.37)	0.19	
	CT		91	22%	65	16%	1.00	Ref		
	TT		6	1%	4	1%	1.47	(1.03–2.10)		
rs3729692	per allele						1.56	(0.43–5.60)		0.03
	AA	6%	358	86%	358	88%	1.42	(1.03–1.95)		
	AC		55	13%	47	12%	1.00	Ref		
	CC		4	1%	2		1.17	(0.77–1.78)		
rs1607237	per allele						1.82	(0.33–10.11)		0.34
	TT	44%	146	35%	128	31%	1.20	(0.82–1.75)		
	CT		190	46%	196	48%	1.00	Ref		
	CC		81	19%	83	20%	0.85	(0.62–1.16)		
rs6786049	per allele						0.87	(0.59–1.28)		0.39
	CC	4%	389	93%	372	92%	0.92	(0.76–1.11)		
	CT		28	7%	33	8%	1.00	Ref		
							0.79	(0.46–1.33)		0.37
PTEN										
rs10887758	TT	16%	294	71%	288	71%	1.00	Ref		0.81
	CT		108	26%	105	26%	0.99	(0.72–1.36)		
	CC		15	4%	13	3%	1.13	(0.53–2.43)		
	per allele						1.02	(0.79–1.32)	0.88	
rs10788569	TT	20%	267	64%	265	65%	1.00	Ref		0.69
	CT		125	30%	121	30%	1.02	(0.75–1.38)		
	CC		25	6%	21	5%	1.17	(0.64–2.15)		
	per allele						1.05	(0.83–1.32)		
rs1234221	AA	24%	250	60%	228	56%	1.00	Ref		0.22
	AC		151	36%	156	39%	0.88	(0.66–1.18)		
	CC		16	4%	21	5%	0.69	(0.35–1.36)		
	per allele						0.86	(0.68–1.09)		
rs1234219	AA	6%	366	88%	362	89%	1.00	Ref		

Gene	htSNP	MAF	Cases (N=417)		Controls (N=407)		OR [†]	95% CI	P-value [‡]	Gene-based P-value [§]
	AG		N	%	N	%				
rs2299939	GG		47	11%	45	11%	1.02	(0.66–1.57)	0.42	
	CC	26%	4	1%	0					
	AC		240	58%	227	56%	1.00	Ref		
	AA		152	36%	149	37%	0.97	(0.72–1.30)		
	per allele		25	6%	30	7%	0.78	(0.44–1.37)		
rs1234223	GG	9%	344	82%	337	83%	1.00	Ref		0.47
	CG		66	16%	66	16%	0.97	(0.67–1.41)		
	CC		7	2%	4	1%	1.63	(0.47–5.64)		
	per allele						1.04	(0.75–1.45)		0.80
rs17106896	AA	3%	385	92%	381	94%	1.00	Ref		
	AT		31	7%	26	6%	1.19	(0.69–2.05)		0.39
	TT		1		0					
rs1234213	GG	28%	211	51%	216	53%	1.00	Ref		
	AG		164	39%	154	38%	1.08	(0.81–1.45)		
	AA		42	10%	37	9%	1.14	(0.70–1.84)		
	per allele						1.07	(0.87–1.32)		0.51
	GG	7%	364	87%	353	87%	1.00	Ref		
rs12357281	CG		52	12%	51	13%	0.98	(0.65–1.48)		
	CC		1		2		0.54	(0.05–6.26)		
	per allele						0.95	(0.64–1.41)		0.79
rs2299941	AA	10%	336	81%	330	81%	1.00	Ref		
	AG		77	18%	71	17%	1.06	(0.74–1.51)		
	GG		4	1%	6	1%	0.65	(0.18–2.32)		
	per allele						0.99	(0.72–1.36)		0.95
rs2735343	GG	28%	211	51%	215	53%	1.00	Ref		
	CG		163	39%	154	38%	1.07	(0.80–1.44)		
	CC		42	10%	37	9%	1.13	(0.70–1.83)		
	per allele						1.07	(0.86–1.31)		0.55
rs2736627	TT	12%	309	74%	314	77%	1.00	Ref		

Gene	htSNP	MAF	Cases (N=417)		Controls (N=407)		OR [†]	95% CI	P-value [‡]	Gene-based P-value [§]
			N	%	N	%				
rs11202607	CT		99	24%	86	21%	1.19	(0.85–1.65)		
	CC		9	2%	7	2%	1.29	(0.47–3.50)		
	per allele						1.17	(0.88–1.56)	0.28	
	CC	5%	366	88%	365	90%	1.00	Ref		
	CT		47	11%	42	10%	1.10	(0.71–1.71)	0.25	
rs478839	TT		4	1%	0	0%				
	AA	47%	128	31%	111	27%	1.00	Ref		
	AG		213	51%	208	51%	0.90	(0.65–1.23)		
	GG		76	18%	88	22%	0.75	(0.50–1.11)		
	per allele						0.87	(0.71–1.06)	0.16	

Note: Numbers of cases and controls genotyped for each tagSNP varies due to missing values for genotypes

* SNPs are written 5' to 3' for each gene.

