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Authors

Charbonneau, Bridget
Block, Matthew S
Bamlet, William R
[et al.](#)

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Risk of Ovarian Cancer and the NF- κ B Pathway: Genetic association with *IL1A* and *TNFSF10*

A full list of authors and affiliations appears at the end of the article.

Abstract

A missense single nucleotide polymorphism (SNP) in the immune modulatory gene *IL1A* has been associated with ovarian cancer risk (rs17561). While the exact mechanism through which this SNP alters risk of ovarian cancer is not clearly understood, rs17561 has also been associated with risk of endometriosis, an epidemiologic risk factor for ovarian cancer. IL-1 α is both regulated by and able to activate NF- κ B, a transcription factor family that induces transcription of many pro-inflammatory genes and may be an important mediator in carcinogenesis. We therefore tagged SNPs in over 200 genes in the NF- κ B pathway for a total of 2,282 SNPs (including rs17561) for genotype analysis of 15,604 cases of ovarian cancer in patients of European descent, including 6,179 of high grade serous (HGS), 2,100 endometrioid, 1,591 mucinous, 1,034 clear cell and 1,016 low grade serous (LGS), including 23,235 control cases spanning 40 studies in the Ovarian Cancer Association Consortium (OCAC). In this large population, we confirmed the association between rs17561 and clear cell ovarian cancer (OR=0.84, 95% CI: 0.76–0.93; p=0.00075), which remained intact even after excluding participants in the prior study (OR=0.85, 95% CI: 0.75–0.95; p=0.006). Considering a multiple-testing-corrected significance threshold of p< 2.5 \times 10⁻⁵, only one other variant, the *TNFSF10* SNP rs6785617, was associated significantly with a risk of ovarian cancer (low malignant potential (LMP) tumors OR=0.85, 95% CI: 0.79–0.91; p=0.00002). Our results extend the evidence that borderline tumors may have a distinct genetic etiology. Further investigation of how these SNPs might modify ovarian cancer associations with other inflammation related risk factors is warranted.

Keywords

clear cell; endometrioid; case-control; single nucleotide polymorphism; IL-1 α

INTRODUCTION

Inflammation is a known mediator of carcinogenesis and a number of risk factors associated with ovarian cancer are also linked to inflammatory processes (1). The inverse relationship between parity (2, 3) and oral contraceptive (OC) use (3–6) and ovarian cancer risk is thought to be due to increased ovulations in women with fewer pregnancies or shorter duration of OC use. The damage and repair cycle associated with each ovulation recruits immune mediators with potential to promote ovarian cancer initiation and growth (1, 7). Evidence for a relationship between pelvic inflammatory disease (PID) and ovarian cancer risk has also been observed in a few studies (8, 9). Furthermore, endometriosis, another condition associated with elevated inflammatory markers (10), has been found to increase risk of clear-cell, invasive endometrioid, and low-grade serous tumors (11). Studies of

*Address correspondence to: Ellen L. Goode, Ph.D., M.P.H., Department of Health Sciences Research, Mayo Clinic, College of Medicine, 200 First Street SW, Rochester, MN 55905, USA, Phone: 507/266-7997, Fax: 507/266-2478, egoode@mayo.edu.

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perineal talcum powder use additionally suggest an association with ovarian cancer risk (12), presumably due to its pro-inflammatory properties (13). Use of nonsteroidal anti-inflammatory drugs (NSAIDs) has also been linked to reduced risk of ovarian cancer, particularly aspirin use in invasive ovarian cancer risk (14, 15).

In a previous study by our group, interrogation of SNPs in several inflammation-related genes revealed an association between ovarian cancer risk and SNPs in *IL1A* and *ALOX5* (16); most notable was a missense SNP in *IL1A*, rs17561, which had the strongest associations with the rarer histologic subtypes. IL-1 α , the cytokine encoded by this gene, mediates a number of inflammatory and immune responses, including response to tissue injury (17, 18). In the present study, we assessed this SNP for overall and histologic subtype associations in a much larger population of ovarian cancer cases and controls to evaluate replication. We additionally investigated SNPs in other NF- κ B pathway genes, as IL-1 α is not only produced following NF- κ B activation (19), but signaling of IL-1 α through its receptor results in downstream activation of NF- κ B (20), which leads to transcription of a number of genes whose products promote inflammation (21).

In addition to the prior association, there is strong biological support for further study of polymorphisms in the NF- κ B pathway in ovarian cancer (21). This pathway appears to play a crucial role in the process that links inflammation to cancer (22). Activation of this family of transcription factors leads to transcription and expression of a number of pro-inflammatory cytokines (23) with the ability to promote tumor growth (24). Specifically, activation of NF- κ B through IKK ϵ was shown to be associated with more aggressive behavior in ovarian cancer cell lines (25). Additionally, NF- κ B activation can inhibit apoptosis (26). Finally, NF- κ B activation has been associated with aberrant cellular activities in endometriosis (27). Therefore, we expanded our investigation of inflammation-related SNPs to include variants in over 200 NF- κ B pathway related genes in a large collection of ovarian cancer patients and controls from the Ovarian Cancer Association Consortium (OCAC).

METHODS

Study participants

Participants from 40 OCAC studies of primarily European ancestry were included in this project (28). For nine studies that were case-only (GRR, HSK, LAX, ORE, PVD, RMH, SOC, SRO, UKR), cases were pooled with case-control studies from the same geographic region, resulting in 31 total case-control sets. Study characteristics are summarized in Supplementary Table 1 and the number of cases by histological subtype is shown in Supplementary Table 2. A total of 47,092 women were included.

SNP selection

As reviewed previously (21), we identified a number of genes known to encode NF- κ B subunits or molecules key to NF- κ B activation (in signaling cascade), inhibition (inhibitory role), degradation (involved in proteasomal degradation), and nuclear function (nuclear proteins involved in transcription) and narrowed the list to the top 210 most important genes in the pathway. In early 2010, tagSNPs within 5 kb of these genes with $r^2 \geq 0.8$ and MAF ≥ 0.05 in European individuals were identified using the most informative source for each gene from among HapMap Project Phase II Release 24 (29), the 1000 Genomes Project Low-coverage Pilot (30), SeattleSNPs (31), Innate Immunity PGA (32), and NIEHS SNPs (33). Additional putative-functional SNPs were also included, regardless of linkage disequilibrium (LD), with European MAF ≥ 0.05 which were 1 kb upstream, non-synonymous or resided in a 3' UTR, 5' UTR, splice site, or miRNA binding site (34, 35).

