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

Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2014 July ; 23(7): 1421–1427. doi:10.1158/1055-9965.EPI-13-0962.

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Abstract

Survival in epithelial ovarian cancer (EOC) is influenced by the host immune response, yet the key genetic determinants of inflammation and immunity that impact prognosis are not known. The nuclear factor-kappa B (NF-κB) 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-κB 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—$p < 2.5\times10^{-5}$). Results were statistically significant when assessed for patients of a single histology. Key associations were with *CARD11* (caspase recruitment domain family, member 11) rs41324349 in patients with mucinous EOC (HR 1.82, 95% CI 1.41–2.35, $p=4.13\times10^{-6}$) and *TNFRSF13B* (tumor necrosis factor receptor superfamily, member 13B) rs7501462 in patients with endometrioid EOC (HR 0.68, 95% CI 0.56–0.82, $p=2.33\times10^{-5}$). Other associations of note included *TRAF2* (TNF receptor-associated factor 2) rs17250239 in patients with high-grade serous EOC (HR 0.84, 95% CI 0.77–0.92, $p=6.49\times10^{-5}$) and *PLCG1* (phospholipase C, gamma 1) rs11696662 in patients with clear cell EOC (HR 0.43, 95% CI 0.26–0.73, $p=4.56\times10^{-4}$). These associations highlight the potential importance of genes associated with host inflammation and immunity in modulating clinical outcomes in distinct EOC histologies.

Keywords

single nucleotide polymorphism; recurrence; survival; ovarian neoplasms

INTRODUCTION

Epithelial ovarian cancer (EOC) is the sixth leading cause of cancer death among women in developed countries (1), with a five-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 EOC patients 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),

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Conflict of interest: There are no conflicts of interest to be disclosed with the exception of: Bridget 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.

extent of residual disease following initial cytoreductive surgery (optimal versus suboptimal) (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 tumorigenesis (8); inflammation from multiple causes, including talc use (9) and endometriosis (9, 10), and the presence of non-specific 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 hypoalbuminemia, has been shown to independently predict poor prognosis in advanced EOC (12).

The nuclear factor-kappa B (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 kappa B kinase (IKK) complex, which leads to phosphorylation and proteosomal degradation of the inhibitor of kappa B (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 (SNPs) 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 (Supplemental Table 1) based in Europe, North America, and Australia which conducted follow-up for vital status, including 12 studies (AUS, BAV, HAW, HSK, LAX, MAL, MAY, NCO, NEC, ORE, PVD, and SRO) followed for disease recurrence or progression.

SNP Selection

We identified 210 key genes (Supplemental Table 2) 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) (6). TagSNPs within 5 kb based on $r^2 \geq 0.8$, minor allele frequency (MAF) ≥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/), Innate Immunity PGA (http://innateimmunity.net/), and NIEHS

SNPs (http://egp.gs.washington.edu) (17). 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′ untranslated region (UTR), 5′ UTR, splice site, or miRNA binding site (http://www.microrna.org/microrna/home.do, http://www.targetscan.org/). Finally, SNPs with an Illumina design score <0.4 or in LD (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 ≥ 0.05 were adequately tagged if we used HapMap as our reference.

Genotyping and Quality Control

Germline genotyping was conducted using an Illumina Infinium iSelect BeadChip as part of the Collaborative Oncological Gene-environment Study (COGS) (16). Centralized genotyping used raw intensity data files and a cluster file generated with HapMap2 European, African, and Asian samples. Samples were excluded with 1) conversion rate <95%, 2) heterozygosity > five standard deviations from the European mean heterozygosity, 3) ambiguous sex, 4) lowest call rate from an observed first-degree relative pair, or 5) duplicate samples that were non-concordant for genotype or genotypic duplicates that were not concordant for phenotype. SNPs were excluded with 1) no genotype call, 2) monomorphism, 3) call rate <95% with MAF >0.05 or call rate <99% with MAF <0.05, 4) deviation from Hardy-Weinberg equilibrium (p <10^{-7}), or 5) >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 intercontinental ancestry; among Europeans (>90% European ancestry), we used 37,000 unlinked non-NF-κB markers in population stratification principal components (PC) analysis (16). Cox regression accounting for left truncation and right censoring at 10 years estimated hazard ratios (HRs) and 95% confidence intervals (CIs) for association with overall survival, 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 calculated 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 four most common histologic subtypes (high grade serous, mucinous, endometrioid, and clear cell). Analyses adjusted for study site and the first five population substructure PCs, as well as the following covariates which associated with survival in these data (p <0.05, Supplemental Table 3): age (continuous), tumor stage summarized from FIGO or SEER stage (localized, regional, distant), tumor grade (well, moderately, poorly, or undifferentiated), oral contraceptive use (ever, never), and, for analysis of all cases only, histology (serous, mucinous, endometrioid, clear cell, mixed cell, undifferentiated, unknown). Sensitivity analyses included covariates only for age, five population substructure PCs, and study site. Analyses were also conducted with a recurrence endpoint defined as time to disease

