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Research Article

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Large-Scale Evaluation of Common Variation in Regulatory T Cell–Related Genes and Ovarian Cancer Outcome

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Abstract

The presence of regulatory T cells (Treg) in solid tumors is known to play a role in patient survival in ovarian cancer and other malignancies. We assessed inherited genetic variations via 749 tag single-nucleotide polymorphisms (SNP) in 25 Treg-associated genes (*CD28*, *CTLA4*, *FOXP3*, *IDO1*, *IL10*, *IL10RA*, *IL15*, *1L17RA*, *IL23A*, *IL23R*, *IL2RA*, *IL6*, *IL6R*, *IL8*, *LGALS1*, *LGALS9*, *MAP3K8*, *STAT5A*, *STAT5B*, *TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGRBR2*, and *TGFBR3*) in relation to ovarian cancer survival. We analyzed genotype and overall survival in 10,084 women with invasive epithelial ovarian cancer, including 5,248 high-grade serous, 1,452 endometrioid, 795 clear cell, and 661 mucinous carcinoma cases of European descent across 28 studies from the Ovarian Cancer Association Consortium (OCAC). The strongest associations were found for endometrioid carcinoma and *IL2RA* SNPs rs11256497 [HR, 1.42; 95% confidence interval (CI), 1.22–1.64; $P = 5.7 \times 10^{-6}$], rs791587 (HR, 1.36; 95% CI, 1.17–1.57; $P = 6.2 \times 10^{-5}$), rs2476491 (HR, = 1.40; 95% CI, 1.19–1.64; $P = 5.6 \times 10^{-5}$), and rs10795763 (HR, 1.35; 95% CI, 1.17–1.57; $P = 7.9 \times 10^{-5}$), and for clear cell carcinoma and *CTLA4* SNP rs231775 (HR, 0.67; 95% CI, 0.54–0.82; $P = 9.3 \times 10^{-5}$) after adjustment for age, study site, population stratification, stage, grade, and oral contraceptive use. The rs231775 allele associated with improved survival in our study also results in an amino acid change in CTLA4 and previously has been reported to be associated with autoimmune conditions. Thus, we found evidence that SNPs in genes related to Tregs seem to play a role in ovarian cancer survival, particularly in patients with clear cell and endometrioid epithelial ovarian cancer. *Cancer Immunol Res; 2(4); 332–40. ©2014 AACR.*

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Introduction

There were an estimated 15,500 deaths from ovarian cancer in the United States in 2012 (1), in part because many tumors are diagnosed at late stage and recurrences are common. Invasive epithelial ovarian cancer (EOC) consists of several histologic subtypes with varying behavior and survival (2), and the rare subtypes are often understudied because of limited numbers. Nonetheless, subtype-specific analysis of EOC is needed to better understand prognostic factors. By combining cases across many studies in the Ovarian Cancer Association Consortium (OCAC), these subtype analyses may now be conducted.

Antitumor immunity by cytotoxic T cells has been demonstrated in ovarian cancer (1), and recent meta-analyses concluded that tumor-infiltrating immune cells predict improved survival in EOC (2) and other malignancies (3). However, in the tumor microenvironment, the function of these cells is often suppressed by a mixture of suppressive cytokines produced by the tumor and by the different populations of suppressive immune cells (4). Of particular interest are regulatory T cells (Treg), which develop in the thymus (natural) or periphery (acquired) and typically express CD4 and FOXP3 (5). These cells interact with antigen-presenting cells (APC) via cell surface molecules, such as CTLA4, to inhibit antigen presentation and to induce APCs to express suppressive cytokines (6). Their presence in tumors has been linked to poor prognosis in patients with EOC (7).

On the basis of this knowledge, we hypothesized that variants in genes expressed in suppressive immune cells may associate with EOC survival. Previously, assessment of polymorphisms in 54 genes in the Treg pathway in 994 EOC cases pooled from two sites found associations between SNPs in *RGS1* (clear cell EOC), *LRRC32* and *TNFRSF4/TNFRSF18* (mucinous EOC), and *CD80* (endometrioid and all EOC) and EOC survival (8). In this study, we have expanded the scope to include polymorphisms in additional Treg-related genes in a much larger pooled analysis of 10,084 invasive EOC cases from 28 studies, allowing subtype-specific analyses.