We used SNPPicker (36) to optimally pick tagSNPs for each gene. SNPs which had an Illumina design score <0.4 or which were in LD ($r^2 > 0.80$) with a SNP found to be null ($p > 0.05$) in prior analysis of genome-wide association study (GWAS) data (28) were excluded. Genes and coverage are shown in Supplementary Table 3.

Genotyping and Quality Control

Genotyping of study samples and duplicates, as previously described (28), was carried out as part of a large custom Illumina Infinium iSelect BeadChip (over 200,000 SNPs) at McGill University and G enome Qu ebec (n=19,806) and the Mayo Clinic Medical Genome Facility (n=28,820) on 96-well plates containing 750 ng genomic DNA (or 1,500 ng whole-genome amplified DNA). Along with OCAC samples, HapMap samples for European (CEU, n=60), African (YRI, n=53) and Asian (JPT+CHB, n=88) populations were also genotyped. Raw intensity data files were reviewed for centralized quality control, and genotypes were called using GenCall (37), which showed superior performance over Illuminus (38) and GenoSNP (39) upon manual inspection of representative SNPs.

SNPs were excluded according to the following criteria: (1) no genotype call; (2) monomorphism; (3) call rate less than 95 percent and MAF > 0.05 or call rate less than 99 percent with MAF < 0.05; (4) evidence of deviation from Hardy-Weinberg equilibrium ($p < 10^{-7}$) in controls; (5) greater than 2 percent discordance in duplicate pairs. Overall, 94.5 percent of SNPs passed QC; a total of 2,282 NF- B SNPs were included in analyses.

SNP data were generated on 47,092 unique samples. We used identity-by-state to identify first-degree relative pairs, of which we excluded the one with the lowest call rate. Additional samples were excluded according to the following criteria: 1) call rates < 95 percent; 2) heterozygosity > five standard deviations from the intercontinental ancestry specific mean heterozygosity; 3) ambiguous sex; 4) lowest call rate from a first-degree relative pair; 5) missing case-control status; 6) missing age at diagnosis; 7) non-epithelial cancer, unknown if epithelial cancer or missing histology; 8) Brenner tumors; 9) <90 European ancestry based on LAMP (40). After the above exclusions, a total of 38,839 subjects including 15,604 cases (13,727 invasive) and 23,235 controls were retained for analysis (Supplementary Table 4).

Statistical methods

SNP genotypes were coded as 0, 1, or 2 based on the number of copies of the minor allele. Associations with risk of ovarian cancer were evaluated first using cases combined, and then within strata defined by tumor behavior [low malignant potential (LMP) and invasive] and histology [low grade serous (LGS), high grade serous (HGS), mucinous, endometrioid, and clear cell]. We used a subset of 37,000 non-NF- B markers to perform principal component (PC) analysis within the European subset in order to account for potential residual population stratification (41). For all analyses, SNPs were modeled using a one degree-of-freedom linear term assuming a log-additive, or ordinal, effect. Odds ratios (OR), 95% confidence intervals (CI) and p-values were generated using logistic regression analysis in PLINK (Version 1.07) (42) with adjustment for age, study site, and the first five European PCs as described above. Effect modification by site and epidemiologic risk factors were tested using interaction terms and differences in risk by subtype were tested using multicategorical (polytomous) regression.

SNPs reported in Tables 1–3, were additionally tested for confounding by the following epidemiologic risk factors in the subset of study sites with information on each epidemiologic variable: acetaminophen use [non-regular (<1 /week), regular (1 /week)], aspirin use [non-regular (<1 /week), regular (1 /week)], non-aspirin NSAID use [non-regular (<1 /week), regular (1 /week)], young adult body mass index (BMI) [continuous

(age 18 or 20 years)], recent BMI [continuous (one or five years prior to diagnosis)], history of endometriosis (yes, no), history of breast or ovarian cancer in a first-degree relative (none, one or more relatives), age at menarche (≤ 11 , >11 years), menopausal status at diagnosis (pre/peri, post), ever use of oral contraceptives (yes, no), and ever use of estrogen after age 50 (yes, no). None of these variables changed the estimates by more than 10% for any of the SNPs with sufficient numbers in the subsets to calculate stable estimates.

Pairwise LD among controls was estimated using PLINK (42). Results ($-\log_{10}(\text{p-value})$) for regions of interest were visualized using LocusZoom (Standalone Version) (43), which included user-specified LD as defined above. The SNP examined in a previous study, *IL1A* rs17561, was re-evaluated in this study for replication purposes using a nominal p-value of 0.05. We used a modified Bonferroni adjusted critical value to determine statistical significance of all other newly studied NF- κ B SNPs. To account for LD between SNPs, a qr decomposition of the SNP genotype matrix (44) was used to determine the effective number of independent tests. Genotypes for 2282 NF- κ B pathway SNPs with a MAF > 0.01 from a random sample of 1000 epithelial ovarian cancer cases and 1000 controls were considered. The number of independent tests (i.e. the rank of the SNP genotype matrix) was determined to be 2000, thus yielding a Bonferroni adjusted critical value of 2.5×10^{-5} (0.05/number of independent tests).

RESULTS

Replication of *IL1A* SNP rs17561 in ovarian cancer risk

The missense SNP, rs17561, in the *IL1A* gene, previously reported by our group to be significantly associated with clear cell, mucinous, and endometrioid ovarian cancer risk in a subset of OCAC studies (3972 cases and 3043 controls) (16), was reevaluated using a larger number of participants (15,604 cases and 23,235 controls). This included 6,179 HGS, 2,100 endometrioid, 1,591 mucinous, 1,034 clear cell, and 1,016 LGS ovarian cancer cases. In this larger pooled study, we found no association between rs17561 and risk of all ovarian cancer; however, when we stratified by histologic subtype, we found modest inverse associations with the minor allele of this SNP and risk of endometrioid (OR=0.93, 95% CI: 0.87–1.00; $p=0.053$) and mucinous subsets (OR=0.91, 95% CI: 0.84–0.98; $p=0.018$) and a stronger inverse association with the minor allele of this SNP and clear cell ovarian cancer (OR=0.84, 95% CI: 0.76–0.93; $p=0.00075$) (Figure 1). As the previous report of rs17561 describing an association with clear cell (N=283 cases) ovarian cancer included a subset of the current study population (16), we restricted our analysis to exclude all participants from the prior study and found that the inverse association between the minor allele of this SNP and risk of clear cell (N=734 cases) disease remained (OR=0.85, 95% CI: 0.75–0.95; $p=0.006$).

