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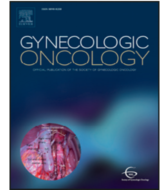
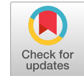


Genome-wide association studies identify susceptibility loci for epithelial ovarian cancer in east Asian women



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HIGHLIGHTS

- This study analyzed genotyping data from >7,000 individuals of Asian descent to find risk loci for epithelial ovarian cancer
- We identified two novel genome-wide significant loci, plus evidence of association at an additional 28 regions
- eQTL analyses in 404 TCGA tumors highlight *ESPNL* as a novel susceptibility gene for ovarian cancer

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ABSTRACT

Objective. Genome-wide association studies (GWASs) for epithelial ovarian cancer (EOC) have focused largely on populations of European ancestry. We aimed to identify common germline variants associated with EOC risk in Asian women.

Methods. Genotyping was performed as part of the OncoArray project. Samples with >60% Asian ancestry were included in the analysis. Genotyping was performed on 533,631 SNPs in 3238 Asian subjects diagnosed with invasive or borderline EOC and 4083 unaffected controls. After imputation, genotypes were available for 11,595,112 SNPs to identify associations.

Results. At chromosome 6p25.2, SNP rs7748275 was associated with risk of serous EOC (odds ratio [OR] = 1.34, $P = 8.7 \times 10^{-9}$) and high-grade serous EOC (HGSOC) (OR = 1.34, $P = 4.3 \times 10^{-9}$). SNP rs6902488 at 6p25.2 ($r^2 = 0.97$ with rs7748275) lies in an active enhancer and is predicted to impact binding of STAT3, P300 and ELF1. We identified additional risk loci with low Bayesian false discovery probability (BFDP) scores, indicating they are likely to be true risk associations (BFDP <10%). At chromosome 20q11.22, rs74272064 was associated with HGSOC risk (OR = 1.27, $P = 9.0 \times 10^{-8}$). Overall EOC risk was associated with rs10260419 at chromosome 7p21.3 (OR = 1.33, $P = 1.2 \times 10^{-7}$) and rs74917072 at chromosome 2q37.3 (OR = 1.25, $P = 4.7 \times 10^{-7}$). At 2q37.3, expression quantitative trait locus analysis in 404 HGSOC tissues identified *ESPNL* as a putative candidate susceptibility gene ($P = 1.2 \times 10^{-7}$).

Conclusion. While some risk loci were shared between East Asian and European populations, others were population-specific, indicating that the landscape of EOC risk in Asian women has both shared and unique features compared to women of European ancestry.

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1. Introduction

Epithelial ovarian cancers (EOC) are a diverse group of tumors occuring predominantly among postmenopausal women. Epidemiological and lifestyle risk factors include a family history of ovarian and breast cancer [1], nulliparity [2], and no oral contraceptive use [3]. Germline mutations in highly penetrant genes, including *BRCA1* [4] and *BRCA2* [5], are associated with a >15% lifetime risk of ovarian cancer [6]; but these mutations are present in only 10–15% of EOC cases. In the general population, common genetic variants identified using genome-wide association studies (GWASs) have been found to confer more modest disease risks with odds ratios generally ≤1.5. To date, GWASs have identified >30 regions of the genome harboring common variants associated with EOC risk (reviewed in Jones et al. [7]), with all but one of these studies reporting risk variants identified in women of European ancestry [8–19]. There is one report of a GWAS performed in 1057 Asian cases and 1799 controls (Han Chinese), with replication in 492 EOC cases and 1004 controls. This study identified two genome-wide significant risk regions at 9q22.33 and 10p11.21; however, neither region appears significantly associated with EOC risk in European populations [9].

EOC incidence varies by race and/or ethnicity; women in Asian, North African and Middle Eastern countries tend to have lower rates of EOC than women in northern European and Baltic countries [20,21]. Women from Asia migrating to the United States and Europe retain their comparatively reduced rates of EOC [22]. In addition, Asian women, particularly those from China, Korea, Vietnam and the Philippines, tend to be significantly younger at diagnosis, and are more likely to be diagnosed with early stage (stage I) disease. Consequently, they have better 5-year survival rates compared to EOC cases in white women of European ancestry [23,24]. The distribution of histological subtypes also differs between Asian and European women. Asian women are more likely than European women to develop clear cell or mucinous EOCs and less likely to develop serous EOC [23,25]. Much of the variation in EOC incidence rates by race or ethnicity may be attributable to differences in reproductive, lifestyle, and environmental risk factors; but variations in the underlying genetics are also likely to contribute to the observed differences.

The international Genetic Associations and Mechanisms in Oncology (GAME-ON) project designed a custom Illumina genotyping array (the 'OncoArray'), containing over 530,000 variants to identify genetic risk factors across multiple tumor types, and in different racial or ethnic groups [26]. The OncoArray includes risk-associated SNPs identified from GWAS meta-analyses of breast, colorectal, lung, ovarian and prostate cancers; risk regions associated with numerous other phenotypes (both cancer and non-cancer); and a genome-wide SNP 'backbone' to enable agnostic GWASs to be performed for each phenotype [26]. The OncoArray has been used to genotype >500,000 subjects, including ~53,000 individuals from EOC case-control studies, of which ~7000 subjects are of East Asian ancestry. Here, we report genetic association analyses of EOC case-control subjects of East Asian ancestry genotyped on the OncoArray.