Materials and Methods

SNP selection

Minor allele frequency (MAF) was defined as the relative frequency of the SNP minor allele in the population. Linkage disequilibrium (defined as the occurrence of paired alleles in a population relative to that expected from random formation of haplotypes) r^2 values were calculated for all pairs of SNPs. Twenty-five genes of relevance to the biology of Tregs (*CD28*, *CTLA4*, *FOXP3*, *IDO1*, *IL10*, *IL10RA*, *IL15*, *1L17RA*, *IL23A*, *IL23R*, *IL2RA*, *IL6*, *IL6R*, *IL8*, *LGALS1*, *LGALS9*, *MAP3K8*, *STAT5A*, *STAT5B*, *TGFB1*, *TGFB2*, *TGFB3*, *TGFBR1*, *TGRBR2*, and *TGFBR3*) were chosen for this study (Supplementary Table S1). The relevance of these genes was established from a PubMed 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 (8). We selected 749 SNPs in these genes, including 5 kb upstream and downstream, in an attempt to tag all common variants using the criteria that all known SNPs with MAF ≥ 0.05 had an $r^2 \geq 0.8$ with at least one tag SNP in the region. Additional SNP information is presented in Supplementary Table S2.

Study participants, genotyping, and quality control

A total of 10,084 invasive EOC cases, of which 5,248 were high-grade serous cases, were examined. Germline DNA (250 ng

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Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

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genomic or 750 ng whole-genome amplified) from participants from 28 studies (Supplementary Table S3) in the OCAC was genotyped on a custom Illumina iSelect BeadArray, using centralized genotype calling and quality control procedures, as described previously (9–13). In brief, we excluded samples with call rate <95% and SNPs with call rate <95% (MAF $\geq$ 0.05) or <99% (MAF < 0.05); we restricted the study to samples with >90% predicted European ancestry, and we estimated principal components representing European substructure (9). Additional exclusions are described by White and colleagues (11).

In silico analysis

Several publicly available *in silico* tools were accessed to determine whether there was any published information related to the identified SNPs, including RegulomeDB, PolyDoms, and the Ensembl Variation. Analysis was carried out on all SNPs that reached a statistical significance of $P < 0.001$. RegulomeDB annotates SNPs with known and predicted regulatory elements in the intergenic and noncoding regions of the *Homo sapiens* genome. Known and predicted regulatory DNA elements include regions of DNase hypersensitivity, binding sites of transcription factors, and promoter regions that have been biochemically characterized to regulate transcription (14). PolyDoms predicts the implications of the nonsynonymous SNPs (nsSNP) using two well-known algorithms [Sort Intolerant from Intolerant (SIFT) and Polymorphism Phenotype (PolyPhen)]. The results are presented onto protein domains and highlight those nsSNPs that are potentially deleterious or have been reported as disease allelic variants (15).

Ensemble Variation (http://useast.ensembl.org/info/genome/variation/index.html) is a database that stores areas of the genome that differ between individual genomes and, if available, stores associated disease and phenotype information for SNPs as well as short nucleotide insertions and/or deletions and longer variants.

Statistical analysis

Cox proportional hazards regression modeling was used to estimate per-allele HRs and 95% confidence intervals (CI) for associations with overall survival (OS). Separate analyses were carried out for all cases combined as well as for each of the four major histologic subtypes (high-grade serous, endometrioid, clear cell, and mucinous), accounting for left truncation and right censoring. Relevant adjustment covariates included lifestyle and clinical variables found to be independently associated with OS in all ovarian cancer cases with available data (Supplementary Table S4). Two different Cox models were created to adjust for relevant covariates: a minimally adjusted Cox model adjusted for age at diagnosis, the first five population substructure principal components, and study site; and a Cox model adjusted additionally for histology (for analyses of all cases only), tumor stage summarized from International Federation of Gynecology and Obstetrics (FIGO) or Surveillance, Epidemiology, and End Results (SEER) stage (localized, regional, distant, unknown), tumor grade (well, moderately, poorly, or undifferentiated, unknown), and oral contraceptive use (yes, no, unknown). The interaction between each SNP and study sites was examined using likelihood ratio testing to identify heterogeneity of HRs across study sites. SNP associations with OS were visually displayed using Kaplan–Meier curves, again accounting for left truncation of data. A Bonferroni-corrected P value (6.2×10^{-4}) was calculated, accounting for linkage disequilibrium between SNPs. Accounting for linkage disequilibrium was done by determining the number of independent bins ($N = 81$), where each bin contained one or more tagSNPs with $r^2 \geq 0.1$ with all other SNPs in the same bin. For the most statistically significant SNPs, we additionally attempted to account for residual disease following surgery by running sensitivity analyses in cases with nonmissing information on tumor debulking status (2,470 total EOC cases, 326 endometrioid EOC cases and 171 clear cell EOC), where we compared unadjusted SNP associations with OS to those adjusted for two-category debulking status (no residual disease versus other). We also used HaploReg v2 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) to identify specific information on potential SNP function for the most statistically significant SNPs (16). Gene expression data were obtained for 200 samples (including 154 serous, 35 endometrioid, 5 clear cell, and 5 mucinous) with matching gene expression and genotype data for rs11256497 and analyzed as described previously (8). Briefly, data were normalized via the Agilent error model and log ratios of signal relative to a reference were used for analyses. After normalization, batch differences caused by Cy5 (case channel) and Cy3 (reference channel) dying dates were adjusted using ComBat, an empirical Bayesian approach (17). Association of rs11256497 genotype with gene expression within the *IL2RA* gene (cis relationship) was assessed by comparing mean $\log_{10}$-transformed normalized gene expression values of GG versus AG/AA genotypes using a two-tailed unpaired t test.

