Risk of Ovarian Cancer and the NF-κB Pathway: Genetic Association with IL1A and TNFSF10


Abstract

A missense single-nucleotide polymorphism (SNP) in the immune modulatory gene IL1A has been associated with ovarian cancer risk (rs17561). Although the exact mechanism through which this SNP alters ovarian cancer risk is not clearly understood, rs17561 has also been associated with risk of endometrioid, an epidemiologic risk factor for ovarian cancer. Interleukin-1α (IL1A) is both regulated by and able to activate NF-κB, a transcription factor family that induces transcription of many proinflammatory genes and may be an important mediator in carcinogenesis. We therefore tagged SNPs in more than 200 genes in the NF-κB transcription factor family that induces transcription of many proinflammatory genes and may be an important mediator in carcinogenesis. We therefore tagged SNPs in more than 200 genes in the NF-κB 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 2,016 low-grade serous, including 23,235 control cases spanning 40 studies in the Ovarian Cancer Association Consortium. In this large population, we confirmed the association between rs17561 and clear cell ovarian cancer [OR, 0.91; 95% confidence interval (CI), 0.85–0.97; 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 × 10⁻⁴, only one other variant, the TNFSF10 SNP rs6785617, was associated significantly with risk of ovarian cancer (low malignant potential tumors OR, 0.95; 95% CI, 0.91–1.01; 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. Cancer Res; 74(3); 852–61. © 2013 AACR.
Introduction

Inflammation is a known mediator of carcinogenesis and several risk factors associated with ovarian cancer are also linked to inflammatory processes (1). The inverse relationship between parity (2, 3) and oral contraceptive use (3–6) and ovarian cancer risk is thought to be due to increased ovulations in women with fewer pregnancies or shorter duration of oral contraceptive 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 (LGS) tumors (11). Studies of perineal talcum powder use additionally suggest an association with ovarian cancer risk (12), presumably due to its proinflammatory properties (13). Use of nonsteroidal anti-inflammatory drugs (NSAID) 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 single-nucleotide polymorphisms (SNP) 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. Interleukin-1α (IL1A), 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-κB pathway genes, as IL1A is not only...
produced following NF-κB activation (19), but signaling of IL1A through its receptor also results in downstream activation of NF-κB (20), which leads to transcription of a number of genes whose products promote inflammation (21).

In addition to the prior association, there is strong biologic support for further study of polymorphisms in the NF-κB pathway in ovarian cancer (21). This pathway seems 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 proinflammatory cytokines (23) with the ability to promote tumor growth (24). Specifically, activation of NF-κB through inhibitor of IκB kinase-ε (IKKε) was shown to be associated with more aggressive behavior in ovarian cancer cell lines (25). In addition, NF-κB activation can inhibit apoptosis (26). Finally, NF-κB activation has been associated with aberrant cellular activities in endometriosis (27). Therefore, we expanded our investigation of inflammation-related SNPs to include variants in more than 200 NF-κB pathway-related genes in a large collection of patients with ovarian cancer and controls from the Ovarian Cancer Association Consortium (OCAC).

Patients and 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, and 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 S1 and the number of cases by histologic subtype is shown in Supplementary Table S2. A total of 47,092 women were included.

SNP selection

As reviewed previously (21), we identified a number of genes known to encode NF-κB subunits or molecules key to NF-κB 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 minor allele frequency (MAF) \( \geq 0.05 \) 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, with European MAF \( \geq 0.05 \) that were 1 kb upstream, nonsynonymous, or resided in a 3′-untranslated region (UTR), 5′-UTR, splice site, or miRNA-binding site (34, 35). We used SNPpicker (36) to optimally pick tagSNPs for each gene. SNPs that had an Illumina design score \(<0.4 \) or that were in linkage disequilibrium \( (r^2 > 0.80) \) 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 S3.