The major allele of rs17561 has also recently been reported to be associated with increased risk of endometriosis in a pooled Japanese case-control study (45). History of endometriosis was obtained for several studies in OCAC via self-report. Given the link between endometriosis and clear cell ovarian cancer (11), we chose to assess the association between endometriosis and rs17561 in the European ancestry OCAC population, where we observed the association between this SNP and clear cell ovarian cancer. While we found a trend in the direction of decreased risk of endometriosis with the minor allele of rs17561 (OR=0.93, 95% CI: 0.82–1.05) among the 10,759 controls with available genotype and endometriosis information, it was not statistically significant ($p=0.25$).

We additionally evaluated whether any of the epidemiologic risk factors for ovarian cancer listed in Supplementary Table 5 modified the association between rs17561 and risk of clear cell ovarian cancer. There was little evidence for interaction between rs17561 and any of

these factors, with the exception of a modest interaction with NSAID use ($p=0.046$). When stratified by NSAID use, the inverse association between rs17561 and clear cell ovarian cancer risk was observed among regular NSAID users (OR=0.71, 95% CI: 0.54–0.95), but null among non-regular NSAID users (OR=1.01, 95% CI: 0.84–1.20).

Overall ovarian cancer risk associations with NF- κ B pathway SNPs

A total of 2,281 additional SNPs in 210 genes in the NF- κ B were also analyzed. When ranked by p-value, the most significant SNPs in the NF- κ B pathway found to be associated with overall (includes LMP) ovarian cancer risk at $p<0.005$ were located in *CARD11*, *FBXW7*, *ILIRAPL2*, *IRAK2*, *MAP3K14*, *NFKB1*, *PRKCA*, *TAF3*, *TLR7*, *TNFRSF1B*, and *TNFSF10* genes (Table 1); however, none of these SNPs reached statistical significance after multiple testing correction. A *CARD11* SNP rs74302019 had the lowest p-value (OR=1.07, 95% CI: 1.03–1.10; $p=8.9110^{-05}$), and four out of 57 SNPs tagged in *CARD11* were associated with ovarian cancer risk at $p<0.005$, although rs41324349 and rs41483047 were in moderate LD with rs74302019 with $r^2=0.61$ and 0.41, respectively.

Tumor behavior associations with NF- κ B pathway SNPs

We also assessed NF- κ B pathway SNPs according to tumor behavior (invasive or LMP). All SNPs associated with tumor behavior at $p<0.005$ are reported in Table 2. SNPs in *ILIRAPL2*, *OTUD7B*, *PLCG1*, *TAF4*, *TLR5*, *TNFSF10*, and *TRAF2* were suggestively associated with LMP ovarian tumors at $p<0.005$. One SNP in *TNFSF10* was statistically significantly associated with LMP risk after adjustment for multiple testing, rs6785617 (OR=0.85, 95% CI: 0.79–0.91; $p=2.0 \times 10^{-5}$). We further evaluated this association for effect modification by epidemiologic risk factors previously reported in association with ovarian cancer, but we found little evidence for interaction (Supplementary Table 5).

No NF- κ B pathway SNPs were associated with risk of invasive ovarian cancer at $p<2.5 \times 10^{-5}$. However, the SNP associated with risk of invasive ovarian cancer with the lowest p-value was rs7071113 (OR=1.06, 95% CI: 1.02–1.10; $p=0.00087$) in *TAF3* and suggestive associations were observed at $p<0.005$ for other SNPs in *TAF3* as well as *CARD11*, *FBXW7*, *ILIRN*, *IRAK2*, *MAP3K7*, *TAB2*, *PRKDC*, and *TNFRSF1B*.

Histologic subtype associations with NF- κ B pathway SNPs

While no SNPs were associated with risk at the corrected level of 2.5×10^{-5} for any histologic subtypes (Table 3), the missense SNP rs17561 (reported above) in the *ILIA* gene, was the NF- κ B pathway SNP that had the lowest p-value in association with clear cell ovarian cancer risk (OR=0.84, 95% CI: 0.76–0.93; $p=0.00075$); four other *ILIA* SNPs, rs1800587, rs1304037, rs2856836, and rs1800794 were also inversely associated with ovarian cancer risk at $p<0.005$ as expected based on near complete LD with rs17561 ($r^2>0.99$). Other SNPs that were suggestively associated with clear cell ovarian cancer at $p<0.005$ were found in *AKT1*, *BCL10*, *CD3E*, *IKBKE*, *ILIRN*, *NFKBIZ*, *PPARG*, *TLR3*, and *TLR7*. For endometrioid ovarian cancer *MTOR* SNP, rs12129467, had the lowest p-value with a suggestive association (OR=1.19, 95% CI: 1.07–1.33; $p=0.0013$); this SNP was the only tagSNP in this gene of 10 genotyped with $p<0.005$ (data not shown). Other SNPs with potential associations for endometrioid ovarian cancer risk at $p<0.005$ were found in the *F2R*, *IKBKAP*, and *HNRNPAB* genes. Mucinous ovarian cancer was potentially ($p<0.005$) associated with SNPs in *CD247*, *ILIA*, *PRKCA*, *PRKCQ*, *PRKCZ*, *PTPN13*, *TLR1*, *TLR10*, and *TNFSF10*. The SNP with the lowest p-value was rs34251715, an intronic SNP in *PRKCA* (OR=0.88, 95% CI: 0.82–0.96; $p=0.0028$).

The SNPs suggestively associated with risk of high-grade serous ovarian cancer at $p<0.005$ were located in *AARB2*, *CARD11*, *ILIRN*, *MAP3K14*, *PIK3R1*, *PRKCA*, *PRKCZ*,

PRKDC, *TLR5*, and *TNFRSF1B*. *CARD11* SNP rs71527417 had the lowest p-value for HGS ovarian cancer risk (OR=0.87, 95% CI: 0.80–0.95; p= 0.0015), although the association was not significant at the multiple comparisons threshold. Two other SNPs in this gene, rs74302019 and rs41324349, were also associated with HGS, at p<0.005 and the association was in the opposite direction (LD with rs71527417: $r^2=0.03$ and 0.05, respectively). For LGS ovarian cancer risk, the association with the lowest p-value was with intronic SNP, rs3136646, located in *NFKB1B* (OR=0.81, 95% CI: 0.72–0.91; p=0.00034). Additional possible associations with LGS at p<0.005 included SNPs from *GSK3B*, *IKBKAP*, and *PRKCA*.