Results

In 10,084 EOC cases (including 5,248 high-grade serous, 1,452 endometrioid, 795 clear cell, and 661 mucinous) pooled from 28 studies, we assessed 749 SNPs in 25 Treg-related genes for associations with OS. The median survival time across the 28 studies included in this analysis ranged from 2.2 to 8.6 years (Supplementary Table S3).

Associations between Treg SNPs and survival among all cases with EOC and by specific tumor histologies

Table 1 includes all SNPs associated at $P < 0.005$ with OS for all EOC cases and by histologic subtype, following adjustment for other prognostic factors. One SNP in *TGFBR2* and 10 SNPs in *IL2RA* were associated with OS in endometrioid EOC at $P < 0.005$, with six *IL2RA* SNPs statistically significantly associated with OS at $P < 6.2 \times 10^{-4}$, including rs11256497 (HR, 1.42; 95% CI, 1.22–1.64; Fig. 1A), rs791587 (HR, 1.36; 95% CI, 1.17–1.57), rs2476491(HR, 1.40; 95% CI, 1.19–1.64), rs10795763 (HR, 1.35; 95% CI, 1.17–1.57), rs2256774 (HR, 1.33; 95% CI, 1.14–1.56), and rs10905669 (HR, 0.71; 95% CI, 0.59–0.85). P values for *IL2RA* SNPs are plotted in Fig. 2A along with linkage disequilibrium with the most strongly associated SNP rs11256497. We observed a moderate amount of linkage disequilibrium between rs11256497 and the other highlighted SNPs, with r^2 values ranging between 0.4 and 0.8. The *CTLA4* SNP rs231775 was

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Table 1. SNPs in Treg genes and association with OS in ovarian cancer ($P < 0.005$)

