Inherited Variants in Regulatory T Cell Genes and Outcome of Ovarian Cancer

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Abstract

Although ovarian cancer is the most lethal of gynecologic malignancies, wide variation in outcome following conventional therapy continues to exist. The presence of tumor-infiltrating regulatory T cells (Tregs) has a role in outcome of this disease, and a growing body of data supports the existence of inherited prognostic factors. However, the role of inherited variants in genes encoding Treg-related immune molecules has not been fully explored. We analyzed expression quantitative trait loci (eQTL) and sequence-based tagging single nucleotide polymorphisms (tagSNPs) for 54 genes associated with Tregs in 3,662 invasive ovarian cancer cases. With adjustment for known prognostic factors, suggestive results were observed among rarer histological subtypes; poorer survival was associated with minor alleles at SNPs in RGS1 (clear cell, rs10921202, \(p = 2.7 \times 10^{-7}\)), LRRC32 and TNFRSF18/TNFRSF4 (mucinous, \(rs3781699, p = 4.5 \times 10^{-6}\), and \(rs3753348, p = 9.0 \times 10^{-4}\), respectively), and CD80 (endometrioid, \(rs13071247, p = 8.0 \times 10^{-7}\)). For the latter, correlation data support a CD80 rs13071247 genotype association with CD80 tumor RNA expression (\(p = 0.006\)). An additional eQTL SNP in CD80 was associated with shorter survival (rs7804190, \(p = 8.1 \times 10^{-6}\)) among all cases combined. As the products of these genes are known to affect induction, trafficking, or immunosuppressive function of Tregs, these results suggest the need for follow-up phenotypic studies.


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Introduction

Ovarian cancer is the fifth leading cause of cancer death among women in the United States [1]. Five-year overall survival is approximately 45%, and, even with modern surgical and chemotherapeutic strategies, most cases with advanced disease relapse and succumb to the disease [2,3]. Rare germline BRCA1 or BRCA2 mutations confer improved survival [4]. Common inherited variants could also influence outcome; genome-wide association studies (GWAS) are underway, but have yet to find

Several studies demonstrate the importance of the immune system in ovarian cancer outcome. For example, in one report, cases with evidence of CD3 T cell tumor infiltration (approximately one-half of the cases studied) showed improved progression-free and overall survival [6]. Subsequent studies have refined our understanding of tumor-infiltrating T cells, including one showing that CD8+ T lymphocytes are the primary sub-population of T cells associated with better survival [7]. Along with the finding that tumor antigen-specific T cell responses can be detected in cases, these results suggest that anti-tumor immunity is elicited against ovarian cancers and impacts the clinical course of the disease [8]. Despite this generation of an immune response, however, anti-tumor immunity is counterbalanced by an immune suppressive microenvironment [9]. Of immune suppressive mechanisms, CD4+ regulatory T cells (Tregs) are a primary means of immune evasion in ovarian cancers; these are CD4+ T lineage cells whose primary function is immune regulation [10]. In collaboration with others, we first suggested a role of tumor-infiltrating Tregs in ovarian cancer pathogenesis, reporting higher levels of CD4+ Tregs, measured with immunofluorescence, among cases with poorer survival [11]. Subsequent work supports the importance of CD4+ Tregs in ovarian cancer pathogenesis and outcome [7,12]. For example, the presence of CD4+ Tregs appears to influence the anti-tumor activity of tumor-infiltrating cytotoxic CD8+ T cells [7]. CD4+ Tregs block both adaptive and innate immune effectors by cell contact mechanisms as well as by soluble mediators [13,14]. Soluble mediators of suppression commonly associated with CD4+ Tregs include IL-10 and TGF-β, both of which block T cell proliferation and cell-mediated immunity [15,16,17]. Other cell surface molecules implicated in suppressing the immune response include B7-H1 (CD274), GITR (TNFRSF18), LAG-3, CTLA-4, and surface-bound TGF-β [18,19,20].

Because of the importance of CD4+ Tregs and a role for inherited factors in outcome, we assessed whether common inherited variation related to CD4+ Treg-related genes was associated with ovarian cancer outcome following standard of care therapy. Specifically, we assessed 54 genes key to the biology of Tregs [18,19,20]. These genes include more than one tagSNP for large groups of correlated SNPs including SNPs with appropriate design scores for an Illumina most informative. Selected tagSNPs (N = 1,451) were optimized to include SNPs with appropriate design scores for an Illumina Goldengate BeadArray Assay, predicted functionality, and to include more than one tagSNP for large groups of correlated SNPs [25]. Additional SNP information is in Table S2.