DISCUSSION

In this large study of 15,604 ovarian cancer cases and 23,235 controls of European descent, we assessed the rs17561 SNP, previously found by our group to be associated with overall ovarian cancer risk. When analyzed by histologic subtypes, there were modest associations with risk of mucinous and endometrioid subtypes and a fairly strong association with risk of clear cell, all of which are consistent with our previous study (16). The clear cell association remained even after exclusion of participants in the prior report. In this same large study population, assessment of additional variants in over 200 genes in the NF- κ B pathway pointed to some suggestive associations with ovarian cancer risk. The most significant SNPs associated with each subtype tended to fall in different genes. However, with the exception of rs6785617 and LMP tumors, none of these SNPs reached our critical p-value of 2.5×10^{-5} .

The missense SNP in *IL1A*, rs17561, results in an amino acid change at position 114 from alanine (major allele) to serine (minor allele). Enhanced cleavage of the IL-1 α precursor (46) has been reported to be the functional consequence of a serine residue at this position and calpain cleaved IL-1 α appears to bind IL-1R1 with higher affinity, resulting in higher cytokine expression than the uncleaved form (47). The major allele (A) of rs17561, has recently been reported to be associated with increased susceptibility to endometriosis in two independent case-control studies in a Japanese population (45). This is consistent with our finding in the present study that the minor allele is associated with decreased risk of clear cell ovarian cancer, and is especially interesting given the previous associations found between endometriosis and clear cell ovarian cancer (11), suggesting a potential shared biological mechanism. When we evaluated this SNP for association with endometriosis in the European ancestry OCAC population, we saw little evidence for an association between rs17561 and endometriosis. The lack of association in the OCAC population could potentially be attributed to other genetic differences between Japanese and European ancestry populations. However, we also note that in the present study we are limited by questionnaire-based self-reported history of endometriosis, while the Japanese study used clinical imaging or biopsy confirmation to ascertain diagnosis of endometriosis.

Recently, Trabert et al reported a statistically significant association between regular aspirin use and a modest non-significant association with non-aspirin NSAID use and decreased risk of invasive ovarian cancer in the OCAC population (15). Interestingly, we find that the association between rs17561 and clear cell risk appears to be modified by non-aspirin NSAID use, where the inverse association with the minor allele is found among regular NSAID users but is null in non-regular NSAID users. The role of IL-1 α on tumor development is complex; depending on whether it has been processed, whether it is membrane-bound or secreted, and which stage in tumorigenesis and cell type it is expressed, it may play a role in immune surveillance or tumor progression (48). One potential mechanism through which NSAIDs may influence the effects of IL-1 α on tumor growth is through inhibition of prostaglandin synthesis by COX-2 (49), which is expressed following

IL-1 α signalling through IL-1R1. NSAID use has also been reported to interact with *IL1* SNP haplotypes in risk of B-cell non-Hodgkin lymphoma (50).

TNFSF10, also known as TRAIL, induces a signaling cascade that leads to apoptosis upon binding either of its cognate death receptors, DR4 and DR5 (51). This ligand has been of particular interest for use in cancer therapy, as many cancer cell types are more sensitive to TNFSF10 induced cell death than normal cells (52). TRAIL is also important in immune surveillance of tumor cells (53) and plays a role in controlling inflammation by inducing apoptosis in macrophages (54) and neutrophils (55). The only novel NF- κ B SNP to pass our significance threshold was *TNFSF10* SNP, rs6785617, in association with LMP tumor behavior. This SNP falls 4.5 kb downstream of this gene and to our knowledge it has not previously been reported to be associated with ovarian tumors or other conditions, nor have consequences of this SNP on expression or function been tested experimentally.

CARD11 is an intermediate protein that assists in NF- κ B activation following B or T cell receptor complex ligation (56–58) or activation of NK cell receptors (59). *CARD11* intronic SNPs, rs74302019, rs41324349, or rs41483047 are in moderate LD with each other and had the lowest p-values associated with overall ovarian cancer risk. To our knowledge none of the have been previously assessed for associations with ovarian cancer or other conditions and their consequences on *CARD11* function or expression are unclear. Although aberrant expression in tumors is possible, *CARD11*, also known as *CARMA1*, is normally expressed in cells of hematopoietic origin (60) suggesting that the role of this polymorphism in ovarian cancer risk may be related to tumor surveillance by immune cells.

This study has several strengths, the most notable of which is the very large sample size which provided greater power than all previous candidate gene studies in ovarian cancer to detect associations between this disease and SNPs with lower MAF. We also had greater power to assess associations between the rare subtypes: endometrioid, clear cell, and mucinous ovarian cancer. We used a SNP tagging approach to comprehensively cover genes in the NF- κ B pathway; however the study was limited by lack of coverage of some genes, mostly due to loss of SNPs that failed QC. Nonetheless, this is the first study to extensively assess variation in genes involved in NF- κ B activation, including signaling, inhibition, degradation, and nuclear function in association with ovarian cancer risk. Because of variation in MAF by race, we restricted our analysis to participants of genetic European descent, which reduces confounding but also generalizability to other populations. Because only one SNP was associated with risk of LMP tumors below the multiple test corrected p-value, we cannot rule out that any of the suggestive associations were actually false positives.

In conclusion, this large study of NF- κ B pathway genes in relation to risk of ovarian cancer risk found several SNPs with suggestive associations that varied by histology and tumor behavior. All SNP associations were modest, but most interesting were the replication of *IL1A* SNP, rs17561, in clear cell risk and the association between *TNFSF10* SNP, rs6785617, and LMP ovarian cancer. Future investigations of interactions between these polymorphisms and environmental factors, the role they play on tumor phenotypes, and how they affect NF- κ B activity in different cell types are needed to better understand the mechanism by which they might be contributing to ovarian cancer pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Authors