Case group	Gene	SNP	Alleles[a]	MAF	HR (95% CI)	P^2
All ($N = 10,084$)	*TGFBR2*	Rs6550005	G>A	0.20	0.92 (0.88–0.97)	3.3×10^{-3}
		Rs6770038	G>A	0.18	0.92 (0.87–0.97)	1.7×10^{-3}
		Rs4522809	A>G	0.47	1.08 (1.03–1.12)	**3.7×10^{-4}**
		Rs9843942	G>A	0.37	1.07 (1.02–1.12)	2.5×10^{-3}
	IL10RA	Rs4252314	A>G	0.04	0.85 (0.76–0.95)	4.1×10^{-3}
High-grade serous ($N = 5,248$)	*TGFBR2*	Rs6550005	G>A	0.20	0.91 (0.85–0.97)	4.6×10^{-3}
		Rs6770038	G>A	0.18	0.88 (0.82–0.94)	**3.2×10^{-4}**
		Rs4522809	A>G	0.48	1.09 (1.04–1.15)	1.0×10^{-3}
Endometrioid ($N = 1,452$)	*TGFBR2*	rs12495646	C>A	0.32	0.79 (0.67–0.93)	4.6×10^{-3}
	IL2RA	rs7072398	G>A	0.44	1.27 (1.10–1.47)	1.2×10^{-3}
		rs6602398	C>A	0.30	1.30 (1.11–1.52)	1.6×10^{-3}
		rs11256497	G>A	0.37	1.42 (1.22–1.64)	**5.7×10^{-6}**
		rs791587	G>A	0.46	1.36 (1.17–1.57)	**6.2×10^{-5}**
		rs10905669	G>A	0.24	0.71 (0.59–0.85)	**2.2×10^{-4}**
		rs2476491	A>T	0.29	1.40 (1.19–1.64)	**5.6×10^{-5}**
		rs2245675	G>A	0.32	1.31 (1.13–1.53)	6.2×10^{-4}
		rs10795763	A>C	0.40	1.35 (1.17–1.57)	**7.9×10^{-5}**
		rs2256774	A>G	0.34	1.33 (1.14–1.56)	**2.9×10^{-4}**
		rs706779	A>G	0.46	1.25 (1.08–1.44)	2.8×10^{-3}
Clear cell ($N = 795$)	*CTLA4*	rs231775	A>G	0.37	0.67 (0.54–0.82)	**9.3×10^{-5}**
	MAP3K8	rs306588	A>G	0.31	1.33 (1.10–1.61)	4.1×10^{-3}
		rs202162340	A>C	0.37	1.32 (1.09–1.59)	4.7×10^{-3}
Mucinous ($N = 661$)	*TGFBR3*	rs284172	T>A	0.14	0.60 (0.42–0.86)	3.0×10^{-3}
		rs12129174	G>A	0.16	1.61 (1.18–2.19)	3.8×10^{-3}
		rs4658265	G>A	0.32	1.56 (1.20–2.05)	1.2×10^{-3}
		rs5019497	C>A	0.43	0.68 (0.51–0.89)	4.5×10^{-3}
	TGFBR2	rs2082224	G>A	0.25	0.61 (0.44–0.85)	2.7×10^{-3}

NOTE: Sorted by chromosomal position; linkage disequilibrium reduced to $r^2 < 0.95$;
Bold indicates $P < 6.2 \times 10^{-4}$ (Bonferroni-corrected P value accounting for linkage disequilibrium between SNPs at $r^2 \geq 0.1$); dbSNP 132.
[a]Listed as major > minor. Adjusted for age at diagnosis, population substructure principal components, study site, and histology (for analyses of all cases only), tumor stage (I, localized; II, regional; III, distant; unknown), tumor grade (1, well; 2, moderately; 3, poorly; 4, undifferentiated, and unknown); and oral contraceptive use (yes, no, unknown).

associated with OS in clear cell EOC (HR, 0.67; 95% CI, 0.54–0.82; Fig. 1B) at $P < 6.2 \times 10^{-4}$. Other SNPs in this gene were not statistically significantly associated with clear cell EOC (Fig. 2B); there was a modest association with two *MAP3K8* SNPs. Three of the *TGFBR2* SNPs were also modestly associated ($P < 0.0005$) with OS in patients with high-grade serous EOC, but only rs6770038 (HR, 0.88; 95% CI, 0.82–0.94) met the threshold for statistical significance. Modest associations were also seen between one *TGFBR2* and four *TGFBR3* SNPs and OS in mucinous EOC. One *IL10RA* and four *TGFBR2* SNPs had suggestive associations with OS in all EOCs, but only the association with rs4522809 was statistically significant (HR, 1.08; 95% CI, 1.03–1.12). Results were generally similar for the minimally adjusted model (data not shown). Although we were limited in the number of cases with nonmissing debulking status (2,470 total EOC cases, including 326 endometrioid EOC cases and 171 clear cell EOC), we carried out sensitivity analyses for the most statistically significant SNPs and found that the estimates did not change substantially between unadjusted and debulking status–adjusted analyses of cases with nonmissing debulking status (data not shown).

Correlations between germline polymorphisms in *IL2RA* and gene expression in the tumor

The most statistically significant association was found between the OS of endometrioid EOC and rs11256497 in *IL2RA*. To determine the functional consequences of this intronic variant, we investigated whether the expression of *IL2RA* in tumors varied by allele in 200 tumors of combined histology with matching genotype and gene expression data available for analysis. There was no evidence for differences in tumor *IL2RA* mRNA expression by *IL2RA* SNP rs11256497 (GG vs. AA/AG; $P = 0.33$ for probe A_23_P237288 and $P = 0.23$ for probe A_24_P230563) in these tumors of combined histology.

