Short Communication

Variation in NF-κB Signaling Pathways and Survival in Invasive Epithelial Ovarian Cancer

Matthew S. Block1, Bridget Charbonneau2, Robert A. Vierkant3, Zachary Fogarty4, William R. Bamlet3, Paul D.P. Pharoah5,6,32,33; Georgia Chenevix-Trench12,41 for ACOCS1,42/ACS Group1; Mary Anne Rossing6,7, Daniel Cramer8,9, Celeste Leigh Pearce10, Joellen Schildkraut17,18, Usha Menon14, Susanne K. Kjaer20,45, Douglas A. Levine21, Nicolas Wentzensen16, Jolanta Kupryjanczyk48, Jenny Chang-Claude55, Elisa V. Bandera23, Estrid Hogdall45,46, Menkiszak50, Allan Jensen45, Simon A. Gayther10, Susan J. Ramus10, Aleksandra Gentry-Maharaj34, Andrew Doherty30, Sharon E. Johnatty41, Anna deFazio44, Honglin Song32, Jonathan Tyrer32, Kimberly R. Kalili1, Brooke L. Fridley31, Julie M. Cunningham5, and Ellen L. Goode6

Abstract

Survival in epithelial ovarian cancer (EOC) is influenced by the host immune response, yet the key genetic determinants of inflammation and immunity that affect prognosis are not known. The nuclear factor-kB (NF-kB) transcription factor family plays an important role in many immune and inflammatory responses, including the response to cancer. We studied common inherited variation in 210 genes in the NF-kB family in 10,084 patients with invasive EOC (5,248 high-grade serous, 1,452 endometrioid, 795 clear cell, and 661 mucinous) from the Ovarian Cancer Association Consortium. Associations between genotype and overall survival were assessed using Cox regression for all patients and by major histology, adjusting for known prognostic factors and correcting for multiple testing (threshold for statistical significance, \( P < 2.5 \times 10^{-5} \)). Results were statistically significant when assessed for patients of a single histology. Key associations were with caspase recruitment domain family, member 11 (CARD11) rs41324349 in patients with mucinous EOC (HR, 1.82; 95% confidence interval (CI), 1.41–2.35; \( P = 4.13 \times 10^{-4}\)) and tumor necrosis factor receptor superfamily, member 13B (TNFRSF13B) rs7501462 in patients with endometrioid EOC (HR, 0.68; 95% CI, 0.56–0.82; \( P = 2.33 \times 10^{-4}\)). Other associations of note included TNF receptor-associated factor 2 (TRAF2) rs17250239 in patients with high-grade serous EOC (HR, 0.84; 95% CI, 0.77–0.92; \( P = 6.49 \times 10^{-5}\)) and phospholipase C, gamma 1 (PLCG1) rs11696662 in patients with clear cell EOC (HR, 0.43; 95% CI, 0.26–0.73; \( P = 4.56 \times 10^{-4}\)). These associations highlight the potential importance of genes associated with host inflammation and immunity in modulating clinical outcomes in distinct EOC histologies. Cancer Epidemiol Biomarkers Prev; 23(7): 1–7. ©2014 AACR.

Authors’ Affiliations: Departments of 1Medical Oncology, 2Health Sciences Research, Division of Epidemiology; 3Health Sciences Research, Division of Biomedical Statistics and Informatics; 4Immunology; and 5Laboratory Medicine and Pathology, Division of Experimental Pathology, Mayo Clinic, Rochester, Minnesota; 6Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center; 7Department of Epidemiology, University of Washington, Seattle, Washington; 8Obstetrics and Gynecology Epidemiology Center, Brigham and Women’s Hospital and Harvard Medical School; 9Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; 10Department of Preventive Medicine, Keck School of Medicine, University of Southern California Norris Comprehensive Cancer Center; 11Department of Medicine, Division of Hematology and Oncology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles; 12Department of Epidemiology, Center for Cancer Genetics Research and Prevention, School of Medicine, University of California Irvine, Irvine; 13Department of Health Research and Policy - Epidemiology, Stanford University School of Medicine, Palo Alto; 14Women’s Cancer Program at the Samuel Oschin Comprehensive Cancer Institute; 15Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinaí Medical Center, Los Angeles, California; 16Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; 17Cancer Prevention, Detection, and Control Research Program, Duke Cancer Institute; 18Department of Community and Family Medicine; 19Department of Obstetrics and Gynecology, Duke...
Introduction