**Materials and Methods**

**Ethics Statement**

Protocols were approved by the appropriate institutional review boards (Mayo Clinic Institutional Review Board, Roswell Park Cancer Institute Institutional Review Boards, Duke University Institutional Review Boards, National Cancer Institute Institutional Review Boards, Moffitt Cancer Center Institutional Review Boards) or ethics panel (Royal Marsden Hospital ethics panel, University of Cambridge ethics panel, University College London ethics panel). All participants gave written informed consent.

**Candidate Gene SNP Array**

Fifty-four genes of key relevance to the biology of Tregs (ACVR2B, AGTR1, CCL11, CCL17, CCL19, CCL2, CCL20, CCL22, CCL3, CCL4, CCL5, CCR4, CCR6, CCR7, CCR8, CD274, CD46, CD90, CD96, CXC10, CXC11, CXC15, DUSP4, EGR2, GPR63, IDO1, IKZF2, IKZF3, IL10RB, IL15RA, IL2RB, IL6ST, IL9, INHA1, INHBB, IRF3, ITGA3, KLF10, LAG3, LRR32, MDHC, NRPI, PDCD1, PLAG1, PRNP, RGAS1, RGAS6, SHBG, SLC1A5, SMAD3, SOCS2, TNFRSF18, TNFRSF4, and TNFRSF9) were chosen for study (Table S1). The relevance of these genes was established from a PubMed [21] database search which revealed published information that either directly showed or suggested a role for the respective gene products in the induction, immune suppressive function, or trafficking of Tregs. Eighty-five SNPs associated with ovarian cancer outcome following standard of care therapy. Specifically, we assessed 54 genes key to the biology of Tregs [18,19,20]. These genes include more than one tagSNP for large groups of correlated SNPs [25]. Additional SNP information is in Table S2.

**Candidate Gene Study Participants and Genotyping**

Cases genotyped on the Treg custom SNP array included women with pathologically-confirmed invasive primary epithelial ovarian, peritoneal, or fallopian tube cancer enrolled at the Mayo Clinic and Roswell Park Cancer Institute (RPCI), Mayo Clinic cases (N = 905) were ascertained between December 1999 and November 2010 into the Mayo Clinic Ovarian Cancer Case-Control Study (MAY) or the Mayo Clinic Case-Only Ovarian Cancer Study (MAY), and included women aged 20 years or above who were enrolled through Mayo Clinic’s Divisions of Gynecologic Surgery and Medical Oncology. Sixty eight percent of these cases were enrolled within a week of diagnosis (median time from diagnosis to recruitment was zero days). RPCI cases (N = 167) were residents of Western Pennsylvania, Eastern Ohio, or Western New York, aged 25 years or above and ascertained between January 2004 and May 2009 within six months of diagnosis through Roswell Park Cancer Institute’s Divisions of Gynecologic Surgery and Oncology. These cases were enrolled with a median time from diagnosis to recruitment of 80 days. At both sites, DNA was extracted from 10 to 15 mL fresh peripheral blood (using Gentra AutoPure LS PurgeL RNA Kit methodology at Mayo Clinic and FlexiGene DNA Kit methodology at RPCI), stored at −80°C, and bar-coded to ensure accurate processing. A total of 1,072 participants were genotyped using a custom Illumina Goldengate BeadArray Assay along with 24 duplicates and 24 HapMap CEU replicates (8 trios). Concordance among study duplicates was 99.998%, no SNPs had unresolved replicate or Mendelian errors, and the mean genotype call rate was 99.88%. Samples with genotyping failure (N = 32) or call rate <97% (N = 11) were excluded as well as samples found to be incorrectly plated (N = 1) or from cases deemed ineligible due to non-epithelial disease (N = 5), borderline tumor behavior (N = 15), or enrollment more than one year prior to diagnosis (N = 5). SNPs with genotyping failure (N = 162), call rate <95% (N = 19), or HWE <0.0001 and poor clustering (N = 15) were excluded. Thus, analyses were based on 994 cases and 1,340 SNPs.