recurrence or death (377 additional events), among cases which were optimally debulked in cytoreductive 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 residual disease).

To address multiple testing concerns, we used spectral decomposition of the observed genotype matrix (19) to account for observed LD and estimated that the effective number of independent tests for each analysis was 2,000. As a result, only SNPs with p-values $<2.50\times10^{-5}$ (0.05/2,000) were considered statistically significant. We used SAS (SAS Institute, Inc., Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria), and, in regions of interest, LocusZoom (Standalone Version) (20) and Haploreg v2 (21) for plotting and annotation respectively.

RESULTS

We analyzed 2,254 SNPs in 210 genes for clinical outcome 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, $p = 4.13\times10^{-6}$, Table 1). In addition, five of the fifty-six genotyped *CARD11* SNPs were associated at $p < 0.005$, including two 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 Figure 1 for both directly genotyped and imputed SNPs. Imputation revealed that the *CARD11* SNP rs2527513, which was in strong LD with rs41324349, was highly correlated with survival. For 1,452 patients with endometrioid EOC, the *TNFRSF13B* 3′ UTR SNP rs7501462 showed the strongest association (HR =0.68, $p = 2.33\times10^{-5}$). Out of eighteen additional *TNFRSF13B* SNPs, two others (rs7212800 and rs11078362) showed association ($p < 0.005$) in endometrioid EOC patients; these additional SNPs were in moderate LD with rs7501462 ($r^2 = 0.26$ and $r^2 = 0.76$, respectively).

For 5,248 high grade serous EOC patients, the *TRAF2* SNP rs17250239 showed the most significant association (HR =0.84, $p = 6.49\times10^{-5}$), although this was just beyond our pathway-wide threshold for statistical significance ($p < 2.50\times10^{-5}$). The rs17250239 SNP is located in an intronic sequence within the *TRAF2* gene. In 795 clear cell EOC patients, *PLCG1* rs11696662 showed the most significant association (HR = 0.43, $p = 4.56 \times 10^{-4}$), but this was not within our pathway-wide threshold for statistical significance. Finally, among all cases, the SNPs rs61764220 and rs518162 (within the genes *MAPK3* and *PGR*, respectively) had the strongest survival associations (HR =0.81, $p = 6.50\times10^{-4}$, and HR =0.87, $p = 8.11\times10^{-4}$, respectively, Table 1). However, these results did not meet our threshold for statistical significance taking into account multiple comparisons ($p < 2.50\times10^{-5}$), 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 to suboptimally debulked patients (available on one-third of participants; data not shown).

DISCUSSION

In this pooled analysis of over 10,000 EOC patients enrolled in 28 different studies within OCAC, we evaluated associations between NF-κB-related SNPs with survival. We did not identify SNPs associating with overall survival among all EOC patients that met our corrected threshold for statistical significance. However, we identified three SNPs, rs41324349, rs2527513, and rs7501462, which associated with overall survival and time to recurrence for EOC subtypes accounting for known prognostic factors. The *CARD11* intronic SNPs rs41324349 and rs2527513 were in high LD 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 overall survival and time to recurrence, and among optimally debulked patients.

CARD11, also known as Carma 1, is an adapter protein that functions as a molecular scaffold in leukocytes (22). CARD11 interacts with the pro-apoptotic 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 seven 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 one 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 impact survival specifically in this subgroup.