Epithelial ovarian cancer (EOC) is the sixth leading cause of cancer death among women in developed countries (1), with a 5-year survival rate of only 37% in the United States (2). A key cause of poor survival is a lack of specific symptoms and screening methods; as such, the majority of patients with EOC present with distant spread of disease. A number of features in addition to stage are known to impact clinical outcome, including age at diagnosis (3), extent of residual disease following initial cytoreductive surgery (optimal vs. suboptimal; ref. 4), and baseline performance status (5). Genetic polymorphisms may also influence EOC survival (6, 7). Understanding the totality of potential prognostic factors is key to discerning pathogenic mechanisms that underlie carcinogenesis and progression in EOC. Inflammation is known to play a role in tumorigenesis (8); inflammation from multiple causes, including talc use (9) and endometriosis (9, 10), and the presence of nonspecific inflammatory markers such as C-reactive protein (CRP) are associated with increased EOC risk (11). Furthermore, the presence of an ongoing inflammatory response, measured by CRP and hypoaalbuminemia, has been shown to independently predict poor prognosis in advanced EOC (12).

The nuclear factor-κB (NF-κB) family of transcription factors regulates the transcription of multiple proteins, including cytokines, chemokines, acute-phase reactants, complement factors, adhesion molecules, and other proteins involved in inflammation, apoptosis, and cell division (13). In canonical NF-κB signaling, binding of NF-κB-associated receptors leads to phosphorylation and activation of the inhibitor of κB (IκK) complex, which leads to phosphorylation and proteosomal degradation of the inhibitor of κB (IκB), thus releasing NF-κB transcription factors into the nucleus to regulate gene transcription (14). Alternatively, receptor binding and IKK activation can lead to processing of the p100 protein into active p52, which binds the NF-κB family member Rel-B, translocates to the nucleus, and regulates gene transcription (14). To assess the role of genetic variation in NF-κB signaling on EOC survival, we evaluated common inherited single nucleotide polymorphisms (SNP) in key genes, which mediate NF-κB activation, inhibit NF-κB function, assist degradation, or regulate nuclear function among patients from the Ovarian Cancer Association Consortium (OCAC).

Materials and Methods

Study participants

A total of 10,084 women with invasive EOC (37,171 person-years follow-up) and greater than 90% estimated European ancestry were analyzed as described previously (15, 16). Participants were from 28 OCAC studies (Supplementary Table S1) based in Europe, North America, and Australia, which conducted follow-up for vital status, including 12 studies (AUS, BAY, HAW, HSK, LAX, MAL, MAY, NCO, NEC, ORE, PVD, and SRO) followed for disease recurrence or progression.

SNP selection

We identified 210 key genes (Supplementary Table S2) known to encode NF-κB subunits or molecules key to NF-κB activation (in signaling cascade), inhibition (inhibitory role), degradation (involved in proteosomal degradation), and nuclear function (nuclear proteins involved in

Lifestyle and Genes, Danish Cancer Society Research Center; 46Molecular Unit, Department of Pathology, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark; 47Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland; Departments of 48Pathology and 49Gynecologic Oncology, The Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; 50Clinic of Gynecological Surgery and Oncology, Pomeranian Medical University, Szczecin, Poland; 51Vesalius Research Center, VIB; 52Laboratory for Translational Genetics, Department of Oncology, University of Leuven; 53Division of Gynecologic Oncology, Department of Obstetrics and Gynecology and Leuven Cancer Institute, University Hospitals Leuven, Leuven, Belgium; 54University Hospital Erlangen, Department of Gynecology and Obstetrics, Friedrich-Alexander-University Erlangen-Nuremberg, Comprehensive Cancer Center Erlangen; 55German Cancer Research Center, Division of Cancer Epidemiology, Heidelberg; 56Department of Gynecology and Gynecologic Oncology, Dr. Horst Schmidt Klinik Wiesbaden; 57Institut fur Humangenetik Wiesbaden, Wiesbaden; 58Department of Gynecology and Gynecologic Oncology, Klinikum Essen-Mitte/Evangelische Huyssens-Stiftung/Knappschaft GmbH, Essen; and 59Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Ellen L. Goode, Department of Health Sciences Research, Mayo Clinic, 200 First Street SW, Rochester, MN 55905; Phone: 507-266-7997; Fax: 507-266-2478; E-mail: egoose@mayo.edu