Bridget Charbonneau¹, Matthew S. Block², William R. Bamlet³, Robert A. Vierkant³, Kimberly R. Kalli², Zachary Fogarty³, David N. Rider³, Thomas A. Sellers⁴, Shelley S. Tworoger^{5,6}, Elizabeth Poole^{5,6}, Harvey A. Risch⁷, Helga B. Salvesen^{8,9}, Lambertus A. Kiemeny^{10,11,12}, Laura Baglietto^{13,14}, Graham G. Giles^{13,14,15}, Gianluca Severi^{13,14}, Britton Trabert¹⁶, Nicolas Wentzensen¹⁶, Georgia Chenevix-Trench¹⁷, for AOCs/ACS group^{17,18}, Alice S. Whittemore¹⁹, Weiva Sieh¹⁹, Jenny Chang-Claude²⁰, Elisa V. Bandera²¹, Irene Orlow²², Kathryn Terry^{6,23}, Marc T. Goodman²⁴, Pamela J Thompson²⁴, Linda S. Cook²⁵, Mary Anne Rossing^{26,27}, Roberta B. Ness²⁸, Steven A. Narod²⁹, Jolanta Kupryjanczyk³⁰, Karen Lu³¹, Ralf Butzow^{32,33}, Thilo Dörk³⁴, Tanja Pejovic^{35,36}, Ian Campbell^{37,38,39}, Nhu D. Le⁴⁰, Clareann H. Bunker⁴¹, Natalia Bogdanova³⁴, Ingo B. Runnebaum⁴², Diana Eccles⁴³, James Paul⁴⁴, Anna H. Wu⁴⁵, Simon A. Gayther⁴⁵, Estrid Hogdall^{46,47}, Florian Heitz^{48,49}, Stanley B. Kaye⁵⁰, Beth Y. Karlan⁵¹, Hoda Anton Culver⁵², Jacek Gronwald⁵³, Claus K. Hogdall⁵⁴, Diether Lambrechts^{55,56}, Peter A. Fasching^{57,58}, Usha Menon⁵⁹, Joellen Schildkraut^{60,61}, Celeste Leigh Pearce⁴⁵, Douglas A. Levine⁶², Susanne Kruger Kjaer^{46,54}, Daniel Cramer^{6,23}, James M. Flanagan⁶³, Catherine M. Phelan⁴, Robert Brown⁶³, Leon F.A.G. Massuger⁶⁴, Honglin Song⁶⁵, Jennifer A. Doherty⁶⁶, Camilla Krakstad^{8,9}, Dong Liang⁶⁷, Kunle Odunsi⁶⁸, Andrew Berchuck⁶⁹, Allan Jensen⁴⁶, Jan Lubiński⁵³, Heli Nevanlinna³², Yukie T. Bean^{35,36}, Galina Lurie⁷⁰, Argyrios Ziogas⁵², Christine Walsh⁵¹, Evelyn Despierre⁷¹, Louise Brinton¹⁶, Alexander Hein⁵⁷, Anja Rudolph²⁰, Agnieszka Dansonka-Mieszkowska³⁰, Sara H. Olson²², Philipp Harter^{48,49}, Jonathan Tyrer⁶⁵, Allison F. Vitonis²³, Angela Brooks-Wilson^{72,73}, Katja K. Aben^{10,12}, Malcolm C. Pike^{22,45}, Susan J. Ramus⁴⁵, Elisabeth Wik^{8,74}, Cezary Cybulski⁵³, Jie Lin⁷⁵, Lara Sucheston⁶⁸, Robert Edwards^{76,77}, Valerie McGuire¹⁹, Jenny Lester⁵¹, Andreas du Bois^{48,49}, Lene Lundvall⁵⁴, Shan Wang-Gohrke⁷⁸, Lukasz M Szafron³⁰, Sandrina Lambrechts⁷¹, Hannah Yang¹⁶, Matthias W. Beckmann⁵⁷, Liisa M. Peltari³², Anne M. Van Altena⁶⁴, David van den Berg⁴⁵, Mari K Halle^{8,9}, Aleksandra Gentry-Maharaj⁵⁹, Ira Schwaab⁷⁹, Urmila Chandran²¹, Janusz Menkiszak⁸⁰, Arif B. Ekici⁸¹, Lynne R Wilkens⁷⁰, Arto Leminen³², Francesmary Modugno^{41,76,77}, Grace Friel⁶⁸, Joseph H. Rothstein¹⁹, Ignace Vergote⁷¹, Montserrat Garcia-Closas⁸², Michelle A.T. Hildebrandt⁷⁵, Piotr Sobiczewski⁸³, Linda E. Kelemen^{84,85}, Paul D.P. Pharoah^{65,86}, Kirsten Moysich⁶⁸, Keith L. Knutson⁸⁷, Julie M. Cunningham⁸⁸, Brooke L. Fridley⁸⁹, and Ellen L. Goode^{1,*}

Affiliations

¹Department of Health Sciences Research, Division of Epidemiology, Mayo Clinic, Rochester, MN, USA ²Department of Medical Oncology, Mayo Clinic, Rochester, MN, USA ³Department of Health Sciences Research, Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA ⁴Department of Cancer Epidemiology, Division of Population Sciences, Moffitt Cancer Center, Tampa, FL, USA ⁵Channing Division of Network Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA ⁶Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA ⁷Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT, USA ⁸Department of Clinical Science, University of Bergen, Bergen, Norway ⁹Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway ¹⁰Department for Health Evidence, Radboud University Medical Centre, Nijmegen, The Netherlands ¹¹Department of Urology, Radboud University Medical Centre, Nijmegen, The Netherlands ¹²Comprehensive Cancer Center The Netherlands, Utrecht, The Netherlands ¹³Cancer Epidemiology Centre, The Cancer Council Victoria,