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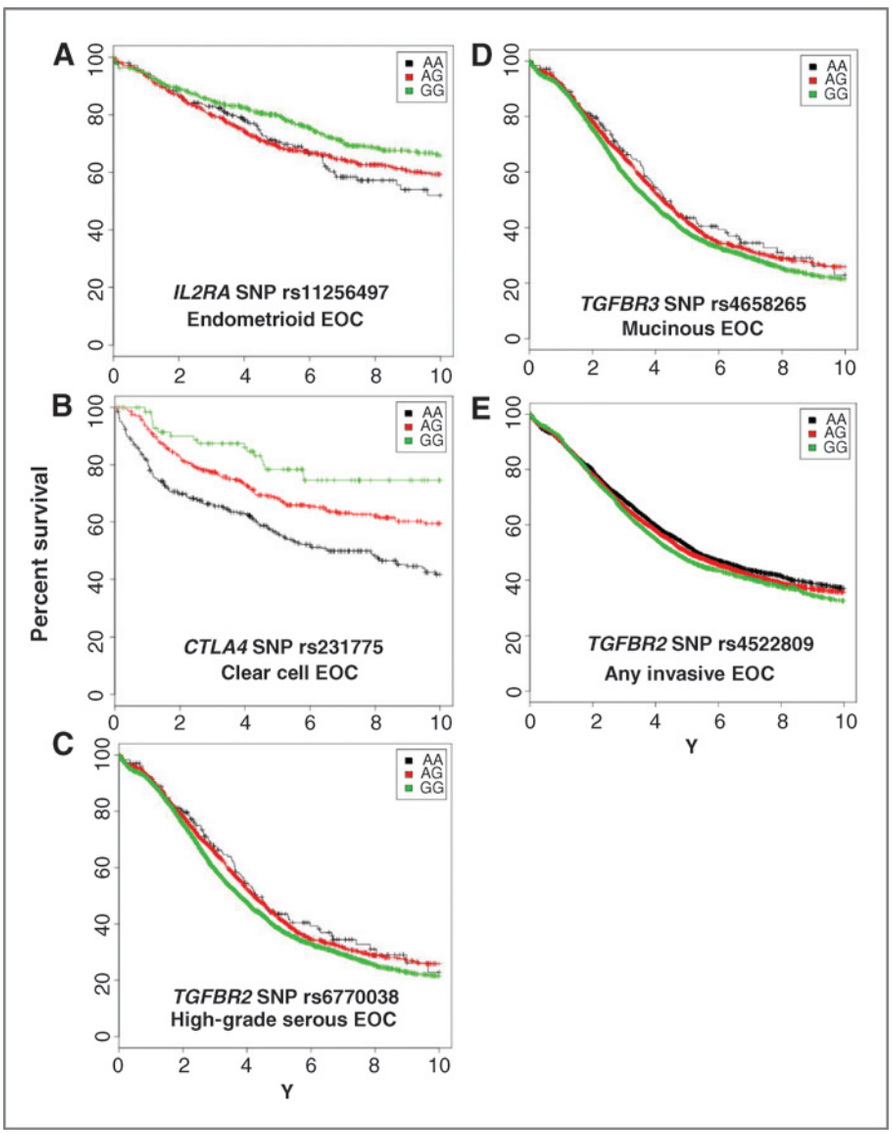

Figure 1. Kaplan–Meier curves accounting for left truncation for the most statistically significantly associated SNPs and OS in women with endometrioid EOC with rs11256497 genotype [GG ($N = 582$), AG ($N = 670$), and AA ($N = 200$); A]; B, clear cell EOC with rs231775 genotype [AA ($N = 313$), AG ($N = 379$), and GG ($N = 103$)]; C, high-grade serous EOC with rs6770038 genotype [GG ($N = 3,484$), AG ($N = 1,604$), and AA ($N = 160$)]; D, mucinous EOC with rs4658265 genotype [GG ($N = 305$), AG ($N = 295$), and AA ($N = 61$)]; and E, any invasive EOC with rs4522809 genotype [AA ($N = 2,782$), AG ($N = 5,069$), and GG ($N = 2,232$)].

However, when we restricted the analysis to endometrioid histology, *IL2RA* expression was lower in the AG/AA group, compared with the GG group ($P = 0.001$ for probe A_23_P237288 and $P = 0.01$ for probe A_24_P230563; Fig. 3).

