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

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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-1α is both regulated by and able to activate NF-κB, 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-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 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 $p< 2.5\times10^{-5}$, 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-1α

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 initition 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

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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-1α, 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 evaulate replication. We additionally investigated SNPs in other NF-κB pathway genes, as IL-1α is not only produced following NF-κB activation (19), but signaling of IL-1α through its receptor 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 biological support for further study of polymorphisms in the NF-κB 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-κB through IKKε was shown to be associated with more aggressive behavior in ovarian cancer cell lines (25). Additionally, 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 over 200 NF-κB 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-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 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).

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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 Génome Québec (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-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: 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-κB 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

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(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 ($-\log_{10}$(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 NFKB 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-κB 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

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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-κB pathway SNPs

A total of 2,281 additional SNPs in 210 genes in the NF-κB were also analyzed. When ranked by p-value, the most significant SNPs in the NF-κB 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= 8.9110^{-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-κB pathway SNPs

We also assessed NF-κB 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, 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-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

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 *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, 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, IL1RN, 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, IL1A, 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 in located in *AARB2, CARD11, IL1RN, MAP3K14, PIK3R1, PRKCA, PRKCZ,*

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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 *NFKBIB* (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-κB 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-1α precursor (46) has been reported to be the functional consequence of a serine residue at this position and calpain cleaved IL-1α 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-1α 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-1α on tumor growth is through inhibition of prostaglandin synthesis by COX-2 (49), which is expressed following

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IL-1α 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-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 consequences of this SNP on expression or function been tested experimentally.

CARD11 is an intermediate protein that assists in NF-κB 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-κB 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-κB activition, 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-κB 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-κB activity in different cell types are needed to better understand the mechanism by which they might be contributing to ovarian cancer pathogenesis.

Supplementary Material

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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)**

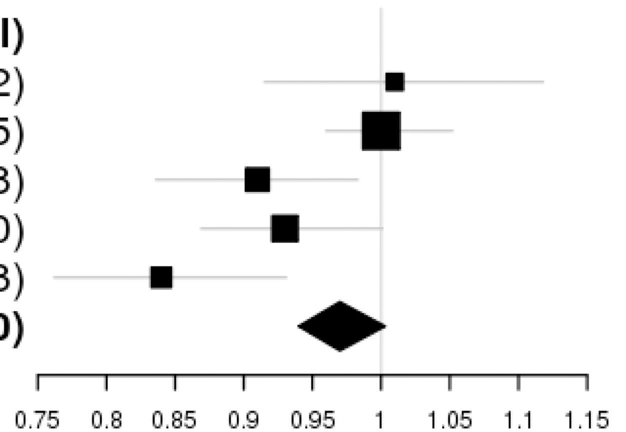

Figure 1.
IL1A SNP, rs17561, associations with risk of ovarian cancer by subtype. Forest plots of OR and 95% CI for HGS (high grade serous), LGS (low grade serous), mucinous, endometrioid, clear cell and overall ovarian cancer.

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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]
TNFRSF1B	rs17884213	1	intron	A	G	26.7	26	1.05	(1.02, 1.09)	0.002
TNFSF10	rs6801105	3	intron	A	G	16.7	17.4	0.94	(0.90, 0.98)	0.002
IRAK2	rs459483	3	intron	G	A	45.2	46.3	0.95	(0.93, 0.98)	0.003
TNFSF10	rs1131535	3	3'UTR	A	G	43.2	44.3	0.96	(0.93, 0.99)	0.004
FBXW7	rs75911772	4	intron	T	A	3.9	3.5	1.14	(1.05, 1.23)	0.002
NFKB1	rs1609993	4	synonymous	A	G	8.8	8	1.08	(1.03, 1.14)	0.003
CARD11	**rs74302019**	7	intron	A	G	32.2	31.1	1.07	(1.03, 1.10)	**8.9×10^{-05}**
CARD11	**rs41324349**	7	intron	A	C	43	41.6	1.06	(1.03, 1.09)	**0.0002**
CARD11	**rs41483047**	7	intron	A	G	18.5	17.6	1.07	(1.03, 1.12)	**0.0004**
CARD11	rs35329971	7	intron	A	G	8.8	9.6	0.92	(0.87, 0.97)	0.002
TAF3	**rs7071113**	10	3' downstream	C	G	31.8	30.8	1.06	(1.03, 1.10)	**0.0003**
TAF3	rs1244229	10	Val696Ala	A	G	30	29.1	1.06	(1.02, 1.09)	0.001
TAF3	rs263417	10	intron	A	C	30.2	29.4	1.05	(1.02, 1.09)	0.002
TAF3	rs1514233	10	intron	T	A	30.3	29.5	1.05	(1.02, 1.09)	0.003
PRKCA	rs7226221	17	intron	G	A	39.1	40.2	0.95	(0.92, 0.98)	0.002
MAP3K14	rs9908462	17	intron	A	G	19	19.8	0.94	(0.91, 0.98)	0.003
IL1RAPL2	rs1384360	23	intron	A	C	26.6	25.7	1.06	(1.02, 1.09)	0.001
TLR7	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

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Table 2

Top NF-κB 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)	*TNFRSF1B*	**rs17884213**	1	intron	A	G	26.8	26	**1.06**	**(1.02,1.10)**	**0.00097**
	IL1RN	rs62161280	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	**rs41324349**	7	intron	A	C	42.9	41.6	**1.05**	**(1.02,1.09)**	**0.0009**
	CARD11	**rs74302019**	7	intron	A	G	32.1	31.1	**1.06**	**(1.02,1.10)**	**0.0009**
	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)**	**0.0009**
	TAF3	rs1244229	10	Val696Ala	A	G	29.9	29.1	1.05	(1.02,1.09)	0.004
LMP (N=1,729)	*OTUD7B*	rs41265172	1	3' UTR	A	G	4.8	3.9	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	**rs6785617**	3	3' downstream	T	A	43.1	46.2	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)	**0.0008**
	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	rs744779	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)	**0.0009**

[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.