Melbourne, Australia ¹⁴Centre for Molecular, Environmental, Genetic and Analytical Epidemiology, University of Melbourne, Australia ¹⁵Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia ¹⁶Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA ¹⁷Cancer Division, Queensland Institute of Medical Research, Herston, QLD, Australia ¹⁸Peter MacCallum Cancer Institute, Melbourne, Australia ¹⁹Department of Health Research and Policy - Epidemiology, Stanford University School of Medicine, Palo Alto, CA, USA ²⁰German Cancer Research Center, Division of Cancer Epidemiology, Heidelberg, Germany ²¹The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ, USA ²²Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA ²³Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA ²⁴Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA ²⁵Division of Epidemiology and Biostatistics, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, USA ²⁶Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA ²⁷Department of Epidemiology, University of Washington, Seattle, WA, USA ²⁸The University of Texas School of Public Health, Houston, TX, USA ²⁹Women's College Research Institute, University of Toronto, Toronto, Ontario, Canada ³⁰Department of Pathology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland ³¹Department of Gynecologic Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA ³²Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland ³³Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland ³⁴Gynaecology Research Unit, Hannover Medical School, Hannover, Germany ³⁵Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR, USA ³⁶Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA ³⁷Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia ³⁸Department of Pathology, University of Melbourne, Parkville, Victoria, Australia ³⁹Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria, Australia ⁴⁰Cancer Control Research, BC Cancer Agency, Vancouver, BC, Canada ⁴¹Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA ⁴²Department of Gynecology, Jena University Hospital - Friedrich Schiller University Jena, Jena, Germany ⁴³Faculty of Medicine, University of Southampton, University Hospital Southampton, UK ⁴⁴The Beatson West of Scotland Cancer Centre, Glasgow, UK ⁴⁵Department of Preventive Medicine, Keck School of Medicine, University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA, USA ⁴⁶Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen, Denmark ⁴⁷Molecular Unit, Department of Pathology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark ⁴⁸Department of Gynecology and Gynecologic Oncology, Dr. Horst Schmidt Kliniken Wiesbaden, Wiesbaden, Germany ⁴⁹Department of Gynecology and Gynecologic Oncology, Kliniken Essen-Mitte/ Evang. Huysens-Stiftung/ Knappschaft GmbH, Essen, Germany ⁵⁰Division of Clinical Studies, The Institute of Cancer Research and the Royal Marsden Hospital, Sutton, UK ⁵¹Women's Cancer Program at the Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA ⁵²Department of Epidemiology, Center for Cancer Genetics Research and Prevention, School of Medicine, University of California Irvine, Irvine, CA, USA ⁵³International Hereditary Cancer Center, Department of Genetics and

Pathology, Pomeranian Medical University, Szczecin, Poland ⁵⁴The Juliane Marie Centre, Department of Obstetrics and Gynecology, Rigshospitalet, Copenhagen, Denmark ⁵⁵Vesalius Research Center, VIB, Leuven, Belgium ⁵⁶Laboratory for Translational Genetics, Department of Oncology, University of Leuven, Leuven, Belgium ⁵⁷University Hospital Erlangen, Department of Gynecology and Obstetrics, Friedrich-Alexander-University Erlangen-Nuremberg, Comprehensive Cancer Center, Erlangen, Germany ⁵⁸Department of Medicine, Division of Hematology and Oncology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA ⁵⁹Gynaecological Cancer Research Centre, Women's Cancer, Institute for Women's Health, University College London, London, UK ⁶⁰Department of Community and Family Medicine, Duke University Medical Center, Durham, NC, USA ⁶¹Cancer Prevention, Detection and Control Research Program, Duke Cancer Institute, Durham, NC, USA ⁶²Memorial Sloan-Kettering Cancer Center, New York, NY, USA ⁶³Department of Surgery and Cancer, Imperial College London, London, UK ⁶⁴Department of Gynaecology, Radboud University Medical Centre, Nijmegen, The Netherlands ⁶⁵Department of Oncology, University of Cambridge, Cambridge, UK ⁶⁶Section of Biostatistics and Epidemiology, The Geisel School of Medicine at Dartmouth, Lebanon, NH, USA ⁶⁷College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX, USA ⁶⁸Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY, USA ⁶⁹Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA ⁷⁰Cancer Epidemiology Program, University of Hawaii Cancer Center, HI, USA ⁷¹Division of Gynecologic Oncology, Department of Obstetrics and Gynecology and Leuven Cancer Institute, University Hospitals Leuven, Belgium ⁷²Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada ⁷³Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada ⁷⁴Department of Pathology, Haukeland University Hospital, Bergen, Norway ⁷⁵Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA ⁷⁶Women's Cancer Research Program, Magee-Women's Research Institute and University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA ⁷⁷Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA ⁷⁸Department of Obstetrics and Gynecology, University of Ulm, Ulm, Germany ⁷⁹Institut für Humangenetik Wiesbaden, Wiesbaden, Germany ⁸⁰Clinic of Gynaecological Surgery and Oncology, Pomeranian Medical University, Szczecin, Poland ⁸¹Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany ⁸²Division of Genetics and Epidemiology, Institute of Cancer Research, Sutton, UK and Breakthrough Breast Cancer Research Centre, London, UK ⁸³Department of Gynecologic Oncology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland ⁸⁴Alberta Health Services-Cancer Care, Department of Population Health Research, Alberta, Canada ⁸⁵Department of Medical Genetics and Oncology, University of Calgary, Calgary, AB, Canada ⁸⁶Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK ⁸⁷Department of Immunology, Mayo Clinic, Rochester, MN, USA ⁸⁸Department of Laboratory Medicine and Pathology, Division of Experimental Pathology, Mayo Clinic, Rochester, MN, USA ⁸⁹Department of Biostatistics, University of Kansas Medical Center, Kansas City, KS, USA

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IL1A rs17561

Histology	# Cases	OR (95% CI)
LGS	1016	1.01 (0.92,1.12)
HGS	6179	1.00 (0.96,1.05)
Mucinous	1591	0.91 (0.84,0.98)
Endometrioid	2100	0.93 (0.87,1.00)
Clear Cell	1034	0.84 (0.76,0.93)
Overall	15601	0.97 (0.94,1.00)

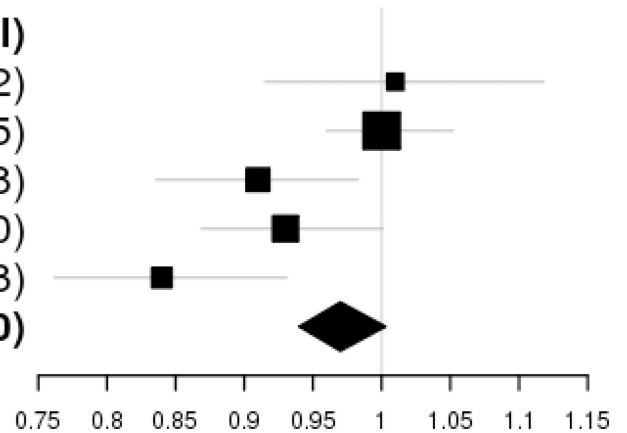


Figure 1.
IL1A SNP, rs17561, associations with risk of ovarian cancer by subtype. Forest plots of OR and 95% CI for HGS (high grade serous), LGS (low grade serous), mucinous, endometrioid, clear cell and overall ovarian cancer.