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 (more than 200,000 SNPs) at McGill University and Genome Québec (Montreal, QC, Canada; \( n = 19,806 \)) and the Mayo Clinic Medical Genome Facility (Rochester, MN; \( n = 28,820 \)) 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: (i) no genotype call; (ii) monomorphism; (iii) call rate less than 95% and MAF > 0.05 or call rate less than 99% with MAF < 0.05; (iv) evidence of deviation from Hardy–Weinberg equilibrium \((P < 10^{-5}) \) in controls; and (v) greater than 2% discordance in duplicate pairs. Overall, 94.5% of SNPs passed quality control; a total of 2,282 NF-κB 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: (i) call rates < 95%; (ii) heterozygosity > five standard deviations from the intercontinental ancestry-specific mean heterozygosity; (iii) ambiguous sex; (iv) lowest call rate from a first-degree relative pair; (v) missing case–control status; (vi) missing age at diagnosis; (vii) nonepithelial cancer, unknown if epithelial cancer or missing histology; (viii) Brenner tumors; and (ix) <90 European ancestry based on local ancestry in admixed populations (LAMP; ref. 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 S4).

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 [LGS, high-grade serous (HGS), mucinous, endometrioid, and clear cell]. We used a subset of 37,000 non-NF-κB markers to perform principal component (PC) analysis within the European subset 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. OR, 95% confidence intervals (CI), and \( P \) values were generated using logistic regression analysis in PLINK (Version 1.07; ref. 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 multivariate (polynomial) 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 [nonregular (<1 ×/week), regular (≥1 ×/week)], aspirin use [nonregular (<1 ×/week), regular (≥1 ×/week)], non-aspirin NSAID use [nonregular (<1 ×/week), regular (≥1 ×/week)], young adult body mass index [BMI: continuous (age 18 or 20 years)], recent BMI [continuous (1 or 5 years before diagnosis)], history of endometriosis (yes, no), history of breast or ovarian cancer in a first-degree relative (none, ≥1 relative), 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 of 50 years (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 linkage disequilibrium among controls was estimated using PLINK (42). Results [−log_{10}(P value)] for regions of interest were visualized using LocusZoom (Stand-alone Version; ref. 43), which included user-specified linkage disequilibrium as defined above. The SNP examined in a previous study, IL1A rs17561, was reevaluated 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-kB SNPs. To account for linkage disequilibrium between SNPs, a qr decomposition of the SNP genotype matrix (44) was used to determine the effective number of independent tests. Genotypes for 2,282 NF-kB pathway SNPs with a MAF > 0.01 from a random sample of 1,000 epithelial ovarian cancer cases and 1,000 controls were considered. The number of independent tests (i.e., the rank of the SNP genotype matrix) was determined to be 2,000, 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 (3,972 cases and 3,043 controls; ref. 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; Fig. 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. Although 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 S5 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 nonregular NSAID users (OR, 1.01; 95% CI, 0.84–1.20).

**Overall ovarian cancer risk associations with NF-kB pathway SNPs**

A total of 2,281 additional SNPs in 210 genes in the NF-kB were also analyzed. When ranked by P value, the most significant SNPs in the NF-kB pathway found to be associated with overall (includes LMP) ovarian cancer risk at P < 0.005 were located in CARD11, FBXW7, IL1RAPL2, 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 = 8.91 × 10^{-6}), and four of 57 SNPs tagged in CARD11 were associated with ovarian cancer risk at P < 0.005, although rs41324349 and rs41483047 were in moderate linkage disequilibrium with rs74302019 with r^2 = 0.61 and 0.41, respectively.

**Tumor behavior associations with NF-kB pathway SNPs**

We also assessed NF-kB 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 IL1RAPL2, OTUD7B, PLCG1, TAF4, TRIB5, 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 S5).

No NF-κB 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, IL1RN, IRAK2, MAP3K7, TAB2, PRKDC, and TNFRSF1B.