**Genome-Wide Association Study**

We also analyzed data from the Follow-up of Ovarian Cancer Genetic Association and Interaction Studies (FOCI) collaboration.
which is part of the National Cancer Institute GAME-ON Post-
GWAS initiative and is described elsewhere [26,27]. In brief,
participants were 2,167 self-reported white invasive ovarian cancer
cases enrolled in the North Carolina Ovarian Cancer Study
(NCO), the NCI Ovarian Case-Control Study in Poland (POL),
the Royal Marsden Cancer Study (RMH), the UK Studies of
Epidemiology and Risk Factors in Cancer Heredity Ovarian
Cancer Study (SEA), the Tampa Bay Ovarian Cancer Study
(TBO), and the UK Familial Ovarian Cancer Registry and the
UK Ovarian Cancer Population Study (UKO+UKR). Genotyping
was performed in Illumina 317 k or 610-Quad Infinium Arrays with
imputation to HapMap v 26 using dosage values obtained from
the MACH software package [28]. Data on 820 SNPs were
available on all cases.

Statistical Analysis

We used Cox proportional hazards regression accounting for
left truncation to estimate hazard ratios (HRs) and 95%
confidence intervals (CIs) for association with overall survival.
Survival time was defined as time from diagnosis of ovarian cancer
until death from any cause or last follow-up. For each SNP, HRs
with 95% CIs were estimated per-allele (i.e., 0, 1, or 2 copies of
minor allele), analogous to the Armitage test for trend for binary
endpoints. Log-additive Cox proportional hazards regression
models were adjusted for study site (MAC+MAC, RPCI, POL,
NCO, RMH, SEA, TBO, UKO+UKR), age at diagnosis (<50
years, 50–69 years, >70 years), tumor stage (I or II, III or IV,
unknown), tumor grade (low, high, unknown), and race (white,
non-white, unknown), modeling direct genotype calls for MAY
and mip and imputed allele dosage values where appropriate
for FOCI participants. Heterogeneity of HRs across
study site was formally examined using study-by-SNP interaction
terms and performing likelihood ratio tests; no correction for
multiple testing was performed.

Tumor Expression Quantitative Trait Locus (eQTL)

Analysis

For 54 genotyped Mayo Clinic cases (33 serous, nine clear cell,
endometrioid, four mucinous), expression analyses were also
performed. Tumor RNA was isolated from fresh frozen samples,
using the Qiagen RNeasy protocol and quantitated using a
Nanodrop Spectrophotometer (Agilent Technologies, Santa Clara,
CA). Total RNA (750 ng) of high quality (RNA integrated number
>2.0) was labeled with cyanine 5-CTP or cyanine 3-CTP, using
the Low RNA Input Fluorescent Linear Amplification Kit (Agilent
Technologies), purified on RNeasy Mini columns (Qiagen), and
hybridized to Agilent whole human genome 4×44 K expression
arrays (using a mixed reference containing 106 tumor samples).
Slides were scanned using the Agilent 25KBA Scanner, and data
were normalized using Agilent’s error model and exported by the
Agilent Feature Extraction Software (version 7.5.1). Data in the
form of the log ratios of signals from individual tumors to signals
from the reference mix were used for analysis. For genes with
SNPs that were associated with survival at p<0.001, association
between genotype and expression probes was assessed using
Wilcoxon rank-sum tests.

Results

The nearly 1,000 invasive ovarian cancer cases genotyped in a
Treg custom SNP array and approximately 2,600 cases in the
FOCI collaboration demonstrated the expected distributions of
mortality, age, and clinical features (Table 1). 1,529 deaths were
observed during a median follow-up of 5.4 years. In combined
association with CD80 tumor RNA expression. Combined analysis of multiple independent datasets provides greater statistical power than separate discovery and replication analyses [29]; thus, we used this study design and examined the possibility of heterogeneity of results across studies. As no statistically significant heterogeneity across studies was observed, our inference is based on this combined, most powerful approach. It is worth noting that we examined a relatively large number of SNPs across 54 Treg-associated genes and across different histologic subtypes, and therefore we acknowledge that multiple testing issues may exist; some of our highlighted results could indeed be false positive associations. The fact that the genes examined were chosen based on an a priori role in ovarian cancer and that we concentrated only on SNP associations with p-values less than 0.001 lessens, but does not entirely eliminate, this possibility. Nonetheless, this work highlights particular Treg-related genes of interest.