Table 1

Top NF-κB pathway SNPs (p<0.005) associated with overall ovarian cancer* risk

Gene	SNP ^a	Chrm	Location	Minor	Major	MAF (Case)	MAF (Control)	OR ^b	95% CI	p-value ^b
<i>TNFRSF1B</i>	rs17884213	1	intron	A	G	26.7	26	1.05	(1.02, 1.09)	0.002
<i>TNFSF10</i>	rs6801105	3	intron	A	G	16.7	17.4	0.94	(0.90, 0.98)	0.002
<i>IRAK2</i>	rs459483	3	intron	G	A	45.2	46.3	0.95	(0.93, 0.98)	0.003
<i>TNFSF10</i>	rs1131535	3	3'UTR	A	G	43.2	44.3	0.96	(0.93, 0.99)	0.004
<i>FBXW7</i>	rs75911772	4	intron	T	A	3.9	3.5	1.14	(1.05, 1.23)	0.002
<i>NFKB1</i>	rs1609993	4	synonymous	A	G	8.8	8	1.08	(1.03, 1.14)	0.003
<i>CARD11</i>	rs74302019	7	intron	A	G	32.2	31.1	1.07	(1.03, 1.10)	8.9×10⁻⁰⁵
<i>CARD11</i>	rs41324349	7	intron	A	C	43	41.6	1.06	(1.03, 1.09)	0.0002
<i>CARD11</i>	rs41483047	7	intron	A	G	18.5	17.6	1.07	(1.03, 1.12)	0.0004
<i>CARD11</i>	rs35329971	7	intron	A	G	8.8	9.6	0.92	(0.87, 0.97)	0.002
<i>TAF3</i>	rs7071113	10	3' downstream	C	G	31.8	30.8	1.06	(1.03, 1.10)	0.0003
<i>TAF3</i>	rs1244229	10	Val696Ala	A	G	30	29.1	1.06	(1.02, 1.09)	0.001
<i>TAF3</i>	rs263417	10	intron	A	C	30.2	29.4	1.05	(1.02, 1.09)	0.002
<i>TAF3</i>	rs1514233	10	intron	T	A	30.3	29.5	1.05	(1.02, 1.09)	0.003
<i>PRKCA</i>	rs7226221	17	intron	G	A	39.1	40.2	0.95	(0.92, 0.98)	0.002
<i>MAP3K14</i>	rs9908462	17	intron	A	G	19	19.8	0.94	(0.91, 0.98)	0.003
<i>IL1RAPL2</i>	rs1384360	23	intron	A	C	26.6	25.7	1.06	(1.02, 1.09)	0.001
<i>TLR7</i>	rs5743733	23	intron	G	C	8.8	8.1	1.09	(1.03, 1.15)	0.003

^a SNPs are listed first by chromosome and then ranked by ordinal p-value within the chromosome.

^b Ordinal OR and p-value, adjusted for first five principal components, age, and study site. Bold values highlight p<0.001.

* Includes invasive and borderline tumor behavior

Table 2

Top NF- κ B pathway SNPs ($p < 0.005$) associated with invasive and low malignant potential tumor behavior

Case Group	Gene	SNP ^a	Chrm	Location	Minor	Major	MAF (Case)	MAF (Control)	OR ^b	95% CI	p-value ^b	
Invasive (N=13,727)	<i>TNFRSF1B</i>	rs17884213	1	intron	A	G	26.8	26	1.06	(1.02,1.10)	0.00097	
	<i>IL1RN</i>	rs62161280	2	3' downstream	G	A	5.7	5.2	1.11	(1.03,1.18)	0.004	
	<i>IRAK2</i>	rs459483	3	intron	G	A	45.2	46.3	0.95	(0.92,0.98)	0.003	
	<i>FBXW7</i>	rs75911772	4	intron	T	A	3.9	3.5	1.13	(1.05,1.23)	0.003	
	<i>MAP3K7</i>	rs80138790	6	3' downstream	A	G	3.7	3.3	1.14	(1.05,1.24)	0.002	
	<i>TAB2</i>	rs573148	6	5' upstream	G	A	37	38.1	0.96	(0.92,0.99)	0.005	
	<i>CARD11</i>	rs41324349	7	intron	A	C	42.9	41.6	1.05	(1.02,1.09)	0.0009	
	<i>CARD11</i>	rs74302019	7	intron	A	G	32.1	31.1	1.06	(1.02,1.10)	0.0009	
	<i>CARD11</i>	rs41483047	7	intron	A	G	18.4	17.6	1.07	(1.03,1.11)	0.001	
	<i>PRKDC</i>	rs74915527	8	intron	A	G	10.5	9.8	1.08	(1.03,1.14)	0.003	
	<i>TAF3</i>	rs7071113	10	3' downstream	C	G	31.8	30.8	1.06	(1.02,1.10)	0.0009	
	<i>TAF3</i>	rs1244229	10	Val696Ala	A	G	29.9	29.1	1.05	(1.02,1.09)	0.004	
	LMP (N=1,729)	<i>OTUD7B</i>	rs41265172	1	3' UTR	A	G	4.8	3.9	1.3	(1.09,1.55)	0.004
		<i>TLR5</i>	rs2241097	1	intron	C	A	27.1	25.4	1.13	(1.04,1.23)	0.004
<i>TNFSF10</i>		rs6785617	3	3' downstream	T	A	43.1	46.2	0.85	(0.79,0.91)	2.0x10⁻⁰⁵	
<i>TNFSF10</i>		rs6801105	3	intron	A	G	15.6	17.4	0.84	(0.76,0.93)	0.0008	
<i>TRAF2</i>		rs17243893	9	intron	G	A	4.8	5.7	0.75	(0.63,0.88)	0.0007	
<i>PLCG1</i>		rs12625708	20	intron	A	C	18.7	21	0.86	(0.78,0.94)	0.001	
<i>TAF4</i>		rs744779	20	intron	A	G	23.8	22	1.14	(1.05,1.25)	0.003	
<i>IL1RAPL2</i>		rs1384360	23	intron	A	C	28.6	25.7	1.15	(1.06,1.25)	0.0009	

^a SNPs are listed first by chromosome and then ranked by ordinal p-value within the chromosome.

^b Ordinal OR and p-value, adjusted for first five principal components, age, and study site. Bold values highlight $p < 0.001$.