TNFRSF13B, more commonly known as TACI (transmembrane activator and calcium-modulating cyclophilin ligand interactor), 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 BAFF (B cell activating factor) and APRIL (a proliferation-inducing ligand) 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, while 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 due to a relatively stringent statistical threshold for significance, as there were several SNPs, most notably rs17250239 (HR =0.84, p =6.49×10^{-5}) which had survival associations not quite meeting our pre-specified threshold for significance ($p < 2.5\times10^{-5}$). 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 two 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 disease was present, as opposed to complete debulking (no visible residual disease); thus, association in certain patient subsets may have been overlooked. In addition, for some population-based studies, there was possible over-enrollment of women with longer survival; this could also bias results to the null if NF-κB SNPs associate only with very poor survival time.

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 functional mechanisms will be a key next step to understanding this deadly disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Irene Orlow[46], Lisa E. Paddock[47], Anja Rudolph[27], Ursula Eilber[27], Agnieszka Dansonka-Mieszkowska[26], Iwona K. Rzepecka[26], Izabela Ziolkowska-Seta[48], Louise A. Brinton[25], Hannah Yang[25], Montserrat Garcia-Closas[49], Evelyn Despierre[50], Sandrina Lambrechts[50], Ignace Vergote[50], Christine S. Walsh[22], Jenny Lester[22], Weiva Sieh[21], Valerie McGuire[21], Joseph H. Rothstein[21], Argyrios Ziogas[20], Jan Lubiński[19], Cezary Cybulski[19], Janusz Menkiszak[51], Allan Jensen[17], Simon A. Gayther[12], Susan J. Ramus[12], Aleksandra Gentry-Maharaj[15], Andrew Berchuck[52], Anna H. Wu[12], Malcolm C. Pike[12,46], David Van Den Berg[12], Kathryn L. Terry[10,11], Allison F. Vitonis[10], Starr M. Ramirez[2], David N. Rider[3], Keith L. Knutson[53], Thomas A. Sellers[54], Catherine M. Phelan[54], Jennifer A. Doherty[55], Sharon E. Johnatty[6], Anna deFazio[56], Honglin Song[4], Jonathan Tyrer[4], Kimberly R. Kalli[1], Brooke L. Fridley[57], Julie M. Cunningham[58], and Ellen L. Goode[2]

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Acknowledgments

We thank all the individuals who took part in this study and all the researchers, clinicians and technical and administrative staff who have made possible the many studies contributing to this work. In particular, we thank: D. Bowtell, A. deFazio, D. Gertig, A. Green, P. Parsons, N. Hayward, P. Webb and D. Whiteman (AUS); G. Peuteman, T. Van Brussel and D. Smeets (BEL); T. Koehler (GER); G.S. Keeney (MAY); A. Samoila Y. Bensman, L. Rodriquez, M. King, U. Chandran, D. Gifkins, and T. Puvananayagam (NJO); M. Sherman, A. Hutchinson, N. Szeszenia- Dabrowska, B. Peplonska, W. Zatonski, A. Soni, P. Chao and M. Stagner (POL); C. Luccarini, P. Harrington, the SEARCH team, and ECRIC (SEA); the Scottish Gynaecological Clinical Trails group and SCOTROC1 investigators (SRO); I. Jacobs, M. Widschwendter, E. Wozniak, N. Balogun, A. Ryan and J. Ford (UKO); C. Pye (UKR); A. Amin Al Olama, K. Michilaidou, and K. Kuchenbäker (COGS). The Australian Ovarian Cancer Study (AOCS) Management Group (D Bowtell, G. Chenevix-Trench, A. deFazio, D. Gertig, A. Green, and P.M. Webb) gratefully acknowledges the contribution of all the clinical and scientific collaborators (see http://www.aocstudy.org/). The Australian Cancer Study (ACS) Management Group comprises A. Green, P. Parsons, N. Hayward, P.M. Webb, and D. Whiteman. This study would not have been possible without the contributions of the following: Per Hall (COGS); Douglas F. Easton (BCAC), Rosalind A. Eeles, Douglas F. Easton, Ali Amin Al Olama, Zsofia Kote-Jarai (PRACTICAL), Georgia Chenevix-Trench, Antonis Antoniou, Fergus Couch and Ken Offit (CIMBA), Joe Dennis, Alison M. Dunning, Andrew Lee, and Ed Dicks (University of Cambridge), Javier Benitez, Anna Gonzalez-Neira and the staff of the CNIO genotyping unit, Jacques Simard and Daniel C. Tessier, Francois Bacot, Daniel Vincent, Sylvie LaBoissière and Frederic Robidoux and the staff of the McGill University and Génome Québec Innovation Centre, Stig E. Bojesen, Sune F. Nielsen, Borge G. Nordestgaard, and the staff of the Copenhagen DNA laboratory, and Sharon A. Windebank, Christopher A. Hilker, Jeffrey Meyer and the staff of Mayo Clinic Genotyping Core Facility.