2. Methods

2.1. Study samples

All subjects included in this analysis were of Asian descent (see below) and provided written informed consent. Data and blood samples were collected under protocols approved by the Institutional Review Boards or Ethics Committee at each institution. All constituent studies and host institutions are listed in Supplementary Table 1. The OCAC OncoArray data set comprised 63 genotyping project/case-controls sets (Supplementary Table 1). Some studies (e.g. SEARCH) contributed samples to more than one genotyping project and some case-control sets are a combination of multiple individual studies. Post-QC sample numbers, by histology, for subjects of Asian ancestry are shown in Table 1.

2.2. Genotype data and quality control (QC)

Genotyping was performed at five locations: University of Cambridge, Center for Inherited Disease Research (CIDR), National Cancer Institute (NCI), Genome Quebec and Mayo Clinic. OncoArray sample QC was similar to that carried out for the other projects (as described in Pharoah et al., 2013 [11,16,17,27]). Samples were excluded if (1) genotyping call rate was <95%, (2) heterozygosity was excessively low or high, (3) if they were not female or had ambiguous sex, or (4) were duplicates (cryptic or intended). Duplicates and close relatives were identified using in-house software that calculates a concordance matrix for all individuals. Samples with concordance >0.86 were flagged as duplicates and samples with concordance between 0.74 and 0.86 were flagged as relatives. The comparison was performed among all the OncoArray samples, and all the previously genotyped samples. Concordance statistics were used to identify cryptic duplicates and expected duplicates whose genotypes did not match, and we attempted to resolve these with the study investigators. If a discrepancy could not be resolved both samples were excluded. For confirmed cryptic duplicates and relatives, we retained one sample in the analysis. For case-control pairs we excluded the control, while for case-case and control-control pairs we excluded the sample with the lower call rate.

SNP QC was carried out according to the OncoArray QC Guidelines [26]. Only SNPs that passed QC for all consortia were used for imputation. We excluded SNPs with a call rate < 95%, SNPs deviating from Hardy-Weinberg equilibrium ($P < 10^{-7}$ in controls and $P < 10^{-12}$ in cases) and SNPs with concordance <98% among 5280 duplicate pairs. For the imputation, we additionally excluded SNPs with a MAF < 1% and a call rate < 98% and SNPs that could not be linked to the 1000 genomes reference or differed significantly in frequency from the 1000 genomes (European frequency) and a further 1128 SNPs where the cluster plot was judged to be inadequate. Of the 533,631 SNPs which were manufactured on the array, 494,813 SNPs passed the initial QC and 470,825 SNPs were used for imputation. Samples with overall heterozygosity <5% or > 40% were excluded.

2.3. Ancestry analysis and imputation

Intercontinental ancestry was calculated using the software package FastPop (http://sourceforge.net/projects/fastpop/) [28]. Consistent with other OncoArray studies, only the samples with >60% Asian ancestry, were included in the analyses reported here. Principal component analysis for the OncoArray data was carried out using data from 33,661 uncorrelated SNPs (pair-wise r2 <0.1) with minor allele frequency >0.05 using an in-house program (available at http://ccge.medschl.cam.ac.uk/software/pccalc/). Principal components analysis for the other genotype data sets was carried out as previously described [16].

We performed imputation separately for each genotyping project data set. We imputed genotypes into the reference panel from the 1000 Genomes Project (v3 October 2014) [29]. We initially used a two-step procedure, which involved pre-phasing in the first step and imputation of the phased data in the second, to improve computation efficiency. We carried out pre-phasing using Shape-IT [30] and the subsequent imputation using IMPUTE2 [31]. We then performed more accurate imputation for any region with a SNP with $P < 10^{-6}$. The boundaries of these regions were set +/− 500 kb from the most significant SNP in the region. The single-step imputation used IMPUTE2 without pre-phasing with some of the default parameters modified. These included an increase of the MCMC iterations to 90 (out of which the first 15 were used as burn-in), an increase of the buffer region to 500 kb and increasing to 100 the number of haplotypes used as templates when phasing observed genotypes.

2.4. Association analyses

We excluded SNPs from the association analysis if their imputation accuracy was $r^2 < 0.3$ or their minor allele frequency (MAF) was <0.01. In total, genotypes for 11,595,112 million variants were available for analysis. We evaluated the association between genotype and disease using the imputed genotype dosage (log-additive genetic models) in a logistic regression model. We carried out initial genome-wide analyses separately for OncoArray, COGS and the five GWAS datasets and pooled the results using a fixed effects meta-analysis. The analyses were adjusted for study and for population substructure by including the eigenvectors of project-specific principal components as covariates in the model (nine for OncoArray, five for COGS, two for UK GWAS, and two

Table 1
Number of participants by analysis stratum. Cases and controls by study stratum. US represents a collection of all US studies with fewer than 50 Asian subjects; EUR represents all European studies with fewer than 50 Asian subjects.