Finally, using *in silico* tools, we determined whether there was information about the role of the SNPs in regulating the function and/or expression of the genes with which they are associated. First, we accessed RegulomeDB to determine whether any of the intronic SNPs with a $P < 0.001$ (Table 1 bold) may be associated with regions involved in the regulation of expression. This included all of the identified SNPs with the exception of rs231775, a coding SNP. The only SNP that was in a region for which binding of regulatory elements was considered likely was rs11256497 within the *IL2RA* gene, which is in agreement with the expression results depicted in Fig. 3. Although dbSNP did not provide additional information with respect to this SNP, using this tool, we found two other *IL2RA* SNPs, rs10905669 and rs10795763, within 2.5 kb of the promoter regions, suggesting a role for these SNPs in regulating expression. The only SNP relevant to the PolyDoms algorithm (i.e., within a coding region) was the *CTLA4* missense SNP rs231775, which was predicted to be a benign variant.

Discussion

Infiltration of ovarian tumors by Tregs is associated with poor patient outcome (7). Previously, we found associations between OS in EOC and SNPs in genes related to Treg activation, migration, and function, including *RGS1* (clear cell), *LRRC32* and *TNFRSF18/TNFRSF4* (mucinous), and *CD80* (endometrioid; ref. 8). In the present study, we assessed the associations between OS in patients with EOC and germline variations in additional Treg-related genes. The most notable associations ($P < 6.2 \times 10^{-4}$) were found

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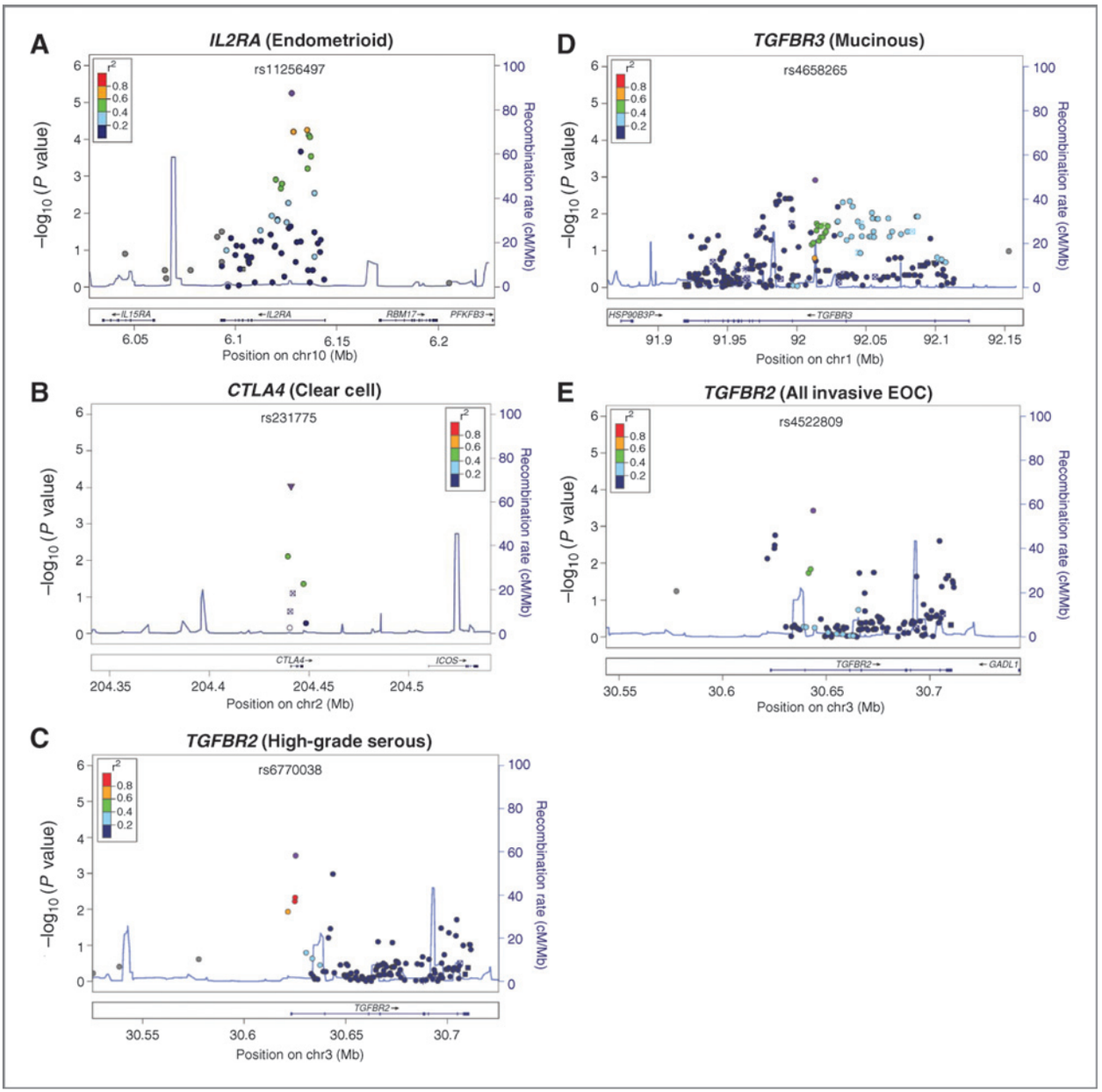