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transcription; ref. 6). TagSNPs within 5 kb based on \( r^2 \) 
\( \geq 0.8 \), minor allele frequency (MAF) \( \geq 0.05 \) in Europeans 
were identified using the most informative source for 
each gene from among HapMap Phase II Release 24 
(http://www.hapmap.org), the 1000 Genomes Project 
Low-Coverage Pilot (http://www.1000genomes.org/), 
SeattleSNPs (http://pga.mbt.washington.edu/), 
Immune System PGA (http://innateimmunity.net/), and 
NIEHS SNPs (http://egp.gs.washington.edu; ref. 17). 
Additional putative-functional SNPs were also included, 
regardless of linkage disequilibrium, with European 
MAF \( \geq 0.05 \), which were 1 kb upstream, non-synonymous, 
or resided in a 3'-untranslated region (UTR), 5'-UTR, 
splice site, or miRNA binding site (http://www.microrna. 

Finally, SNPs with an Illumina design score <0.4 or in 
linkage disequilibrium \( (r^2 > 0.80) \) with a SNP found to 
be null \( (P > 0.05) \) in a small prior analysis \( (16) \) were 
excluded. With this approach, 76% of significant SNPs 
with MAF \( \geq 0.05 \) were adequately tagged if we used 
HapMap as our reference.

Genotyping and quality control

germ line genotyping was conducted using an Illumina 
Infinium iSelect BeadChip as part of the Collaborative 
Oncological Gene-environment Study (COGS; ref. 16). 
Centralized genotyping used raw intensity data files and 
a cluster file generated with HapMap2 European, African, 
and Asian samples. Samples were excluded with (i) 
conversion rate <95%, (ii) heterozygosity >5 SDs from 
the European mean heterozygosity, (iii) ambiguous sex, (iv) 
lowest call rate from an observed first-degree relative pair, 
or (v) duplicate samples that were nonconcordant for 
genotype or genotypic duplicates that were not concur-
dant for phenotype. SNPs were excluded with (i) no 
genotype call, (ii) monomorphism, (iii) call rate <95% with 
MAF >0.05 or call rate <99% with MAF <0.05, (iv) devi-
ation from Hardy–Weinberg equilibrium \( (P < 10^{-5}) \), or (v) 
>2% duplicate discordance.

SNP imputation

Imputation to the 1000 Genomes (1000G) Phase I 
Integrated Release Version 3 haplotypes was carried out 
in MaCH \( (18) \) using all 1,092 1000G samples and excluding 
monomorphic and singleton sites.

Statistical methods

HapMap2 genotypes were used to define interconti-
nental ancestry; among Europeans (90% European 
ancestry), we used 37,000 unlinked non-NF-xB markers 
in population stratification principal components analysis 
\( (16) \). Cox regression accounting for left truncation and 
right censoring at 10 years estimated hazard ratios (HR) 
and 95% confidence intervals (CI) for association with 
overall survival (OS), defined as time to death from any 
cause. Censoring at 10 years was performed to minimize 
competing causes of mortality, which become more 
common after 10 years from EOC diagnosis. HRs were calcu-
lated based on the ordinal number of copies of the minor 
allele for all genotyped SNPs and allele dosage variables 
for all imputed SNPs. Analyses were conducted overall 
and within the 4 most common histologic subtypes (high-
grade serous, mucinous, endometrioid, and clear cell). 
Analyses adjusted for study site and the first 5 population 
substructure principal components, as well as the follow-
ing covariates, which associated with survival in these 
data \( (P < 0.05); \) Supplementary Table S3: age (continuous), 
tumor stage summarized from FIGO or SEER stage (local-
ized, regional, distant), tumor grade (well, moderately, 
poorly, or undifferentiated), oral contraceptive use (ever, 
never), and, for analysis of all cases only, histology 
(serosal, mucinous, endometrioid, clear cell, mixed cell, 
undifferentiated, unknown). Sensitivity analyses includ-
ed covariates only for age, 5 population substructure 
principal components, and study site. Analyses were also 
conducted with a recurrence endpoint defined as time to 
disease recurrence or death \( (377 \) additional events), 
among cases that were optimally debulked in cytoreduc-
tive surgery \( (2,078 \) cases having no residual deposits of 
cancer that were >1 cm) and among cases where surgical 
debulking was suboptimal \( (1,215 \) cases with >1 cm resid-
ual disease).