Analysis stratum	Controls	HGSOC	LGSOC	EnOC	CCOC	MOC	Other invasive	All invasive cases	Serous borderline	Mucinous borderline	All borderline	Total samples
AUS	18	41	2	12	18	3	2	78	4	8	12	108
CAN	49	57	0	15	20	7	8	107	5	8	13	169
CHI	2032	878	12	193	50	92	310	1535	33	30	63	3630
DOV	45	31	0	8	11	1	4	55	6	7	13	113
EUR	32	12	1	4	7	4	3	31	3	0	3	66
HAW	408	103	1	45	31	26	13	219	25	23	48	675
JPN	230	72	2	10	35	13	4	136	5	8	13	379
KRA	682	194	9	25	28	12	16	284	1	15	16	982
LAX	92	53	2	15	22	15	3	110	25	11	36	238
MAS	157	62	0	36	23	14	11	146	2	2	4	307
MEC	28	10	0	2	2	1	4	19	0	0	0	47
STA	60	24	3	9	10	8	7	61	10	4	14	135
SWH	135	45	0	17	10	13	45	130	0	0	0	265
US	115	33	4	13	11	3	6	70	14	8	22	207
Total	4083	1615	36	404	278	212	436	2981	133	124	257	7321

for the US, BWH and POL GWAS, and a single PC for the MAY GWAS). The number of eigenvectors chosen was based on the point of inflection of a scree plot. After one-step imputation of the genotypes in the regions of interest we used genotype dosages in a single logistic regression model with adjustment for each genotyping project/study combination and nineteen principal components. Principal components were set to zero for samples not included in a given project. We used custom written software for the analysis.

To assess the magnitude of confounding caused by cryptic population substructure, we calculated inflation in the test statistics (λ) by dividing the median of the test statistic by 0.455 (the median for the $\chi2$ distribution on 1 degree of freedom). The inflation was converted to an equivalent inflation for a study with 1000 cases and 1000 controls ($\lambda1000$) by adjusting by effective study size:

$$\lambda_{1000} = 1 + \frac{500(\lambda - 1)}{\Sigma_K \left(\frac{1}{n_k} + \frac{1}{m_k}\right)}$$

where n is the number of cases and m is the number of controls in each study stratum, k. There was a small inflation of the test statistics for the all invasive analysis ($\lambda = 1.057$, $\lambda_{1000} = 1.015$).

EOC is a heterogeneous phenotype with five major histotypes for invasive disease – high-grade serous ovarian cancer (HGSOC), low-grade serous ovarian cancer (LGSOC), mucinous ovarian cancer (MOC), endometrioid ovarian cancer (EnOC) and clear cell ovarian cancer (CCOC) – and two histotypes of borderline disease – serous and mucinous. The pattern of association across the different histotypes varies for the known OC risk loci [8]. We therefore carried out the association analysis on the following nine histotypes: all invasive disease; HGSOC; LGSOC; all invasive serous; serous borderline; LGSOC and borderline serous combined; EnOC; CCOC; and mucinous invasive/mucinous borderline combined. QQ plots are shown in Supplementary Fig. 1.

2.5. Identifying candidate causal SNPs in each susceptibility region

To identify a set of variants most likely to contain the true underlying causal association – the candidate causal variants – we excluded SNPs with causality odds of <1:100 by comparing the likelihood of each SNP from the association analysis with the likelihood of the most strongly associated SNP.

2.6. Expression quantitative trait locus (eQTL) analyses

Expression QTL analyses were conducted using three data sets. We used an in-house generated RNA-sequencing data set of 105 primary normal ovarian surface epithelial cell (OSEC) and 60 fallopian tube secretory epithelial cell (FTSEC) cultures (Lawrenson et al., submitted). All samples were sequenced following a ribo-depletion library preparation method, and all specimens were genotyped on the OncoArray. We also performed tumor-specific eQTL analyses using TCGA HGSOC data set [32]. The sample size for women of Asian ancestry was too small in each data set to perform an eQTL analysis restricted specifically to Asians and so we decided to leverage the entire (cosmopolitan ancestry) data sets for eQTL analysis. For the TCGA analysis, we focused on all genes and samples ($n = 404$) that had matched gene expression (measured on the Agilent 1 M microarray), CpG methylation (measured on the Illumina Infinium HumanMethylation27 BeadChip), copy number alteration (called using the Affymetrix SNP 6.0 array), and germline genotype (called using the Affymetrix SNP 6.0 array) data available. Genotypes were imputed into the 1000 Genomes October 2014 (Phase 3, version 5) European reference panel for all three data sets [29]. Expression QTL analyses were performed using linear regression as implemented in the R package Matrix eQTL [33]. Prior to eQTL analyses the effects of tumor copy number and methylation on gene expression were regressed out as previously described [34]. We focused on *cis*-acting eQTL relationships between candidate causal risk SNPs and all genes up to 1 Mb on either side of these SNPs. Two-sided *P*-values are reported.