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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)	*TLR5*	rs116693072	1	5' upstream	G	A	4.5	4	1.18	(1.06,1.30)	0.002
	TNFRSF1B	rs17884213	1	intron	A	G	26.9	26	1.07	(1.02,1.13)	0.004
	PRKCZ	rs9729600	1	intron	A	G	9.5	9.3	1.11	(1.03,1.19)	0.005
	IL1RN	rs62161280	2	3' downstream	G	A	5.8	5.2	1.14	(1.04,1.25)	0.004
	PIK3R1	rs72757693	5	intron	A	G	13.9	13.2	1.1	(1.03,1.17)	0.003
	PIK3R1	rs12755	5	3'UTR	A	C	17.8	16.8	1.09	(1.03,1.15)	0.004
	CARD11	rs71527417	7	intron	A	C	6.3	7.2	0.87	(0.80,0.95)	0.002
	CARD11	rs74302019	7	intron	A	G	32.3	31.1	1.07	(1.03,1.12)	0.003
	CARD11	rs41324349	7	intron	A	C	43.1	41.6	1.06	(1.02,1.11)	0.005
	PRKDC	rs74915527	8	intron	A	G	10.6	9.8	1.11	(1.04,1.19)	0.004
	MAP3K14	rs117642368	17	5' upstream	G	C	11.6	10.5	1.1	(1.03,1.18)	0.004
	ARRB2	rs28365157	17	intron	A	G	9.6	10	0.9	(0.84,0.97)	0.005
	PRKCA	rs28733563	17	intron	A	G	24.7	23.4	1.07	(1.02,1.13)	0.005
Endometrioid (N=2,100)	*MTOR*	rs12129467	1	intron	C	G	10.2	8.7	1.19	(1.07,1.33)	0.001
	F2R	rs253073	5	intron	G	A	46.2	43.7	1.11	(1.04,1.18)	0.002
	HNRNPAB	rs116592017	5	5' upstream	A	C	2.1	2.9	0.72	(0.58,0.89)	0.003
	IKBKAP	rs2230792	9	Gly765Ala	A	G	20.3	18.6	1.13	(1.04,1.22)	0.004
Mucinous (N=1,591)	*PRKCZ*	rs34415348	1	intron	C	A	9.2	10.8	0.83	(0.73,0.94)	0.004
	CD247	rs1773539	1	intron	A	G	4.9	3.9	1.29	(1.08,1.53)	0.004
	IL1A	rs150712565	2	intron	A	T	32.5	29.9	1.12	(1.04,1.22)	0.004
	TNFSF10	rs12488654	3	5' upstream	A	G	18.9	16.7	1.15	(1.05,1.26)	0.004
	PTPN13	rs62308410	4	intron	A	G	46.5	43.9	1.12	(1.04,1.20)	0.004
	TLR1	rs5743551	4	5' upstream	G	A	20.9	23.9	0.88	(0.80,0.96)	0.004
	TLR10	rs4274855	4	5'UTR	A	G	15.3	17.7	0.86	(0.78,0.96)	0.005
	PRKCQ	rs4750528	10	intron	G	A	15.5	17.6	0.86	(0.78,0.95)	0.004
	PRKCA	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)	*IKBKE*	rs41296022	1	intron	A	G	4.7	3.4	1.37	(1.11,1.70)	0.004
	BCL10	rs2735593	1	5' UTR	C	G	24.1	21.5	1.16	(1.05,1.29)	0.005
	IL1A	**rs17561**	2	Ala114Ser	A	C	26.8	30.2	**0.84**	**(0.76,0.93)**	**0.0008**
	IL1A	**rs1800587**	2	5'UTR	A	G	26.8	30.2	**0.84**	**(0.76,0.93)**	**0.0008**
	IL1A	**rs1304037**	2	3'UTR	G	A	26.8	30.1	**0.84**	**(0.76,0.93)**	**0.0008**
	IL1A	**rs2856836**	2	3'UTR	G	A	26.8	30.2	**0.85**	**(0.76,0.93)**	**0.00099**
	IL1A	rs1800794	2	5' upstream	A	G	26.7	30	0.85	(0.77,0.94)	0.001
	IL1RN	rs2071459	2	intron	A	G	10.6	13	0.81	(0.70,0.94)	0.004
	NFKBIZ	rs80099440	3	intron	A	G	6.5	4.9	1.36	(1.13,1.63)	0.001
	PPARG	rs77323418	3	3' downstream	G	A	3.4	4.7	0.7	(0.55,0.89)	0.004
	TLR3	rs66624661	4	intron	A	G	36.5	33.7	1.14	(1.04,1.25)	0.005
	CD3E	rs73014299	11	3' downstream	A	G	8.8	11.1	0.78	(0.67,0.91)	0.002
	AKT1	rs45531934	14	intron	A	G	5.6	7.2	0.74	(0.60,0.90)	0.003
	TLR7	rs5743733	23	intron	G	C	10.1	8.1	1.26	(1.09,1.46)	0.002
LGS (N=1,016)	*GSK3B*	rs13320980	3	intron	G	A	28.7	31.9	0.86	(0.78,0.96)	0.005
	IKBKAP	rs10117384	9	intron	A	G	18.5	21.7	0.84	(0.74,0.94)	0.003
	PRKCA	rs7226221	17	intron	G	A	36.9	40.2	0.87	(0.79,0.96)	0.004
	NFKBIB	**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.

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