**Histologic subtype associations with NF-κB pathway SNPs**

Although no SNPs were associated with risk at the corrected level of $2.5 \times 10^{-5}$ for any histologic subtypes (Table 3), the missense SNP rs17561 (reported above) in the IL1A gene, was the NF-κB 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 IL1A SNPs, rs1800887, rs1304037, rs2856836, and rs1800794 were also inversely associated with ovarian cancer risk at $P < 0.005$ as expected on the basis of near complete linkage disequilibrium 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, IKKBE, IL1RN, NFKB1Z, PPARG, TL1B, and TL87. 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 HNRNPA2 genes. Mucinous ovarian cancer was potentially ($P < 0.005$) associated with SNPs in CD247, IL1A, PRKCA, PRKCC, PRKCZ, PTPIN3, TL1B, TL1R0, 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 HGS ovarian cancer at $P < 0.005$ were located in AARB2, CARD11, IL1RN, MAP3K14, PIK3R1, PRKCA, PRKCC, PRKDC, TRIB5, 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 (linkage disequilibrium 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, rs3366646, 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.

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*\(^a\)Includes invasive and borderline tumor behavior.

*\(^b\)SNPs are listed first by chromosome and then ranked by ordinal $P$ value within the chromosome.

*\(^c\)Ordinal OR and $P$ value, adjusted for first five PCs, age, and study site. Bold values highlight $P < 0.001$. 

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Table 1. Top NF-κB pathway SNPs ($P < 0.005$) associated with overall ovarian cancer risk

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NF-κB SNPs and Ovarian Cancer Risk

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 used clinical imaging or biopsy confirmation to ascertain diagnosis of endometriosis.

Recently, Trabert and colleagues reported a statistically significant association between regular aspirin use and a modest nonsignificant 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 seems 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-aspirin NSAID users. The role of IL1A 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).

Table 2. Top NF-κB pathway SNPs (P < 0.005) associated with invasive and LMP tumor behavior

| Case group | Gene | SNP* | Chrm | Location | Minor | Major | MAF (case) | MAF (control) | OR* (95% CI) | P*
|------------|------|------|------|----------|-------|-------|------------|---------------|--------------|-------
| Invasive (N = 13,727) | TNFRSF1B | rs1784213 | 1 | Intron | A | G | 26.8 | 26 | 1.06 (1.02–1.10) | 0.00097
| LILR1N | rs6161280 | 2 | 3' Downstream | G | A | 5.7 | 5.2 | 1.11 (1.03–1.18) | 0.004
| IRAK2 | rs459483 | 3 | Intron | G | A | 45.2 | 46.3 | 0.95 (0.92–0.98) | 0.003
| FBXW7 | rs75911772 | 4 | Intron | T | A | 3.9 | 3.5 | 1.13 (1.05–1.23) | 0.003
| MAP3K7 | rs80138790 | 6 | 3' Downstream | A | G | 3.7 | 3.3 | 1.14 (1.05–1.24) | 0.002
| TAB2 | rs573148 | 6 | 5' Upstream | G | A | 37 | 38.1 | 0.96 (0.92–0.99) | 0.005
| CARD11 | rs14324349 | 7 | Intron | A | C | 42.9 | 41.6 | 1.05 (1.02–1.09) | 0.00099
| CARD11 | rs74302019 | 7 | Intron | A | G | 32.1 | 31.8 | 1.06 (1.02–1.10) | 0.00099
| CARD11 | rs41483047 | 7 | Intron | A | G | 18.4 | 17.6 | 1.07 (1.03–1.11) | 0.001
| PRKDC | rs74915527 | 8 | Intron | A | G | 10.5 | 9.8 | 1.08 (1.03–1.14) | 0.003
| TAF3 | rs7071113 | 10 | 3' Downstream | C | G | 31.8 | 30.8 | 1.06 (1.02–1.10) | 0.00099
| TAF3 | rs1244229 | 10 | Val696Ala | A | G | 29.9 | 29.1 | 1.05 (1.02–1.09) | 0.004
| OTUD7B | rs41265172 | 1 | Intron | A | G | 4.8 | 4.3 | 1.3 (1.09–1.55) | 0.004
| TLR5 | rs2241097 | 1 | Intron | C | A | 27.1 | 25.4 | 1.13 (1.04–1.23) | 0.004
| TNFSF10 | rs6756617 | 3 | 3' Downstream | T | A | 43.1 | 42.6 | 0.85 (0.79–0.91) | 2.0 × 10^-05
| TNFSF10 | rs6801105 | 3 | Intron | A | G | 15.6 | 17.4 | 0.84 (0.76–0.93) | 0.0006
| TRAF2 | rs17243893 | 9 | Intron | G | A | 4.8 | 5.7 | 0.75 (0.63–0.88) | 0.0007
| PLCG1 | rs12625708 | 20 | Intron | A | C | 18.7 | 21 | 0.86 (0.78–0.94) | 0.001
| TAF4 | rs744777 | 20 | Intron | A | G | 23.8 | 22 | 1.14 (1.05–1.25) | 0.003
| IL1RAPL2 | rs1384360 | 23 | Intron | A | C | 28.6 | 25.7 | 1.15 (1.06–1.25) | 0.00099