To address multiple testing concerns, we used spec-
tral decomposition of the observed genotype matrix \( (19) \) 
to account for observed linkage disequilibrium and 
estimated that the effective number of independent tests 
for each analysis was 2,000. As a result, only SNPs with 
P-values \( < 2.50 \times 10^{-5} \) \( (0.05/2,000) \) were considered 
statistically significant. We used SAS (SAS Institute Inc.) 
and R (R Foundation for Statistical Computing), and, in 
regions of interest, LocusZoom (Standalone Version; 
ref. 20) and Haploreg v2 \( (21) \) for plotting and annota-
tion, respectively.

Results

We analyzed 2,254 SNPs in 210 genes for clinical out-
come among 10,084 EOC cases. The strongest survival 
association in any of the histology subgroups was seen in 
661 mucinous EOC with the CARD11 intronic SNP 
rs41324349 \( (HR, 1.82; \) Table 1). In addition, 
5 of the 56 genotyped CARD11 SNPs were associated 
with \( P < 0.05 \), including 2 independent SNPs \( (r^2 > 0.20) \) 
with \( P < 0.001 \) \( (Table 1) \). The distribution of P-values 
and correlation with rs41324349 across CARD11 are 
shown in Fig. 1 for both directly genotyped and imput-
ed SNPs. Imputation revealed that the CARD11 SNP 
rS2527513, which was in strong linkage disequilibrium 
with rs41324349, was highly correlated with survival. For 
1,452 patients with endometrioid EOC, the 
association \( (P = 2.33 \times 10^{-5}) \). Of 18 additional 
TNFRSF13B SNPs, 2 others (rs7212800 and rs11078362) showed 
association \( (P < 0.005) \) in patients with endometrioid EOC; 
these additional SNPs were in moderate linkage disequ-
librium with rs7501462 \( (r^2 = 0.26 \) and 0.76, respectively).
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Table 1. SNP association with EOC OS (P < 0.001, r² < 0.20)