CD80 has been extensively studied for its role in immune responses, yet no focused analysis of SNPs and ovarian cancer outcome has been reported, to our knowledge. It acts as a ligand for both CD28 and CTLA4, leading to proliferation and anergy in

### Table 1. Distributions of Ovarian Cancer Clinical Characteristics by Study.

<table>
<thead>
<tr>
<th></th>
<th>MAY+MAC (N = 873)</th>
<th>RPCI (N = 121)</th>
<th>NCO (N = 492)</th>
<th>POL (N = 210)</th>
<th>RMH (N = 143)</th>
<th>SEA (N = 1,087)</th>
<th>TBO (N = 212)</th>
<th>UKO+UKR (N = 527)</th>
<th>Total (N = 3,665)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital status at last follow up</td>
<td></td>
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</tr>
<tr>
<td>Alive</td>
<td>450 (52%)</td>
<td>74 (61%)</td>
<td>264 (54%)</td>
<td>95 (45%)</td>
<td>57 (40%)</td>
<td>719 (66%)</td>
<td>118 (56%)</td>
<td>359 (68%)</td>
<td>2,136 (58%)</td>
</tr>
<tr>
<td>Deceased</td>
<td>423 (48%)</td>
<td>47 (39%)</td>
<td>228 (46%)</td>
<td>115 (55%)</td>
<td>86 (60%)</td>
<td>368 (34%)</td>
<td>94 (44%)</td>
<td>168 (32%)</td>
<td>1,529 (42%)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
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<tr>
<td>&lt;50 years</td>
<td>126 (14%)</td>
<td>19 (16%)</td>
<td>119 (24%)</td>
<td>66 (31%)</td>
<td>45 (31%)</td>
<td>260 (24%)</td>
<td>35 (17%)</td>
<td>94 (18%)</td>
<td>764 (21%)</td>
</tr>
<tr>
<td>50–69 years</td>
<td>484 (55%)</td>
<td>71 (59%)</td>
<td>299 (61%)</td>
<td>117 (56%)</td>
<td>97 (68%)</td>
<td>780 (72%)</td>
<td>134 (63%)</td>
<td>332 (63%)</td>
<td>2,314 (63%)</td>
</tr>
<tr>
<td>70+ years</td>
<td>263 (30%)</td>
<td>31 (26%)</td>
<td>74 (15%)</td>
<td>27 (13%)</td>
<td>1 (1%)</td>
<td>47 (4%)</td>
<td>43 (20%)</td>
<td>101 (19%)</td>
<td>587 (16%)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
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<tr>
<td>Serous</td>
<td>638 (79%)</td>
<td>89 (82%)</td>
<td>294 (65%)</td>
<td>93 (65%)</td>
<td>55 (48%)</td>
<td>502 (53%)</td>
<td>139 (74%)</td>
<td>282 (60%)</td>
<td>2,092 (65%)</td>
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<tr>
<td>Endometrioid</td>
<td>106 (13%)</td>
<td>11 (10%)</td>
<td>80 (18%)</td>
<td>28 (20%)</td>
<td>26 (23%)</td>
<td>201 (21%)</td>
<td>27 (14%)</td>
<td>91 (19%)</td>
<td>570 (18%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>46 (6%)</td>
<td>1 (1%)</td>
<td>58 (13%)</td>
<td>9 (6%)</td>
<td>17 (15%)</td>
<td>109 (12%)</td>
<td>11 (6%)</td>
<td>46 (10%)</td>
<td>297 (9%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>22 (3%)</td>
<td>7 (6%)</td>
<td>20 (4%)</td>
<td>13 (9%)</td>
<td>16 (14%)</td>
<td>129 (14%)</td>
<td>12 (6%)</td>
<td>53 (11%)</td>
<td>272 (8%)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>61 13 40 67 29 146 23 55 434</td>
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<td>Stage</td>
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<tr>
<td>Stage I/II</td>
<td>176 (20%)</td>
<td>25 (23%)</td>
<td>160 (33%)</td>
<td>56 (39%)</td>
<td>0</td>
<td>512 (61%)</td>
<td>50 (24%)</td>
<td>216 (47%)</td>
<td>1,195 (38%)</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>692 (80%)</td>
<td>84 (77%)</td>
<td>330 (67%)</td>
<td>86 (61%)</td>
<td>0</td>
<td>326 (39%)</td>
<td>155 (76%)</td>
<td>247 (53%)</td>
<td>1,920 (62%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 12 2 68 143 249 7 64 55 434</td>
<td></td>
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<tr>
<td>Low grade</td>
<td>116 (14%)</td>
<td>37 (33%)</td>
<td>202 (42%)</td>
<td>61 (47%)</td>
<td>43 (54%)</td>
<td>442 (55%)</td>
<td>51 (24%)</td>
<td>168 (43%)</td>
<td>1,120 (37%)</td>
</tr>
<tr>
<td>High grade</td>
<td>724 (86%)</td>
<td>76 (67%)</td>
<td>278 (58%)</td>
<td>69 (53%)</td>
<td>36 (46%)</td>
<td>365 (45%)</td>
<td>156 (75%)</td>
<td>225 (57%)</td>
<td>1,929 (63%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>33 8 12 80 64 280 5 134 616</td>
<td></td>
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<td>Self-reported race</td>
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</tr>
<tr>
<td>White</td>
<td>767 (98%)</td>
<td>112 (93%)</td>
<td>492 (100%)</td>
<td>210 (100%)</td>
<td>143 (100%)</td>
<td>1,087 (100%)</td>
<td>212 (100%)</td>
<td>527 (100%)</td>
<td>3,550 (99%)</td>
</tr>
<tr>
<td>Non-white</td>
<td>13 (2%)</td>
<td>8 (7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21 (1%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>93 1 0 0 0 0 0 0 94</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Adjusted for study site (MAY+MAC, RPCI, POL, UKO+UKR, TBO, NCO, RMH, SEA), age at diagnosis (<50 years, 50–69 years, ≥70 years), tumor stage (I or II, III or IV, unknown), race (white, non-white, unknown), and tumor grade (low, high, unknown); linkage disequilibrium reduced to r^2<0.95; MAF, minor allele frequency.