Figure 2. SNPs most statistically significantly associated with OS in endometrioid and clear cell EOC. Association *P* values [$-\log_{10}$ (*P*)] using LocusZoom (30) of all SNPs in *IL2RA* for endometrioid (rs11256497 represented by purple circle; A); B, *CTLA4* for clear cell (rs231775 represented by purple triangle); C, *TGFBR2* for high-grade serous (rs6770038 represented by purple circle); D, *TGFBR3* for mucinous (rs4658265 represented by purple circle); and E, TGFBR2 for all invasive EOC (rs4522809 represented by purple circle). ▲, framestop or splice; ▼, nonsynonymous; ■, synonymous or UTR; ●, no; x, conserved in placental mammals.

between six SNPs in *IL2RA* and OS in women with endometrioid cancer, a *CTLA4* SNP and OS in women with clear cell carcinoma, a *TGFBR2* SNP and OS in women with high-grade serous EOC, and a *TFGBR2* SNP and OS in women with any EOC. There were also a few modest associations between OS and Treg-related SNPs in *TGFBR2* and *IL10RA* for all EOC, *TGFBR2* for high-grade serous, *TGFBR2* for endometrioid, *MAP3K8* for clear cell, and *TGFBR2* and *TGFBR3* for patients with mucinous EOC.

The most statistically significant association found was between OS in women with endometrioid EOC and *IL2RA* SNP, rs11256497. IL-2Rα, also known as CD25, forms a portion of the high affinity interleukin (IL)-2 receptor, and is expressed by most Tregs. IL-2 signaling through this receptor plays an important role in Treg homeostasis (18). IL-2 treatment has been shown to enhance Treg numbers and function (19), whereas anti-CD25 antibodies can be used to deplete Tregs (20). The rs11256497 SNP, associated with OS in our study, is

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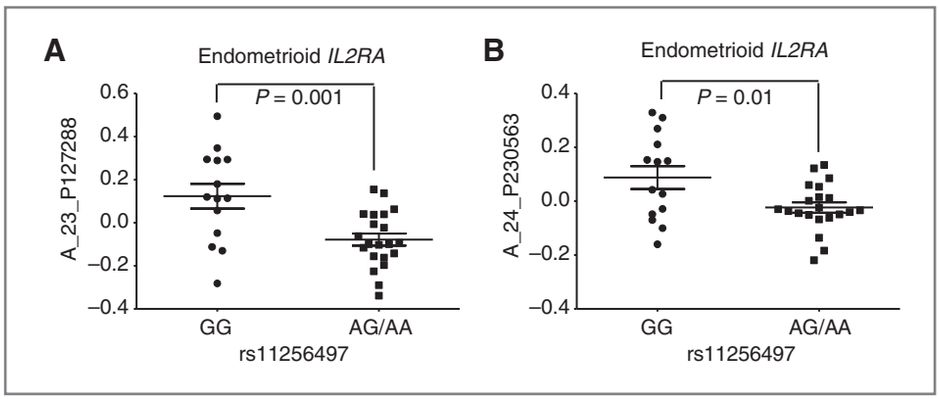

Figure 3. *IL2RA* mRNA expression (normalized and $\log_{10}$ transformed) by rs11256497 genotype in endometrioid EOC tumors. Two different Agilent *IL2RA* probes are presented: A, A_23_P237288; and B, A_24_P230563. Two-tailed, unpaired *t* test *P* value is reported.