2.7. Functional annotation of variants

We used shell scripts with bedtools (http://bedtools.readthedocs.org/en/latest/) to generate overlap data between all variants in each associated region including likely causal SNPs and bed file versions of all the biofeature data used. The overlap data thus obtained were converted to matrix form by means of python scripts and then sorted in Microsoft Excel.

To test for locus-specific tissue enrichment of variants, H3K27 acetylation peaks were collated from public sources or from in-house data (all listed in Supplementary Table 2). Overlaps were counted for the all SNPs against which genotypes were imputed in 1000 genomes for each H3K27ac dataset. The fraction of causal SNPs with overlaps was then tested for significance against this background for each cell type in the H3K27ac datasets using the hypergeometric distribution. Finally, *p* values were adjusted for multiple comparisons using Bonferroni's method.

2.8. Data availability statement

The summary results for all imputed SNPs reported in this paper are available at: https://doi.org/10.17863/CAM.25845

3. Results

3.1. Association analyses

Of the samples genotyped on the OncoArray, 21,879 EOC cases and 29,224 controls from OCAC met our quality control (QC) criteria (see Methods). Intercontinental ancestry was determined using FastPop [28] to select samples with >60% Asian ancestry for inclusion in association analyses (Fig. 1a). This analysis inferred Asian ancestry in 2981 women with invasive EOC (1615 high-grade serous, 36 low-grade serous, 404 endometrioid, 278 clear cell and 212 mucinous EOC; 436 'other'), 257 women with borderline EOC (133 serous and 124 mucinous), and 4083 controls. Most samples were collected through population-based studies conducted in eastern Asia (Fig. 1b), with around 25% of specimens collected in studies conducted in the US, Europe and Australia. The number of participants by analysis stratum is listed in Table 1. Data from the 1000 Genomes Project reference panel [29] were used to impute genotypes for 11,403,952 common variants (minor allele frequency, MAF > 1%). Histology was ascertained for 2802 (86.5%) Asian EOC cases in total. The Asian populations had higher frequencies of the clear cell (9.9%) and mucinous (12.0%) histological subtypes ('histotypes') and lower frequency of the high-grade serous histotype (57.6%) compared to the non-Asian populations (7.0%, 7.2% and 66.4%, respectively) (Fig. 1c).

Association analyses were performed for all East Asian subjects considering histotypes combined, all invasive cases, all borderline cases, and for each histotype separately (Table 1). There was little evidence of inflation of the test statistic (λ_{1000} for all invasive analysis = 1.015). We identified three risk associations at two different loci reaching genome-wide significance (effect allele frequency, EAF ≥0.05, $P < 5 \times 10^{-8}$) (Table 2 and Fig. 1 d-f). There was no evidence of heterogeneity by study for these associations. At chromosome 6p25.2, rs7748275 was associated with risk of serous EOC (OR = 1.4, $P = 8.7 \times 10^{-9}$). The Bayesian false-discovery probability (BFDP [35]) for this association was 9% assuming a maximum likely OR of 1.2 and a prior of 1:10,000, or 1% assuming a prior of 1:1000). This SNP was also associated with risk of high-grade serous ovarian cancer (HGSOC) specifically (OR = 1.4, $P = 4.3 \times 10^{-9}$, BFDP = 24% or 3% assuming a prior of 1:10,000 or 1:1000, respectively).