[†] Unconditional logistic regression, adjusted for frequency matching variables age (± 5 years) and site (Lodz, Warsaw)

[‡] Test for trend per allele calculated using a single variable for the number of minor alleles present

[§] ARTP method

Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between rs2677764 and endometrial cancer in PECS and SEARCH

Table 3

		PECS					SEARCH								
		Cases (N=417)					Controls (N=1141)								
		N	%	N	%	OR [*]	95% CI	P-value [†]	N	%	N	%	OR [*]	95% CI	P-value [†]
<i>PIK3CA</i>															
rs2677764	CC	320	77%	337	83%	1.00	Ref		955	84%	1889	83%	1.00	Ref	
	CT	91	22%	65	16%	1.47	(1.03–2.10)		170	15%	361	16%	0.93	(0.77–1.13)	
	TT	6	1%	4	1%	1.56	(0.43–5.60)		16	1%	25	1%	1.27	(0.68–2.39)	
per allele						1.42	(1.03–1.95)	0.03					0.98	(0.82–1.17)	0.81

* Unconditional logistic regression, adjusted for frequency matching variables age (± 5 years) and site (Lodz, Warsaw)

† Test for trend per allele calculated using a single variable for the number of minor alleles present

Odds ratios (ORs), adjusted for age and site, and 95% confidence intervals (CIs) for associations with endometrial cancer in a population based case-control study in Poland

Table 4

Gene	Haplotype*	% of Cases (N= 417)	% of Controls (N=407)	OR†	95% CI
<i>AKT1</i>	00000	51%	53%	1.00	Ref
	00101	35%	32%	1.16	(0.93–1.45)
	01011	7%	6%	1.17	(0.77–1.79)
	Rare haplotypes‡			0.81	(0.56–1.19)
	Global p-value§	0.53			
<i>MLH1</i>	11010001111011100	30%	31%	1.00	Ref
	00100000000100001	7%	8%	0.94	(0.63–1.41)
	00100010000000001	21%	19%	1.16	(0.88–1.52)
	00101000000000011	21%	21%	1.05	(0.80–1.38)
	11010000111011100	12%	12%	1.02	(0.73–1.43)
	Rare haplotypes‡			0.97	(0.21–4.56)
	Global p-value§	0.94			
<i>MSH2</i>	0000000000010000000000000000000	24%	23%	1.00	Ref
	0000000000000000000000000000000	9%	10%	0.90	(0.62–1.31)
	0000000000010000000000000000000	11%	11%	0.98	(0.68–1.41)
	01100010101011011011110100101	16%	15%	0.99	(0.70–1.39)
	01110010001011011011111001001	9%	9%	0.99	(0.67–1.45)
	Rare haplotypes‡			0.89	(0.19–4.19)
	Global p-value§	0.97			
<i>PIK3CA</i>	000000000010	42%	44%	1.00	Ref
	000000000000	7%	7%	0.95	(0.64–1.43)
	10000011000	12%	9%	1.39	(1.00–1.93)
	10000110000	18%	18%	1.02	(0.78–1.35)
	Rare haplotypes‡			1.04	(0.80–1.34)

		% of Cases (N= 417)		% of Controls (N=407)		OR [†]		95% CI	
Gene	Haplotype*								
	Global p-value [§]	0.55							
<i>PTEN</i>	000010000000001	24%		25%		1.00		Ref	
	000000000000000	13%		13%		1.08		(0.76–1.53)	
	000000000000100	14%		12%		1.24		(0.89–1.74)	
	00010101001010	6%		5%		1.32		(0.83–2.08)	
	001000000000001	13%		14%		0.97		(0.70–1.36)	
	001000001000001	6%		7%		0.98		(0.21–4.63)	
Rare haplotypes [‡]	11000001011000	10%		10%		1.12		(0.79–1.58)	
	11000001011100					1.03		(0.74–1.44)	
						0.99		(0.63–1.57)	
	Global p-value [§]	0.67							

* Zero indicates common allele and one indicates variant allele. Reference haplotype is the most common haplotype. SNPs are written 5' to 3'. Positions of SNPs, in order. *AKT1*: rs2494738, rs2494746, rs1130214, rs4983387, rs4983559; *MLH1*: rs4678922, rs6789043, rs9311149, rs1046512, rs1800734, rs4647225, rs1540354, rs1799977, rs2286939, rs3774338, rs3774332, rs748766, rs9876116, rs2241031, rs749072, rs3774327; *MSH2*: rs4952886, rs7584256, rs3924917, rs6744343, rs1863332, rs4952887, rs2347794, rs6753135, rs13408008, rs17036577, rs6726691, rs1981929, rs6724876, rs6757035, rs6741393, rs3771281, rs3732182, rs10183143, rs3764959, rs3764960, rs3821227, rs2059520, rs2303428, rs4608577, rs4638843, rs2042649, rs6544991, rs7355698, rs6544992; *PIK3CA*: rs2699905, rs7641889, rs7641983, rs7651265, rs6443624, rs7640662, rs2677760, rs2677764, rs3729692, rs1607237, rs6786049; *PTEN*: rs10887758, rs10788569, rs1234221, rs1234219, rs2299939, rs1234223, rs17106896, rs1234213, rs12357281, rs2299941, rs2735343, rs2736627, rs11

† Unconditional generalized linear model, adjusted for matching variables age (± 5yr) and site (Lodz, Warsaw). Estimates are based on an additive model

‡ Rare haplotypes include haplotypes with frequencies <0.01

§ Global test for entire set of haplotypes, adjusting for matching variables age and site