<table>
<thead>
<tr>
<th>Histologic subtype</th>
<th>SNP</th>
<th>Alleles</th>
<th>MAF</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous (N = 661)</td>
<td>rs41324349</td>
<td>C &gt; A</td>
<td>0.44</td>
<td>1.82 (1.41–2.35)</td>
<td>4.13 x 10⁻⁶</td>
</tr>
<tr>
<td>CARD11</td>
<td>rs6944821</td>
<td>A &gt; G</td>
<td>0.31</td>
<td>1.64 (1.26–2.13)</td>
<td>2.47 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>rs34251392</td>
<td>A &gt; G</td>
<td>0.34</td>
<td>0.63 (0.48–0.82)</td>
<td>5.08 x 10⁻⁴</td>
</tr>
<tr>
<td>Traf5</td>
<td>rs79776636</td>
<td>G &gt; C</td>
<td>0.04</td>
<td>2.89 (1.70–4.92)</td>
<td>4.01 x 10⁻⁴</td>
</tr>
<tr>
<td>IKBKE</td>
<td>rs10836</td>
<td>G &gt; C</td>
<td>0.47</td>
<td>0.62 (0.47–0.82)</td>
<td>6.04 x 10⁻⁴</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>rs10940158</td>
<td>G &gt; A</td>
<td>0.52</td>
<td>1.47 (1.17–1.85)</td>
<td>8.47 x 10⁻⁴</td>
</tr>
<tr>
<td>Endometrioid (N = 1,452)</td>
<td>TNFRSF13B</td>
<td>A &gt; G</td>
<td>0.26</td>
<td>0.68 (0.56–0.82)</td>
<td>2.33 x 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>rs1152468</td>
<td>G &gt; C</td>
<td>0.40</td>
<td>0.75 (0.64–0.87)</td>
<td>1.86 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>rs72847071</td>
<td>G &gt; A</td>
<td>0.09</td>
<td>1.61 (1.26–2.05)</td>
<td>2.66 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>rs40401</td>
<td>G &gt; A</td>
<td>0.22</td>
<td>0.72 (0.59–0.87)</td>
<td>6.56 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>rs5744157</td>
<td>G &gt; C</td>
<td>0.12</td>
<td>0.66 (0.52–0.85)</td>
<td>8.28 x 10⁻⁴</td>
</tr>
<tr>
<td>High-grade serous (N = 5,248)</td>
<td>Traf2</td>
<td>G &gt; A</td>
<td>0.11</td>
<td>0.84 (0.77–0.92)</td>
<td>6.49 x 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>rs17250239</td>
<td>G &gt; A</td>
<td>0.11</td>
<td>0.84 (0.77–0.92)</td>
<td>6.49 x 10⁻⁵</td>
</tr>
<tr>
<td>PRKCA</td>
<td>rs9894564</td>
<td>A &gt; G</td>
<td>0.24</td>
<td>0.90 (0.84–0.95)</td>
<td>5.83 x 10⁻⁴</td>
</tr>
<tr>
<td>Clear cell (N = 795)</td>
<td>PLCG1</td>
<td>G &gt; A</td>
<td>0.07</td>
<td>0.43 (0.26–0.73)</td>
<td>4.56 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>rs11696662</td>
<td>G &gt; A</td>
<td>0.07</td>
<td>0.43 (0.26–0.73)</td>
<td>4.56 x 10⁻⁴</td>
</tr>
<tr>
<td>MAPK1</td>
<td>rs72847071</td>
<td>T &gt; A</td>
<td>0.43</td>
<td>0.70 (0.57–0.86)</td>
<td>6.10 x 10⁻⁴</td>
</tr>
<tr>
<td>All (N = 10,084)</td>
<td>MAPK3</td>
<td>rs61764220</td>
<td>A &gt; G</td>
<td>0.03</td>
<td>0.81 (0.71–0.92)</td>
</tr>
<tr>
<td></td>
<td>rs518162</td>
<td>G &gt; A</td>
<td>0.08</td>
<td>0.87 (0.81–0.95)</td>
<td>8.11 x 10⁻⁴</td>
</tr>
</tbody>
</table>

NOTE: Bold indicates P < 2.5 x 10⁻⁵; adjusted for study site, first 5 European ancestry population substructure principal components, age at diagnosis, tumor stage, tumor grade, oral contraceptive use, and ethnicity (for analyses of all cases only); SNPs with P < 0.001, but correlated at r² > 0.20 SNPs above are not shown; SNP id is dbSNP 137 rsid; minor allele designation based on allele frequencies in all cases.

Discussion

In this pooled analysis of more than 10,000 patients with EOC enrolled in 28 different studies within OCAC, we evaluated associations between NF-κB–related SNPs with survival. We did not identify SNPs associating with OS among all patients with EOC that met our corrected threshold for statistical significance. However, we identified 3 SNPs, rs41324349, rs2527513, and rs7501462, which associated with OS and time to recurrence for EOC subtypes accounting for known prognostic factors. The CARD11 intronic SNPs rs41324349 and rs2527513 were in high linkage disequilibrium with each other and were associated with shortened survival in patients with mucinous EOC, whereas TNFRSF13B 3'UTR rs7501462 associated with improved outcome among patients with endometrioid EOC. Sensitivity analyses showed concordance between HRs for OS and time to recurrence, and among optimally debulked patients.

For 5,248 patients with high-grade serous EOC, the Traf2 SNP rs17250239 showed the most significant association (HR, 0.84; P = 6.49 x 10⁻⁵), although this was just beyond our pathway-wide threshold for statistical significance (P < 2.50 x 10⁻⁵). The rs17250239 SNP is located in an intronic sequence within the Traf2 gene. In 795 patients with clear cell EOC, the SNPs rs61764220 and rs518162 (within the genes MAPK3 and PGR, respectively) had the strongest survival associations (HR, 0.81; P = 6.50 x 10⁻⁴ and HR, 0.87; P = 8.11 x 10⁻⁴, respectively; Table 1). However, these results did not meet our threshold for statistical significance taking into account multiple comparisons (P < 2.50 x 10⁻⁵), and so there were not clear associations between polymorphisms in MAPK3 and PGR and survival in EOC.