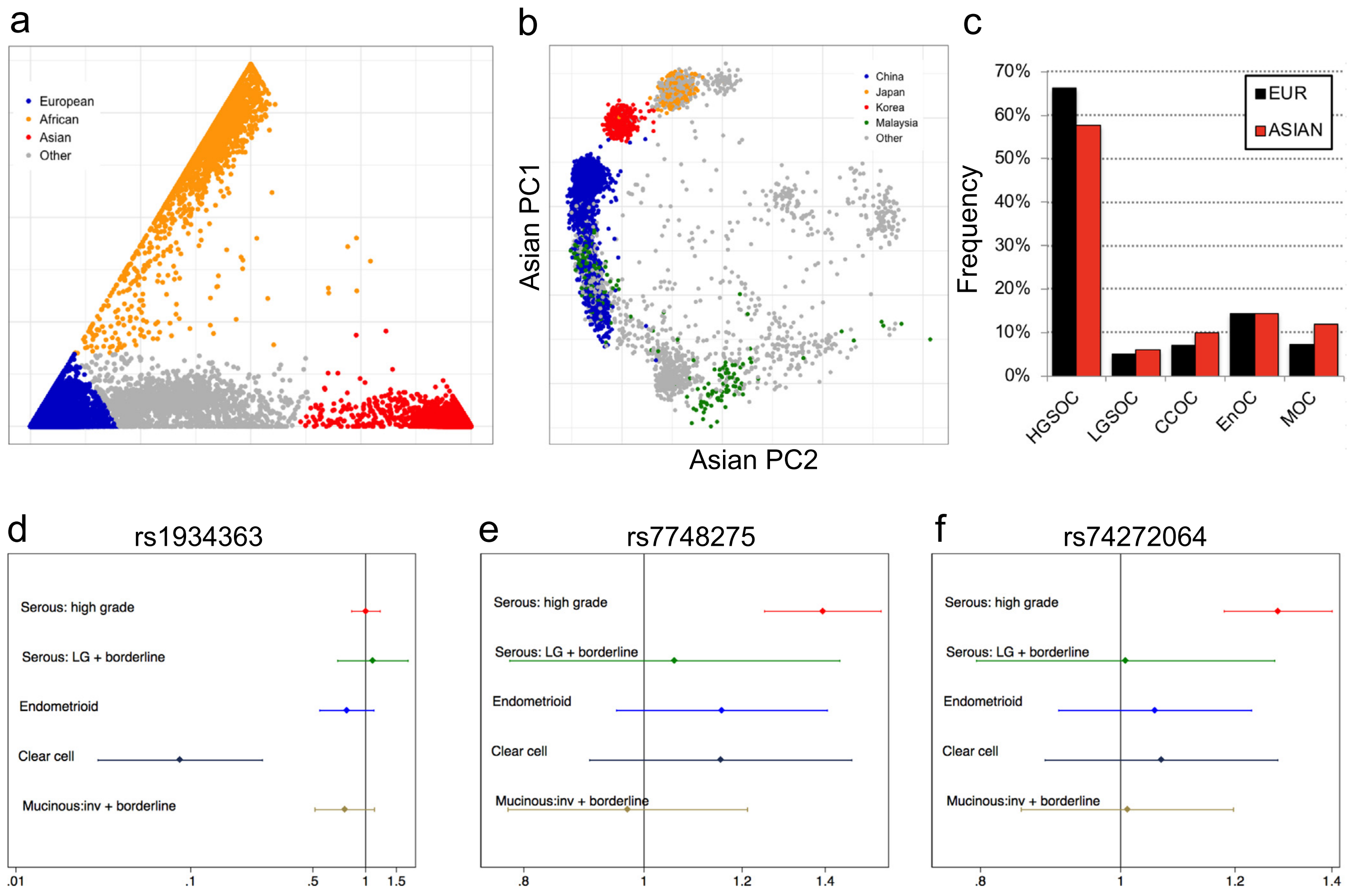


Fig. 1. Novel loci associated with EOC risk in Asian women. (a-b) Principal component analysis (PCA) of 21,879 EOC cases and 29,224 controls (a) identified a total of 7321 subjects with Asian ancestry which were stratified by country-of-origin (b); (c) Clear cell and mucinous EOC is more common in Asian women than women of European ancestry. (d-f) Forest plots showing the three genome-wide significant risk regions for EOC identified in women of Asian ancestry.

At chromosome 10p12.1, SNP rs1934363 was associated with risk of clear cell ovarian cancer (CCOC) (odds ratio (OR) = 10.9, $P = 3.0 \times 10^{-10}$); however, the Bayesian false-discovery probability (BFDP [35]) for this association was close to 100% (Table 2) strongly suggesting that it is likely to be a false positive result. SNPs rs1934363 and rs7748275 were not significantly associated with EOC risk in Europeans genotyped on the OncoArray (Supplementary Table 3).

We also identified one-hundred and twenty-six loci that showed marginal evidence of risk associations (P-values 1×10^{-5} to 5×10^{-8}) (Supplementary Table 4). Twenty-eight of these loci are more likely than not to be true associations based on a BFDP score < 50% for the index SNP (Supplementary Table 5). This included a locus at chromosome 20q11.22, where SNP rs74272064 (kgp7556451) was associated with both HGSOC (OR = 1.27, $P = 9.0 \times 10^{-8}$, BFDP = 3% assuming a prior of 1:1000), and serous EOC ($P = 1.5 \times 10^{-7}$); and a locus at chromosome 7p21.3, where SNP rs10260419 was associated with overall EOC risk (OR = 1.33, $P = 1.2 \times 10^{-7}$, BFDP = 5% assuming a prior of 1:1000); and a locus at chromosome 2q37.3, where SNP rs74917072 was associated with overall risk of invasive EOC (OR = 1.25, $P = 4.7 \times 10^{-7}$, BFDP = 7% assuming a prior of 1:1000).

Finally, we compared EOC risk associations in European and East Asian populations [8]. Of the 28 East Asian risk loci with a BFDP score < 50%, only one showed evidence of an association in European women (rs10260419, $P = 3.4 \times 10^{-4}$ for HGSOC risk) (Supplementary Table 5). However, of the 30 loci previously identified in European women, associations in this East Asian population were in the same direction for 22, 17 of which have a one-sided P-value <0.2. While the P-values for these loci were modest in the Asian population, the BFDPs indicate they are all likely to be true associations (BFDP range 0.5–21%, Supplementary Table 6). The most significant association was for rs10069690 at chromosome 5p15, which lies within an intron of *TERT*, identified in European subjects (OncoArray East Asian study OR = 1.21, $P = 3.18 \times 10^{-4}$) [18]. To explore

Table 2
New genome-wide histotype-specific EOC risk associations in Asian women.
[a] Average imputation r^2 across the six data sets; [b] From analysis of imputed genotyped derived from one-step imputation (see methods); [c] test for heterogeneity of effect between study strata in OCAC; chr, chromosome; RAF, risk allele frequency; LCL, lower 95% confidence limit; UCL, upper 95% confidence limit; HGSOC, high grade ovarian cancer; CCOC, clear cell ovarian cancer; BFDP, Bayes false discovery probability, assuming a prior of 1:1000 or 1:10,000 and maximum likely true odds ratio of 1.2. Position is genome build 37.