located in an NF-κB and EBF1 binding site (HaploReg v2; described in ref. 16 and is predicted to impact binding by RegulomeDB), but the effect of this SNP on transcription factor binding is unknown. Analysis of endometrioid (N = 35 EOC) tumors with matching tumor mRNA expression and rs11256497 genotype data revealed elevated *IL2RA* mRNA expression in the tumors of patients with the GG genotype, which was also associated with improved survival, compared with patients with the AA/AG genotype. This finding seems counterintuitive, as higher *IL2RA* would be predicted to associate with higher Treg counts and, therefore, reduced survival; however, prior studies have identified the complexity of IL-2/IL-2Rα signaling and its importance in effector T-cell response (21). We also note that sample size was limited for endometrioid cases with overlapping genotype and gene expression data. These data also do not permit an evaluation of whether or not these SNPs alter expression in specific cell types such as Treg or other immune cells. Future studies are needed to explore whether this variant affects Treg maintenance and associates with infiltrating Tregs in the tumor.

The minor (G) allele of the *CTLA4* SNP rs231775 is associated with increased risk of systemic lupus erythematosus, primary biliary cirrhosis, and type I diabetes (22–24). The increased risk associated with these autoimmune diseases suggests that this SNP reduces CTLA4 function, thereby increasing effector T-cell response against self-antigens. Therefore, it is plausible that this missense (Thr>Ala) SNP would be associated with improved EOC survival as presented in our study, as it may relate to enhanced effector T-cell response to tumors. In a prior study assessing the function of this variant, T cells from individuals with the GG genotype had increased proliferation with suboptimal stimulation, lower CTLA4 expression following activation, and different intracellular distribution of CTLA4 than T cells from individuals with the AA genotype (25). Ipilimumab, an anti-CTLA4 monoclonal antibody, has been used with some success in inducing tumor regression of melanoma and renal clear cell carcinoma (26). Recent studies in rodent models have demonstrated that treatment with anti-CTLA4 antibodies results in enhanced tumor rejection and higher intratumoral ratio of effector T cell/Treg (27, 28). Although further experimental studies should be carried out to evaluate how this genetic association translates into clinical application, our findings, combined with those from the previous reports of clear cell EOC being molecularly similar to renal clear cell carcinoma (29), indicate that blocking CTLA4 with an agent such as ipilimumab might be reasonable to investigate in clinical trials for clear cell EOC. A phase II study of this drug currently is under way in recurrent platinum-sensitive ovarian cancer (www.clinicaltrials.gov).

The strengths of this study include centralized quality control and large sample size, thus providing the opportunity to examine associations between survival and SNPs with more modest effects in patients with the common high-grade serous histology and those with the rare histologic subtypes. Our study includes samples from 28 EOC studies with different designs and goals, and we controlled for potentially confounding factors by adjusting for study site and several clinical covariates for which information was available in a large portion of the OCAC population. Although we were limited by the number of cases with nonmissing debulking status and, therefore, did not perform this adjustment in our primary analysis, we did perform sensitivity analyses in cases with nonmissing debulking status. We used a fairly comprehensive approach (SNP tagging) to identify variations in Treg-related genes; however, due to quality control failures, some genes were not as well covered as others. Differences in enrollment time, particularly for population-based studies with delayed enrollment, may also affect the results due to a failure to enroll subjects, who died very soon after diagnosis. Although adjustment for left truncation removes some of the biases, an increased survival time was still evident. Finally, due to variation in racial differences in allele frequencies and the limited number of racial minorities, the analysis was restricted to participants of European descent, which reduces generalizability.

In conclusion, we provide evidence that Treg-related SNPs are associated with survival in subtypes of EOC. In particular, several SNPs in *IL2RA* are associated with survival in endometrioid EOC. We found that the minor allele of a missense *CTLA4* SNP, previously reported to be associated with impaired CTLA4 function and several autoimmune disorders, is associated with improved survival in clear cell EOC. This finding may have important clinical implications, as anti-CTLA4 therapy has already been used with some success in the treatment of melanoma and renal clear cell carcinoma.

Further research on the effects of inhibiting CTLA4 in clear cell EOC is warranted.

Disclosure of Potential Conflicts of Interest

B. Charbonneau is employed and has an ownership interest (including patents) in Eli Lilly and Company. A. deFazio has received honoraria from Roche. R. Brown is employed as team leader in The Institute for Cancer Research. F. Heitz has received honoraria from the speakers' bureau of Roche. U. Menon has ownership interest (including patents) in Abcodia. No potential conflicts of interest were disclosed by the other authors.

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