In addition to OS, we performed sensitivity analyses for time to recurrence, examined results from minimally adjusted analyses, and assessed optimally debulked and suboptimally debulked patients separately. The HRs for recurrence were similar to HRs for survival with and without full covariate adjustment for each of the SNPs that we had considered to have the most significant associations with survival (P < 0.0001) and among optimally debulked compared with suboptimally debulked patients (available on one-third of participants; data not shown).
CARD11, also known as Carma 1, is an adapter protein that functions as a molecular scaffold in leukocytes (22). CARD11 interacts with the proapoptotic protein BCL10, and overexpression of CARD11 leads to increased NF-κB activation (23). Oncogenic mutations in CARD11 have been reported in association with several types of lymphoma (24). The expression of CARD11 in leukocytes suggests that it may influence immune/inflammatory responses to EOC. rs41324349 lies within 7 regulatory motifs that would be altered by the base change, which could potentially alter transcription; however, this SNP is not in a conserved domain. Six additional intronic and 1 synonymous SNPs located in regulatory motifs were correlated with this SNP ($r^2 \geq 0.6$). Primary mucinous EOC is relatively uncommon, and mechanisms responsible for tumorigenesis, invasion, and metastasis that are specific for mucinous subtype have not yet been clearly demonstrated. Thus, it is not clear how a change in expression or function of CARD11 would affect survival specifically in this subgroup.

TNFRSF13B, more commonly known as transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI), is a member of the tumor necrosis factor (TNF) receptor superfamily and is found on B lymphocytes (25). TACI interacts with the TNF family members B-cell–activating factor (BAFF) and a proliferation-inducing ligand (APRIL) to activate NF-κB and other transcription factors in B cells. It is not known whether rs7501462 affects TNFRSF13B expression, and it is not located in an evolutionarily conserved domain; however, it falls in a strong enhancer region and POL2 binding site in B-lymphoblastoid cell lines. As the primary pathologic process associated with endometrioid ovarian carcinomas is endometriosis, alterations in TNFRSF13B that affect inflammatory responses to endometriosis may modulate the aggressiveness of endometriosis-associated carcinomas.

Interestingly, although SNPs associated with survival were identified for relatively rare histologies (mucinous and endometrioid histologies), there were no SNP associations identified for the most common EOC histology (high-grade serous). This may simply reflect underdetection of SNPs because of a relatively stringent statistical threshold for significance, as there were several SNPs, most notably rs17250239 (HR, 0.84; $P = 6.49 \times 10^{-5}$), which had survival associations not quite meeting our prespecified threshold for significance ($P < 2.5 \times 10^{-5}$). However, this may also reflect that survival high-grade serous EOC, which is characterized by dramatic alterations in DNA macrostructure, may be more closely associated with certain amplified or deleted regions of DNA rather than alterations at the single nucleotide level.

The search for inherited variants associated with EOC outcome has proven challenging, with no published variants reaching genome-wide significance to date (15, 26). Here, by testing a candidate pathway within a consortium, we identified 2 SNPs from NF-κB–related genes that associated with survival in patients with distinct histologic subtypes of EOC using a pathway-wide statistical significance threshold. Strengths of this report include large sample size and use of centralized genotyping; limitations include missing data on surgical debulking status. For example, analysis by debulking status classified patients based on whether <1 cm or ≥1 cm residual...
interest were disclosed by the other authors.

As additional outcome-associated variants come to light, further work will address the potential prognostic utility of a broad panel of outcome-associated SNPs. For now, we provide evidence that the genetics of the immune/inflammatory response to EOC may impact clinical outcome and suggest that characterization of these mechanisms will be a key next step to understanding this deadly disease.

Disclosure of Potential Conflicts of Interest

B. Charbonneau is an employee at Eli Lilly and Company and also has ownership interest (including patents) in Abcodya. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


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