Histotype	SNP rsID	Chr	Position	Risk Allele	RAF	r^{2a}	OR	LCL	UCL	P-value[b]	P-het[c]	BFDP (%)	
												1:10,000	1:1000
CCOC	rs1934363	10	28,773,478	C	0.94	0.90	11	5.3	24	3.0×10^{-10}	0.98	100	100
Serous	rs7748275	6	3,581,089	A	0.16	0.97	1.4	1.2	1.5	8.7×10^{-9}	0.19	9	1
HGSOC	rs7748275	6	3,581,089	A	0.16	0.97	1.4	1.2	1.5	4.3×10^{-8}	0.19	24	3

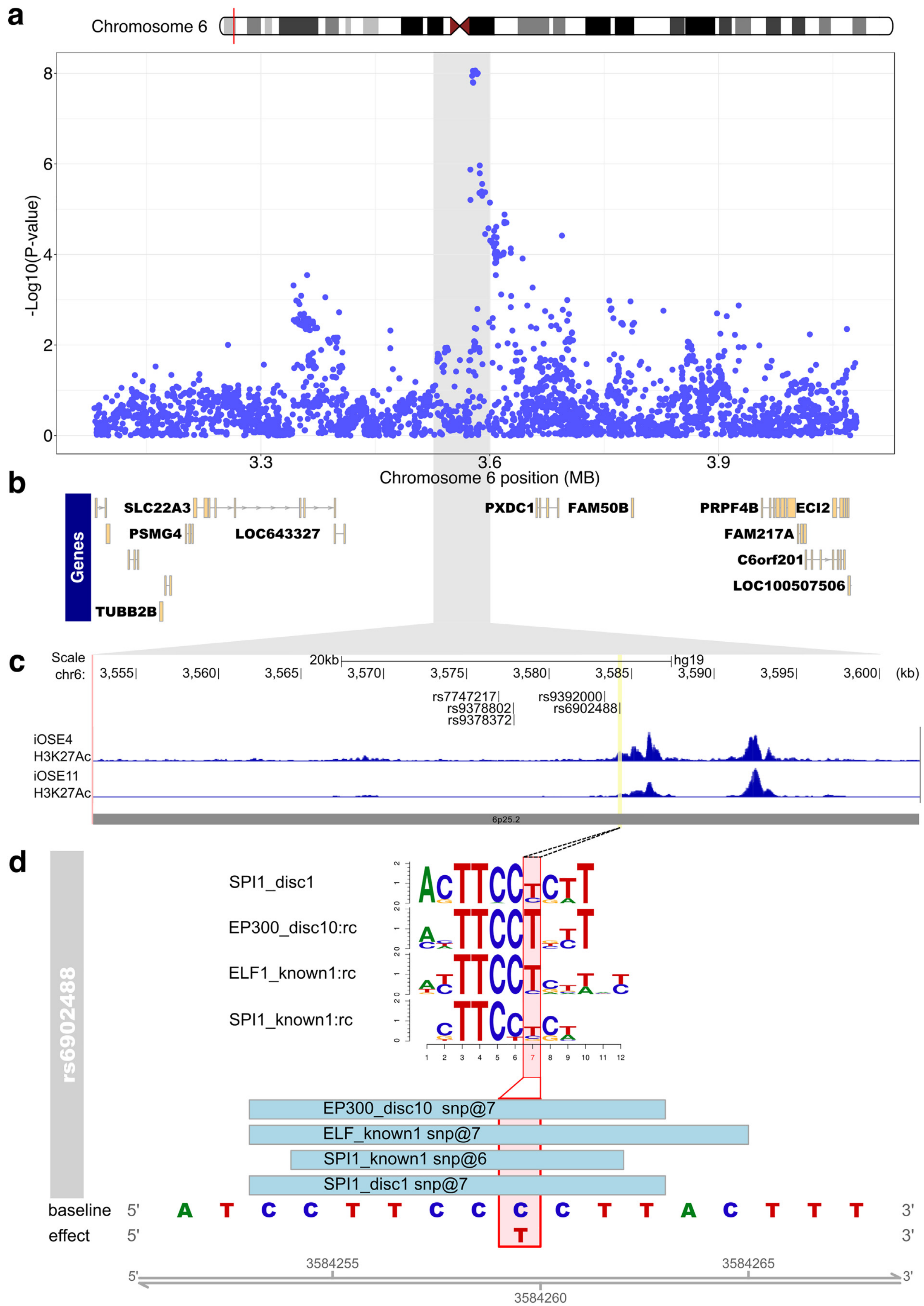


Fig. 2. Functional annotation of risk variants at the 6p25.2 risk locus. (a) Regional association plot for serous cancers, centered on rs7748275, with genes in the regions indicated in panel (b). The grey highlighted region corresponds indicates the interval on chromosome 6 in panel a that is shown in panel (c). (c) ChIP-seq in OC-relevant cell types. The locations of the top 6 candidate causal alleles are indicated. (d) SNP rs6902488 significantly alters binding sites for 4 transcription factors. Position weight matrices are shown for each, the height of each letter indicates the importance of that base in the motif.