doi:10.1371/journal.pone.0053903.t001

### Table 2. Regulatory T Cell SNPs Associated with Overall Survival (p<0.001).

<table>
<thead>
<tr>
<th>Case Group</th>
<th>Gene</th>
<th>SNP</th>
<th>MAF</th>
<th>Location</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Cell (N = 217)</td>
<td>RGS5</td>
<td>rs10921202</td>
<td>0.07</td>
<td>Intron</td>
<td>2.93 (1.77–4.84)</td>
<td>2.7×10⁻⁵</td>
</tr>
<tr>
<td>Mucinous (N = 272)</td>
<td>LRRC32</td>
<td>rs3781699</td>
<td>0.35</td>
<td>3’ UTR</td>
<td>2.32 (1.45–3.71)</td>
<td>4.5×10⁻⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs7944357</td>
<td>0.44</td>
<td>Intron</td>
<td>2.04 (1.34–3.10)</td>
<td>8.3×10⁻⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNFRSF4/TNFRSF18</td>
<td>0.05</td>
<td>Intergenic</td>
<td>3.41 (1.65–7.05)</td>
<td>9.0×10⁻⁴</td>
</tr>
<tr>
<td>Endometrioid (N = 570)</td>
<td>CD80</td>
<td>rs13071247</td>
<td>0.14</td>
<td>Intron</td>
<td>1.73 (1.26–2.39)</td>
<td>8.0×10⁻⁴</td>
</tr>
<tr>
<td>All Cases (N = 3,655)</td>
<td>CD80</td>
<td>rs7804190</td>
<td>0.37</td>
<td>MADI1</td>
<td>1.14 (1.06–1.23)</td>
<td>8.1×10⁻⁴</td>
</tr>
</tbody>
</table>

Adjusted for study site (MAY+MAC, RPCI, POL, UKO+UKR, TBO, NCO, RMH, SEA), age at diagnosis (<50 years, 50–69 years, ≥70 years), tumor stage (I or II, III or IV, unknown), race (white, non-white, unknown), and tumor grade (low, high, unknown); linkage disequilibrium reduced to r^2<0.95; MAF, minor allele frequency.

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naïve T cells, respectively [30,31,32]. CTLA4 is constitutively expressed in Tregs, where it is important in suppressing immune responses through a variety of proposed mechanisms, including activation of the indolamine-2,3-dioxygenase pathway in dendritic cells (DCs) and inhibition of interactions between activated T cells and DCs [33,34,35]. We observed that an intronic tagging CD80 SNP was associated with poorer survival of endometrioid cases and with increased tumor CD80 expression, and, among all cases, we observed that an eQTL SNP on another chromosome which associated with CD80 lymphocyte expression was also associated with poorer survival. Altogether, these data suggest that CD80 expression may be in part driven by inherited factors and may lead to increased immune suppression and poorer outcome.