Financial Support: This study was supported by funding from several sources including the Ovarian Cancer Research Fund thanks to donations by the family and friends of Kathryn Sladek Smith (PPD/RPCI.07); the Genetic Associations and Mechanisms in Oncology (GAME-ON): a NCI Cancer Post-GWAS Initiative (U19-CA148112 and U19-CA148537); the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175); the Canadian Institutes for Health Research (CIHR) MOP-86727 and the CIHR Team in Familial Risks of Breast Cancer; the American Cancer Society (CRTG-00-196-01-CCE); the California Cancer Research Program (00-01389V-20170, N01-CN25403, 2II0200); the Canadian Institutes for Health Research; Cancer Council Victoria; Cancer Council Queensland; Cancer Council New South Wales; Cancer Council South Australia; Cancer Council Tasmania; Cancer Foundation of Western Australia; the Cancer Institute of New Jersey; Cancer Research UK (C490/A6187, C490/A10119, C490/A10124, C536/A13086, C536/A6689, C1287/A10118, C1287/A 10710, C12292/A11174, C5047/A8384, C5047/A15007, C5047/A10692); the Celma Mastry Ovarian Cancer Foundation; the Danish Cancer Society (94-222-52); the ELAN Program of the University of Erlangen-Nuremberg; the Eve Appeal; the Helsinki University Central Hospital Research Fund; Helse Vest; Imperial Experimental Cancer Research Centre (C1312/A15589); the Norwegian Cancer Society; the Norwegian Research Council; the Ovarian Cancer Research Fund; Nationaal Kankerplan of Belgium; the L. & S. Milken Foundation; the Polish Ministry of Science and Higher Education; the US National Cancer Institute (K07-CA095666, K07-CA143047, K22-CA138563, N01-CN55424, N01-PC067010, N01-PC035137, P01-CA017054, P01-CA087696, P20-GM103418, P30-CA072720, P30-CA15083, P30-CA168524, P50-CA105009, P50-CA136393, R01-CA014089, R01-CA016056, R01-CA017054, R01-CA049449, R01-CA050385, R01-CA054419, R01-CA058598, R01-CA058860, R01-CA061107, R01-CA061132, R01-CA063682, R01-CA064277, R01-CA067262, R01-CA071766, R01-CA074850, R01-CA076016, R01-CA080742, R01-CA080978, R01-CA128978, R01-CA083918, R01-CA087538, R01-CA092044, R01-095023, R01-CA106414, R01-CA122443, R01-CA61107, R01-CA112523, R01-CA114343, R01-CA126841, R01-CA136924, R01-CA149429, R03-CA113148, R03-CA115195, R21-GM86689, R37-CA070867, R37-CA70867, U01-CA069417, U01-CA071966, and Intramural research funds); the US Army Medical Research and Material Command (DAMD17-98-1-8659, DAMD17-01-1-0729, DAMD17-02-1-0666, DAMD17-02-1-0669, W81XWH-10-1-0280, W81XWH-10-1-0341); the National Health and Medical Research Council of Australia (199600, 400413, and 400281); the German Federal Ministry of Education and Research of Germany Programme of Clinical Biomedical Research (01 GB 9401); the state of Baden-Württemberg through Medical Faculty of the University of Ulm (P.685); the Minnesota Ovarian Cancer Alliance; the Mayo Foundation; the Fred C. and Katherine B. Andersen Foundation; the Lon V. Smith Foundation (LVS-39420); the Polish Committee for Scientific Research (4P05C 028 14 and 2P05A 068 27); the Oak Foundation; the OHSU Foundation; the Mermaid I project; the Rudolf-Bartling Foundation; the UK National Institute for Health Research Biomedical Research Centres at the University of Cambridge; Imperial College

London; University College Hospital “Womens Health Theme” and the Royal Marsden Hospital; WorkSafeBC; Komen Foundation for the Cure; and the Breast Cancer Research Foundation.