*aSNPs are listed first by chromosome and then ranked by ordinal P value within the chromosome.

**Ordinal OR and P value, adjusted for first five PCs, age, and study site. Bold values highlight P < 0.001.
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 (TNFRSF10A) and DR5 (TNFRSF10B; ref. 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-κB 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

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

| Case group  | Gene  | SNPa | Chrom Location | Minor Major | MAF (case) | MAF (control) | OR* (95% CI) | *P*
<table>
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</thead>
<tbody>
<tr>
<td>HGS (N = 6,179)</td>
<td>TLR5</td>
<td>rs116693072 1 5’ Upstream</td>
<td>G A</td>
<td>4.5 4</td>
<td>1.18 (1.06–1.30)</td>
<td>0.002</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TNFRSF18</td>
<td>rs17884213 1 5’ Upstream</td>
<td>A G</td>
<td>26.9 26</td>
<td>1.07 (1.02–1.13)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRKCZ</td>
<td>rs9729600 1 5’ Upstream</td>
<td>A G</td>
<td>9.5 9.3</td>
<td>1.11 (1.03–1.19)</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL1RN</td>
<td>rs6161280 2 3’ Downstream</td>
<td>G A</td>
<td>5.8 5.2</td>
<td>1.14 (1.04–1.25)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PIKR1</td>
<td>rs7257693 5 Intron</td>
<td>A G</td>
<td>13.9 13.2</td>
<td>1.1 (1.03–1.17)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CARD11</td>
<td>rs71527417 7 Intron</td>
<td>A C</td>
<td>6.3 7.2</td>
<td>0.87 (0.80–0.95)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CARD11</td>
<td>rs74302019 7 Intron</td>
<td>A G</td>
<td>32.3 31.1</td>
<td>1.07 (1.03–1.12)</td>
<td>0.003</td>
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<td></td>
<td>CARD11</td>
<td>rs41324349 7 Intron</td>
<td>A G</td>
<td>43.1 41.6</td>
<td>1.06 (1.02–1.11)</td>
<td>0.005</td>
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<tr>
<td></td>
<td>PRKDC</td>
<td>rs74915527 8 Intron</td>
<td>A G</td>
<td>10.6 9.8</td>
<td>1.11 (1.04–1.19)</td>
<td>0.004</td>
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<td></td>
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<tr>
<td>Endometrioid (N = 2,100)</td>
<td>MTOR</td>
<td>rs12129467 1 Intron</td>
<td>C G</td>
<td>10.2 8.7</td>
<td>1.19 (1.07–1.33)</td>
<td>0.001</td>
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<td>F3R</td>
<td>rs253073 5 Intron</td>
<td>G A</td>
<td>46.2 43.7</td>
<td>1.11 (1.04–1.18)</td>
<td>0.002</td>
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<td>HRNRNPAB</td>
<td>rs116592017 5 5’ Upstream</td>
<td>A C</td>
<td>2.1 2.9</td>
<td>0.72 (0.58–0.89)</td>
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<td>IKKBPAK</td>
<td>rs22307927 9 Gly</td>
<td>765AAla</td>
<td>20.3 18.6</td>
<td>1.13 (1.04–1.22)</td>
<td>0.004</td>
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<td>Mucinous (N = 1,591)</td>
<td>PRKCZ</td>
<td>rs34415348 1 Intron</td>
<td>C A</td>
<td>9.2 10.8</td>
<td>0.83 (0.73–0.94)</td>
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<td>CD247</td>
<td>rs1773539 1 Intron</td>
<td>A G</td>
<td>4.9 3.9</td>
<td>1.29 (1.08–1.53)</td>
<td>0.004</td>
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<td>IL1A</td>
<td>rs150712565 2 Intron</td>
<td>A T</td>
<td>32.5 29.9</td>
<td>1.12 (1.04–1.22)</td>
<td>0.004</td>
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<td>TNFSF10</td>
<td>rs12488654 3 5’ Upstream</td>
<td>A G</td>
<td>18.9 16.7</td>
<td>1.15 (1.05–1.26)</td>
<td>0.004</td>
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<td>TNFSF10</td>
<td>rs62308410 4 Intron</td>
<td>A G</td>
<td>46.5 43.9</td>
<td>1.12 (1.04–1.20)</td>
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<td>TLR1</td>
<td>rs5743551 4 Intron</td>
<td>G A</td>
<td>20.9 23.9</td>