Of interest to clear cell ovarian cancer, the gene product of RGS1, RGS1 or BL34, is a member of the RGS protein family whose members are involved in regulation of G-protein signaling. Specifically, they are GTPase-activating proteins that limit the duration of G-protein signaling [36,37]. Lymphocyte migration to chemotactic signals are mediated by G-protein signals and previous studies have found that Tregs do not respond as well to chemokine signaling as naïve T cells [38]. Furthermore, RGS1 is more highly expressed in Tregs and expression is inversely correlated with migration [38]. Interestingly, RGS1 gene expression is increased in activated Tregs, and this is mediated by binding of the Treg transcription factor FOXP3 to the RGS1 gene [39]. Associations of RGS1 SNPs have also been observed in numerous T cell-mediated autoimmune diseases, including type 1 diabetes, celiac disease, and multiple sclerosis [40,41,42,43]. Based on these prior studies, it is tempting to speculate that genetically determined RGS1 levels may regulate Treg infiltration into clear cell ovarian cancers and thus contribute to outcome.

A SNP of possible relevance to mucinous disease is in LRRC32 which encodes GARP, a transmembrane protein expressed specifically on naturally occurring activated Tregs, but not resting Tregs [44,45]. GARP has been shown to be a receptor for inactive, latency-associated peptide (LAP) bound TGF-β [46,47]. GARP does not induce activation of latent TGF-β; however, it may function in infectious tolerance, converting FOXP3− cells to suppressive FOXP3+ cells [46,49]. Additionally, GARP is part of a positive feedback loop with FOXP3 in Tregs, which are known to maintain a suppressive tumor microenvironment and prevent an effective immunological response [45]. Our finding of poorer survival among mucinous cases with minor alleles at an LRRC32 SNP suggests that these cases may have increased immune suppression.

We report an association between risk of death due to mucinous ovarian cancer and a SNP in a gene cluster containing TNFRSF18 and TNFRSF4. TNFRSF18 encodes GITR, a co-stimulatory molecule present constitutively on Treg cells and upregulated on naïve T cells after stimulation. Reports are conflicting as to the role of GITR, as it has been reported both to increase [49,50] and to abolish the suppressive function of Tregs [51,52]. While the specific mechanism of GITR action remains controversial, it is clear that it modulates Tregs. TNFRSF4 encodes OX40 (CD134), and signaling through OX40 reduces the ability of Tregs to act as suppressor cells by decreasing expression of FOXP3 [53,54]. Reduced FOXP3 results in decreased miR155 and a subsequent increase in SOCS1 (suppressor of cytokine signaling 1). SOCS1 is a component of a negative feedback loop.
for IL-2 signaling cascade; increased expression of SOCS1 results in a need for increased IL-2 levels for survival of Tregs [55]. Thus, OX40 signaling in Tregs can modulate suppressor functions both by decreasing their effector function and by requiring higher amounts of IL-2 for continued survival and activation. The intragenic SNP associated with overall survival in cases with mucinous ovarian cancer may act through either a GTR or OX40 mechanism. Dissecting the role of the germline variants may help to identify mechanisms that could explain why the immune system is not able to mount an effective response to ovarian cancers.

In conclusion, our analysis of 3,662 invasive ovarian cancer cases suggests that inherited variants related to Tregs are associated with ovarian cancer outcome in a subtype-specific manner, even after adjustment for known prognostic features. Our findings underscore the importance of subtype-specific analyses in clinical and epidemiological studies of ovarian cancer, given the established disease heterogeneity, with each histologic subtype expressing different patterns of genetic, epidemiologic and clinical characteristics [reviewed by Karst and Drapkin [56]]. Future work should include examination of additional study populations, immunological studies, and correlation of inherited variants with other tumor features, such as levels of Treg infiltration.

**References**


**Supporting Information**

| Table S1 | Gene information. (XLS) |
| Table S2 | SNP information. (XLSX) |

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**Author Contributions**

Conceived and designed the experiments: ELG KRK ALO LCH KRM BLK. Performed the experiments: JMC BW GR. Analyzed the data: MJM BLF SMA DJS PR KG RAV DNR H Sicotte CW. Contributed reagents/materials/analysis tools: CMP JMS RPW EI AB RS MJB SH LP SAG SJR NW HPY MG H Sicotte H Song JT PDPD TAS RBN LES KO. Wrote the paper: ELG MD KLK.