G. Chenevix-Trench and P.M. Webb are supported by the Australian National Health and Medical Research Council; B. Karlan holds an American Cancer Society Early Detection Professorship (SIOP-06-258-01-COUN); and A. Berchuck holds the Barbara Thomason Ovarian Cancer Research Professorship from the American Cancer Society (SIOP-06-090-06).

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61:69–90. [PubMed: 21296855]
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62:10–29. [PubMed: 22237781]
3. Riggs JE. Rising ovarian cancer mortality in the elderly: a manifestation of differential survival. Gynecol Oncol. 1995; 58:64–67. [PubMed: 7789892]
4. du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer. 2009; 115:1234–1244. [PubMed: 19189349]
5. Friedlander ML. Prognostic factors in ovarian cancer. Semin Oncol. 1998; 25:305–314. [PubMed: 9633842]
6. White KL, Rider DN, Kalli KR, Knutson KL, Jarvik GP, Goode EL. Genomics of the NF-kappaB signaling pathway: hypothesized role in ovarian cancer. Cancer Causes Control. 2011; 22:785–801. [PubMed: 21359843]
7. Fasching PA, Gayther S, Pearce L, Schildkraut JM, Goode E, Thiel F, et al. Role of genetic polymorphisms and ovarian cancer susceptibility. Mol Oncol. 2009; 3:171–181. [PubMed: 19383379]
8. Virchow, R. Aetiologie der neoplastischen Geschwulst/Pathogenie de neoplastischen Geschwulste. In: Hirschwald, VvA, editor. Die Krankhaften Geschwulste. Berlin: 1863. p. 57-101.
9. Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. Markers of inflammation and risk of ovarian cancer in Los Angeles County. Int J Cancer. 2009; 124:1409–1415. [PubMed: 19065661]
10. Brinton LA, Gridley G, Persson I, Baron J, Bergqvist A. Cancer risk after a hospital discharge diagnosis of endometriosis. Am J Obstet Gynecol. 1997; 176:572–579. [PubMed: 9077609]
11. Toriola AT, Grankvist K, Agborsangaya CB, Lukanova A, Lehtinen M, Surcel H-M. Changes in pre-diagnostic serum C-reactive protein concentrations and ovarian cancer risk: a longitudinal study. Annals Oncol. 2011; 22:1916–1921.
12. Sharma R, Hook J, Kumar M, Gabra H. Evaluation of an inflammation-based prognostic score in patients with advanced ovarian cancer. Eur J Cancer. 2008; 44:251–256. [PubMed: 18155897]
13. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene. 1999; 18:6853–6866. [PubMed: 10602461]
14. Hayden MS, Ghosh S. Signaling to NF-kappaB. Genes Dev. 2004; 18:2195–2224. [PubMed: 15371334]
15. White KL, Vierkant RA, Fogarty ZC, Charbonneau B, Block MS, Pharoah PD, et al. Analysis of over 10,000 cases finds no association between previously reported candidate polymorphisms and ovarian cancer outcome. Cancer Epidemiol Biomarkers Prev. 2013; 22:987–992. [PubMed: 23513043]
16. Pharoah PDP, Tsai Y-Y, Ramus SJ, Phelan CM, Goode EL, Lawrenson K, et al. GWAS meta-analysis and replication identifies three new susceptibility loci for ovarian cancer. Nat Genet. 2013; 45:362–370. [PubMed: 23535730]
17. Sicotte H, Rider D, Poland G, Dhiman N, Kocher J-P. SNPPicker: High quality tag SNP selection across multiple populations. BMC Bioinformatics. 2011; 12:129. [PubMed: 21535878]

18. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34:816–834. [PubMed: 21058334]

19. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004; 74:765–769. [PubMed: 14997420]

20. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics. 2010; 26:2336–2337. [PubMed: 20634204]

21. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 2012; 40:D930–D934. [PubMed: 22064851]

22. Bertin J, Wang L, Guo Y, Jacobson MD, Poyet JL, Srinivasula SM, et al. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. J Biol Chem. 2001; 276:11877–11882. [PubMed: 11278692]

23. Gaide O, Martinon F, Micheau O, Bonnet D, Thome M, Tschopp J. Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF-kappaB activation. FEBS Lett. 2001; 496:121–127. [PubMed: 11356195]

24. Chan W, Schaffer TB, Pomerantz JL. A quantitative signaling screen identifies CARD11 mutations in the CARD and LATCH domains that induce Bcl10 ubiquitination and human lymphoma cell survival. Mol Cell Biol. 2013; 33:429–443. [PubMed: 23149938]

25. Wu Y, Bressette D, Carrell JA, Kaufman T, Feng P, Taylor K, et al. Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLyS. J Biol Chem. 2000; 275:35478–35485. [PubMed: 10956646]

26. Bolton KL, Tyrer J, Song H, Ramus SJ, Notaridou M, Jones C, et al. Common variants at 19p13 are associated with susceptibility to ovarian cancer. Nat Genet. 2010; 42:880–884. [PubMed: 20852633]