<td>0.88 (0.80–0.96)</td>
<td>0.004</td>
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<td>TLR10</td>
<td>rs4274855 4 5’-UTR</td>
<td>A G</td>
<td>15.3 17.7</td>
<td>0.86 (0.78–0.96)</td>
<td>0.005</td>
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<td>PRKCQ</td>
<td>rs4705028 10 Intron</td>
<td>G A</td>
<td>15.5 17.6</td>
<td>0.86 (0.78–0.95)</td>
<td>0.004</td>
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<td>PRKCA</td>
<td>rs34251715 17 Intron</td>
<td>G A</td>
<td>28.9 31.8</td>
<td>0.88 (0.82–0.96)</td>
<td>0.003</td>
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<tr>
<td>Clear cell (N = 1,034)</td>
<td>IKKBK</td>
<td>rs41296022 1 Intron</td>
<td>A G</td>
<td>4.7 3.4</td>
<td>1.37 (1.11–1.70)</td>
<td>0.004</td>
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<td>BCL10</td>
<td>rs2735593 1 5’-UTR</td>
<td>C G</td>
<td>24.1 21.5</td>
<td>1.16 (1.05–1.29)</td>
<td>0.005</td>
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<td>IL1A</td>
<td>rs17561 2 Aflal14Ser</td>
<td>A C</td>
<td>26.8 30.2</td>
<td>0.94 (0.76–0.93)</td>
<td>0.0008</td>
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<td>IL1A</td>
<td>rs1800587 2 5’-UTR</td>
<td>A G</td>
<td>26.8 30.2</td>
<td>0.94 (0.76–0.93)</td>
<td>0.0008</td>
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<td>IL1A</td>
<td>rs1304037 2 3’-UTR</td>
<td>G A</td>
<td>26.8 30.1</td>
<td>0.94 (0.76–0.93)</td>
<td>0.0008</td>
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<td>IL1A</td>
<td>rs2856836 2 3’-UTR</td>
<td>G A</td>
<td>26.8 30.2</td>
<td>0.95 (0.76–0.93)</td>
<td>0.0009</td>
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<td>IL1A</td>
<td>rs1800794 2 5’ Upstream</td>
<td>A G</td>
<td>26.7 30</td>
<td>0.85 (0.77–0.94)</td>
<td>0.001</td>
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<td>IL1RN</td>
<td>rs2071459 2 Intron</td>
<td>A G</td>
<td>10.6 13</td>
<td>0.81 (0.70–0.94)</td>
<td>0.004</td>
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<td>NFKBIZ</td>
<td>rs80099440 3 Intron</td>
<td>A G</td>
<td>6.5 4.9</td>
<td>1.36 (1.13–1.63)</td>
<td>0.001</td>
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<td>PPARG</td>
<td>rs77323418 3 3’ Downstream</td>
<td>G A</td>
<td>3.4 4.7</td>
<td>0.7 (0.55–0.89)</td>
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<td>TLR3</td>
<td>rs66624561 4 Intron</td>
<td>A G</td>
<td>36.5 33.7</td>
<td>1.14 (1.04–1.25)</td>
<td>0.005</td>
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<td>CD3E</td>
<td>rs73014299 11 3’ Downstream</td>
<td>A G</td>
<td>8.8 11.1</td>
<td>0.78 (0.67–0.91)</td>
<td>0.002</td>
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<td>AKT1</td>
<td>rs45531934 14 Intron</td>
<td>A G</td>
<td>6.5 7.2</td>
<td>0.74 (0.60–0.90)</td>
<td>0.003</td>
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<td>TLR7</td>
<td>rs5743733 23 Intron</td>
<td>G C</td>
<td>10.1 8.1</td>
<td>1.26 (1.09–1.46)</td>
<td>0.002</td>
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<tr>
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<td>GSK3B</td>
<td>rs13202980 3 Intron</td>
<td>A G</td>
<td>28.7 31.9</td>
<td>0.86 (0.78–0.96)</td>
<td>0.005</td>
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<tr>
<td></td>
<td>IKKBPAK</td>
<td>rs10117384 9 Intron</td>
<td>A G</td>
<td>18.5 21.7</td>
<td>0.84 (0.74–0.94)</td>
<td>0.003</td>
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<td>PRKCA</td>
<td>rs7226221 17 Intron</td>
<td>G A</td>
<td>36.9 40.2</td>
<td>0.87 (0.79–0.96)</td>
<td>0.004</td>
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<tr>
<td></td>
<td>NFKBIB</td>
<td>rs3136646 19 Intron</td>
<td>A G</td>
<td>20.3 23.8</td>
<td>0.81 (0.72–0.91)</td>
<td>0.0003</td>
<td></td>
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</tr>
</tbody>
</table>