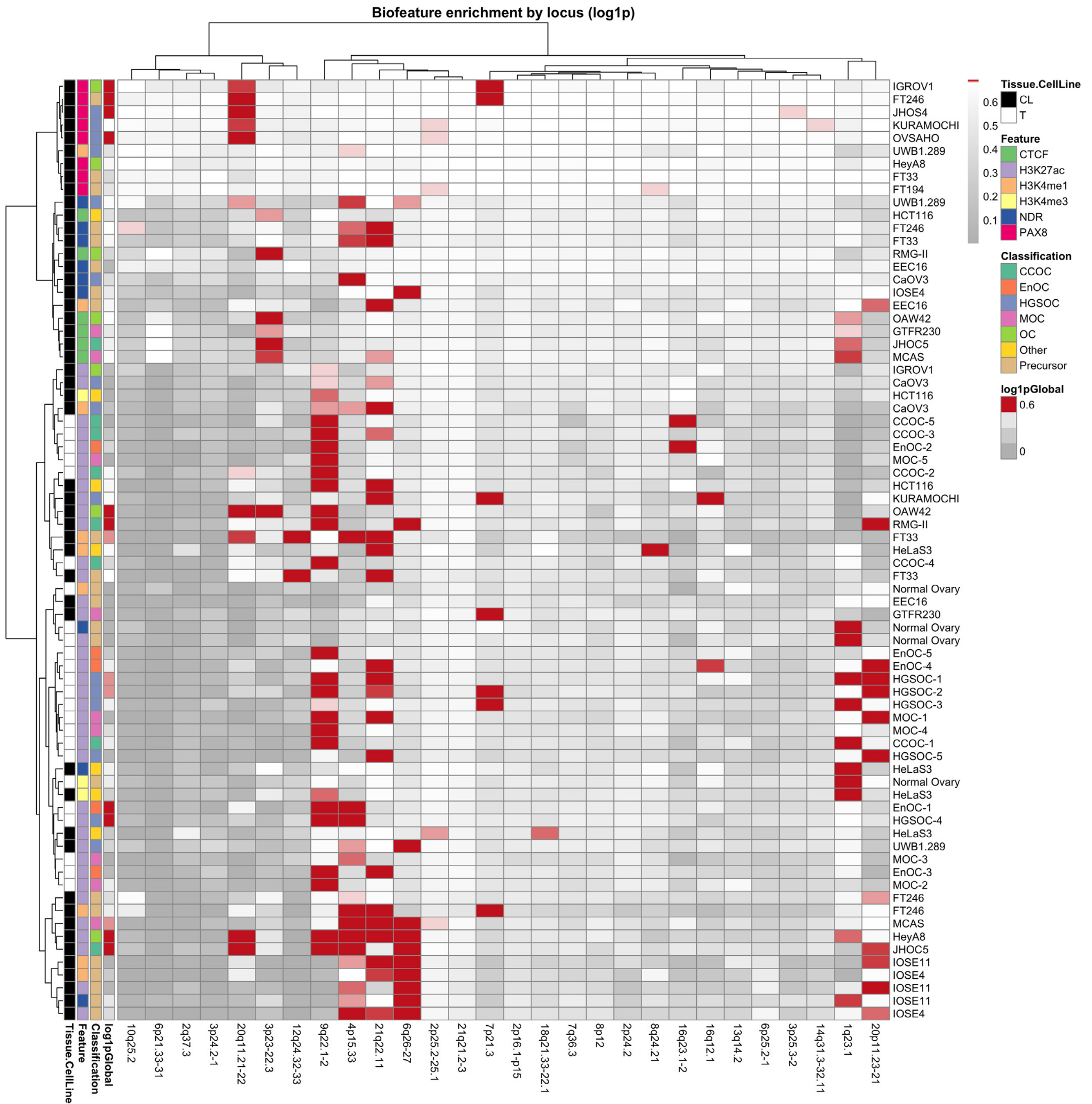


Fig. 3. Risk loci can be stratified by biofeature enrichment patterns. All loci with BFDP <0.5 were intersected with 73 biofeature data sets for EOC-relevant cell types. Red color indicates a statistically significant enrichment of SNPs within a certain biofeature (rows) for a particular locus (columns). Global enrichment for all loci collectively is indicated on the far left of the heatmap. Clustering was performed to aggregate loci that exhibit similar patterns of enrichment, using the Ward method. CL, cell line; NDR, nucleosome depleted region; T, tumor.

the global genetic architecture in the two populations we calculated a polygenic risk score using published European hits (excluding mucinous associations) and found that European risk scores do predict cancer in East Asians (HGSOC OR = 1.76 per unit increase in polygenic risk score, $P = 8.6 \times 10^{-6}$), confirming that European risk loci contribute to EOC risk in the East Asian population. Finally, we compared our results to the previous Han Chinese ovarian cancer GWAS [9]. Neither of the risk regions identified in this report (at 9q22.33 and 10p11.21) were associated with EOC risk in our study (Supplementary Table 7). Conversely, none of the 126 regions identified in our study showed evidence of association in the prior GWAS (Supplementary Table 4).