Table 3
 Top *NF-κB* pathway SNPs ($p < 0.005$) associated with risk of ovarian cancer histologic subtypes*

Case Group	Gene	SNP ^a	Chrm	Location	Minor	Major	MAF (Case)	MAF (Control)	OR ^b	95% CI	p-value ^b	
HGS (N=6,179)	<i>TLR5</i>	rs116693072	1	5' upstream	G	A	4.5	4	1.18	(1.06,1.30)	0.002	
	<i>TNFRSF1B</i>	rs17884213	1	intron	A	G	26.9	26	1.07	(1.02,1.13)	0.004	
	<i>PRKCZ</i>	rs9729600	1	intron	A	G	9.5	9.3	1.11	(1.03,1.19)	0.005	
	<i>IL1RN</i>	rs62161280	2	3' downstream	G	A	5.8	5.2	1.14	(1.04,1.25)	0.004	
	<i>PIK3R1</i>	rs72757693	5	intron	A	G	13.9	13.2	1.1	(1.03,1.17)	0.003	
	<i>PIK3R1</i>	rs12755	5	3'UTR	A	C	17.8	16.8	1.09	(1.03,1.15)	0.004	
	<i>CARD11</i>	rs71527417	7	intron	A	C	6.3	7.2	0.87	(0.80,0.95)	0.002	
	<i>CARD11</i>	rs74302019	7	intron	A	G	32.3	31.1	1.07	(1.03,1.12)	0.003	
	<i>CARD11</i>	rs41324349	7	intron	A	C	43.1	41.6	1.06	(1.02,1.11)	0.005	
	<i>PRKDC</i>	rs74915527	8	intron	A	G	10.6	9.8	1.11	(1.04,1.19)	0.004	
	<i>MAP3K14</i>	rs117642368	17	5' upstream	G	C	11.6	10.5	1.1	(1.03,1.18)	0.004	
	<i>ARRB2</i>	rs28365157	17	intron	A	G	9.6	10	0.9	(0.84,0.97)	0.005	
	<i>PRKCA</i>	rs28733563	17	intron	A	G	24.7	23.4	1.07	(1.02,1.13)	0.005	
	Endometrioid (N=2,100)	<i>MTOR</i>	rs12129467	1	intron	C	G	10.2	8.7	1.19	(1.07,1.33)	0.001
		<i>F2R</i>	rs253073	5	intron	G	A	46.2	43.7	1.11	(1.04,1.18)	0.002
		<i>HNRNPAB</i>	rs116592017	5	5' upstream	A	C	2.1	2.9	0.72	(0.58,0.89)	0.003
		<i>IKBKAP</i>	rs2230792	9	Gly765Ala	A	G	20.3	18.6	1.13	(1.04,1.22)	0.004
Mucinous (N=1,591)	<i>PRKCZ</i>	rs34415348	1	intron	C	A	9.2	10.8	0.83	(0.73,0.94)	0.004	
	<i>CD247</i>	rs1773539	1	intron	A	G	4.9	3.9	1.29	(1.08,1.53)	0.004	
	<i>IL1A</i>	rs150712565	2	intron	A	T	32.5	29.9	1.12	(1.04,1.22)	0.004	
	<i>TNFSF10</i>	rs12488654	3	5' upstream	A	G	18.9	16.7	1.15	(1.05,1.26)	0.004	
	<i>PTPN13</i>	rs62308410	4	intron	A	G	46.5	43.9	1.12	(1.04,1.20)	0.004	
	<i>TLR1</i>	rs743551	4	5' upstream	G	A	20.9	23.9	0.88	(0.80,0.96)	0.004	
	<i>TLR10</i>	rs4274855	4	5'UTR	A	G	15.3	17.7	0.86	(0.78,0.96)	0.005	
	<i>PRKCQ</i>	rs4750528	10	intron	G	A	15.5	17.6	0.86	(0.78,0.95)	0.004	
	<i>PRKCA</i>	rs34251715	17	intron	G	A	28.9	31.8	0.88	(0.82,0.96)	0.003	

Case Group	Gene	SNP ^a	Chrm	Location	Minor	Major	MAF (Case)	MAF (Control)	OR ^b	95% CI	p-value ^b
Clear Cell (N=1,034)	<i>IKBKE</i>	rs41296022	1	intron	A	G	4.7	3.4	1.37	(1.11,1.70)	0.004
	<i>BCL10</i>	rs2735593	1	5' UTR	C	G	24.1	21.5	1.16	(1.05,1.29)	0.005
	<i>IL1A</i>	rs17561	2	Ala114Ser	A	C	26.8	30.2	0.84	(0.76,0.93)	0.0008
	<i>IL1A</i>	rs1800587	2	5'UTR	A	G	26.8	30.2	0.84	(0.76,0.93)	0.0008
	<i>IL1A</i>	rs1304037	2	3'UTR	G	A	26.8	30.1	0.84	(0.76,0.93)	0.0008
	<i>IL1A</i>	rs2856836	2	3'UTR	G	A	26.8	30.2	0.85	(0.76,0.93)	0.00099
	<i>IL1A</i>	rs1800794	2	5' upstream	A	G	26.7	30	0.85	(0.77,0.94)	0.001
	<i>IL1RN</i>	rs2071459	2	intron	A	G	10.6	13	0.81	(0.70,0.94)	0.004
	<i>NFKB1Z</i>	rs80099440	3	intron	A	G	6.5	4.9	1.36	(1.13,1.63)	0.001
	<i>PPARG</i>	rs77323418	3	3' downstream	G	A	3.4	4.7	0.7	(0.55,0.89)	0.004
	<i>TLR3</i>	rs66624661	4	intron	A	G	36.5	33.7	1.14	(1.04,1.25)	0.005
	<i>CD3E</i>	rs73014299	11	3' downstream	A	G	8.8	11.1	0.78	(0.67,0.91)	0.002
	<i>AKT1</i>	rs45531934	14	intron	A	G	5.6	7.2	0.74	(0.60,0.90)	0.003
	<i>TLR7</i>	rs5743733	23	intron	G	C	10.1	8.1	1.26	(1.09,1.46)	0.002
LGS (N=1,016)	<i>GSK3B</i>	rs13320980	3	intron	G	A	28.7	31.9	0.86	(0.78,0.96)	0.005
	<i>IKBKAP</i>	rs10117384	9	intron	A	G	18.5	21.7	0.84	(0.74,0.94)	0.003
	<i>PRKCA</i>	rs7226221	17	intron	G	A	36.9	40.2	0.87	(0.79,0.96)	0.004
	<i>NFKB1B</i>	rs3136646	19	intron	A	G	20.3	23.8	0.81	(0.72,0.91)	0.0003

* Subtype analyses included invasive and LMP cases.

^a SNPs are listed first by chromosome and then ranked by ordinal p-value within the chromosome.

^b Ordinal OR and p-value, adjusted for first five principal components, age, and study site. Bold values highlight p<0.001.