aSubtype analyses included invasive and LMP cases.

bSNPs are listed first by chromosome and then ranked by ordinal *P* value within the chromosome.

cOrdinal OR and *P* value, adjusted for first five PCs, age, and study site. Bold values highlight *P* < 0.001.
consequences of this SNP on expression or function have been tested experimentally.

CARD11 is an intermediate protein that assists in NF-kB activation following B- or T-cell receptor complex ligation (56–58) or activation of natural killer (NK) cell receptors (59). CARD11 intronic SNPs, rs74302019, rs41324349, and rs41483047 are in moderate linkage disequilibrium 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 that 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 less common subtypes: endometrioid, clear cell, and mucinous ovarian cancer. We used a SNP tagging approach to comprehensively cover genes in the NF-kB pathway; however, the study was limited by lack of coverage of some genes, mostly due to loss of SNPs that failed quality control. Nonetheless, this is the first study to extensively assess variation in genes involved in NF-kB 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-kB pathway genes in relation to risk of ovarian cancer 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, rs6758617, 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-kB activity in different cell types are needed to better understand the mechanism by which they might be contributing to ovarian cancer pathogenesis.

Disclosure of Potential Conflicts of Interest

U. Menon has an expert testimony from Abcodia Ltd. B. Charbonneau was an employee of Mayo Clinic at the time this manuscript was drafted and is currently an employee of and owns stock in Eli Lilly and Company. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Development of methodology: M.S. Block, R.A. Vierkant, H.A. Culver, L.R. Wilkens, I. Vergote, K.B. Moysich, E.L. Goode


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Risk of Ovarian Cancer and the NF-κB Pathway: Genetic Association with IL1A and TNFSF10

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