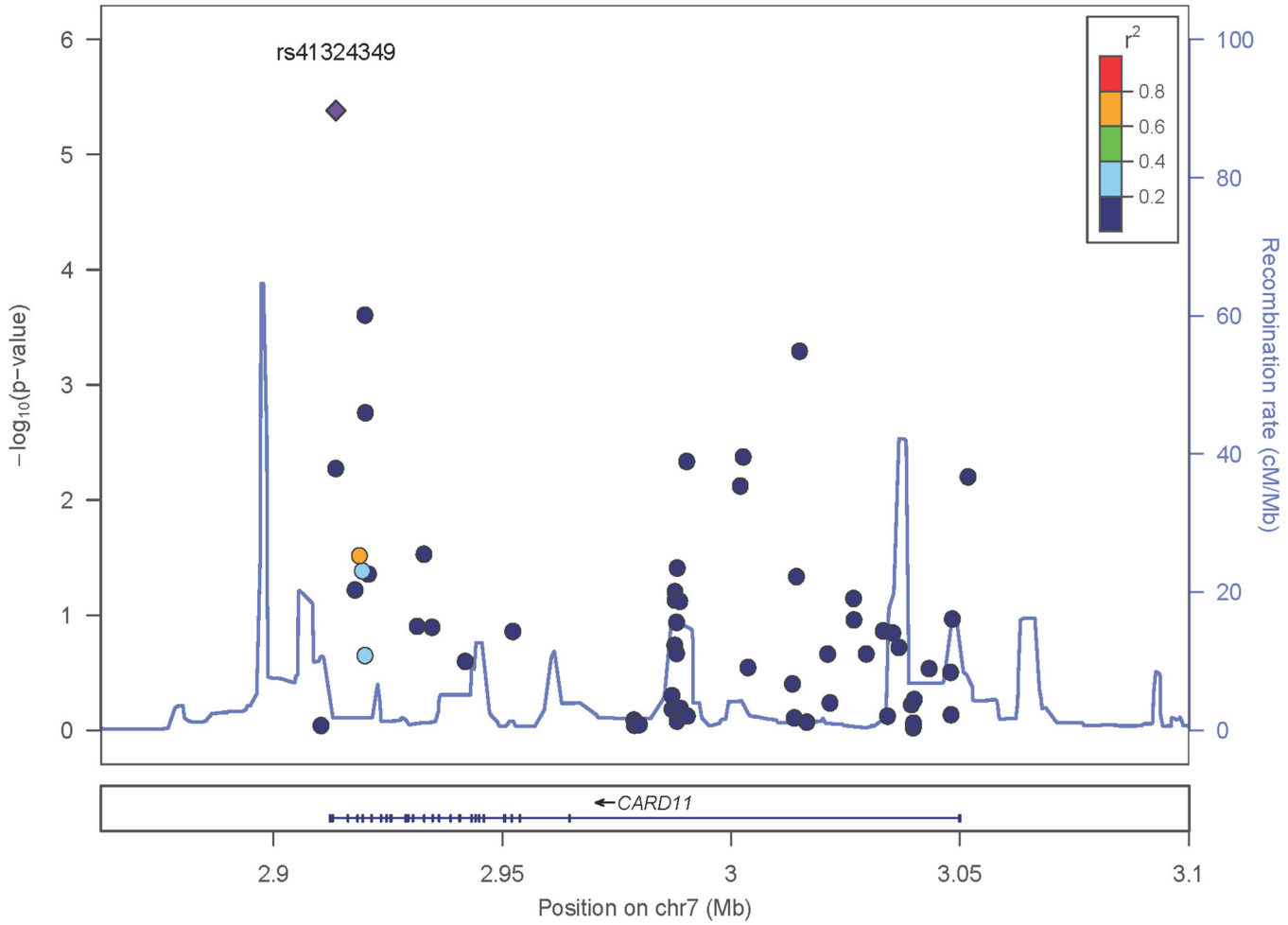

Figure 1. Strength of association between *CARD11* genotypes and survival of women with mucinous EOC (N=661)

Adjusted for study site, first five European ancestry population substructure PCs, age at diagnosis, tumor stage, tumor grade, and oral contraceptive use. Circles represent imputed SNPs, while triangles represent genotyped SNPs.

Table 1

SNP association with EOC overall survival ($p<0.001$, $r^2<0.20$)

Histologic Subtype	Gene	SNP	Alleles	MAF	HR (95% CI)	p-value
Mucinous (N=661)	*CARD11*	**rs41324349**	**C>A**	**0.44**	**1.82 (1.41–2.35)**	**4.13×10^{-6}**
		rs6944821	A>G	0.31	1.64 (1.26–2.13)	2.47×10^{-4}
		rs34251392	A>G	0.34	0.63 (0.48–0.82)	5.08×10^{-4}
	TRAF5	rs79776636	G>C	0.04	2.89 (1.70–4.92)	4.01×10^{-4}
	IKBKE	rs10836	G>C	0.47	0.62 (0.47–0.82)	6.04×10^{-4}
	PIK3R1	rs10940158	G>A	0.52	1.47 (1.17–1.85)	8.47×10^{-4}
Endometrioid (N=1,452)	*TNFRSF13B*	**rs7501462**	**A>G**	**0.26**	**0.68 (0.56–0.82)**	**2.33×10^{-5}**
	PELI2	rs1152468	G>C	0.40	0.75 (0.64–0.87)	1.86×10^{-4}
	MAP2K6	rs72847071	G>A	0.09	1.61 (1.26–2.05)	2.66×10^{-4}
	IL3	rs40401	G>A	0.22	0.72 (0.59–0.87)	5.65×10^{-4}
	TLR5	rs5744157	G>C	0.12	0.66 (0.52–0.85)	8.28×10^{-4}
High grade serous (N=5,248)	*TRAF2*	rs17250239	G>A	0.11	0.84 (0.77–0.92)	6.49×10^{-5}
	PRKCA	rs9894564	A>G	0.24	0.90 (0.84–0.95)	5.83×10^{-4}
Clear cell (N=795)	*PLCG1*	rs11696662	G>A	0.07	0.43 (0.26–0.73)	4.56×10^{-4}
	MAPK1	rs72847071	T>A	0.43	0.70 (0.57–0.86)	6.10×10^{-4}
All (N=10,084)	*MAPK3*	rs61764220	A>G	0.03	0.81 (0.71–0.92)	6.50×10^{-4}
	PGR	rs518162	G>A	0.08	0.87 (0.81–0.95)	8.11×10^{-4}

Bold indicates $p< 2.5\times10^{-5}$; adjusted for study site, first five European ancestry population substructure PCs, age at diagnosis, tumor stage, tumor grade, oral contraceptive use, and histology (for analyses of all cases only); SNPs with $p<0.001$, but correlated at $r^2>0.20$ SNPs above are not shown; SNP id is dbSNP 137 rsid; MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval; minor allele designation based on allele frequencies in all cases.