3.2. Functional annotation of risk variants

We performed functional annotation for the genome-wide significant 6p25.2 risk region and the 28 loci with a BFDP score < 50% (Supplementary Tables 2 & 8). These loci were associated with overall EOC, serous EOC or HGSOC-specific risk. For each region we used imputation-based fine mapping to identify SNP sets that represent the most likely candidate causal risk variants (SNPs with likelihood odds >1:100 of being the causal variant at each locus; see Methods and Supplementary Table 8). In total we identified 1283 candidate causal risk SNPs across the 29 regions. These variants were intersected with

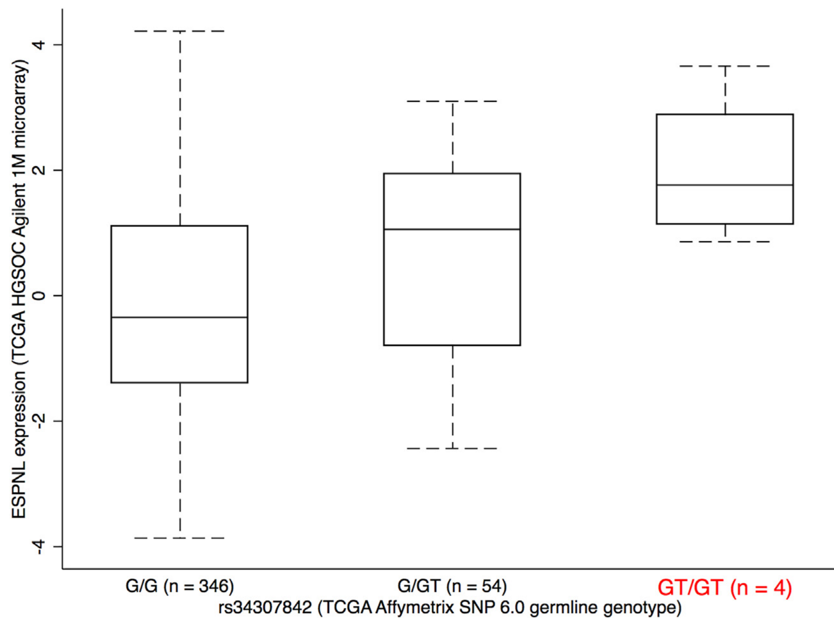


Fig. 4. *ESPNL* expression is associated with rs34307842 genotype. Increased expression is associated with the GT genotype, which is associated with increased risk. Other variants that are also eQTLs for this gene are listed in Supplementary Table 10.

catalogues of regulatory biofeature data generated using chromatin immunoprecipitation sequencing (ChIP-seq) of primary EOC tissues and normal EOC precursor cells (fallopian tube secretory epithelial cells (FTSECs) and ovarian surface epithelial cells (OSECs)). The biofeature annotated in the most cell types was active chromatin, indicated by H3K27ac ChIP-seq signal. Other marks included H3K4me1, CCCTC-binding factor (CTCF), associated with insulators, PAX8, a transcription factor overexpressed in many EOCs [36,37], and regions of open chromatin catalogued using Formaldehyde-Assisted Isolation of Regulatory Elements sequencing (FAIRE-seq) [38]. The full list of EOC-relevant epigenetic data sets used is provided in Supplementary Table 2.

Twenty-nine percent of SNPs (368/1283) overlapped one or more biofeatures present in relevant cell types (Supplementary Table 8). SNPs rs6058070 and rs6087592 at chromosome 20q11.22 (linked with $r^2 = 0.97$) coincided with 44 and 43 unique biofeature data sets, respectively. Rs6058070 and rs6087592 show expression quantitative trait locus (eQTL) associations for the *MAP1LC3A* (microtubule-associated protein 1 light chain 3 alpha) gene in GTEx tissues, but not in EOC related samples (OSEC, FTSEC or HGSOC). At the 6p25.2 locus, out of seven candidate causal SNPs, only one, rs6902488, coincided with biofeatures in EOC-relevant cells, specifically marks of active chromatin (H3K27ac and H3K4me1) present in two independent immortalized OSEC lines [38,39]. SNP rs6902488 lies within a putative enhancer close to the center of a 265 kb intergenic region between *SLC22A23* and *PXDC1*. We used MotifbreakR [40] to predict the function of rs6902488 and found this SNP alters the binding of transcription factors (TFs) STAT3, p300 and ELF1. Stronger binding was associated with the risk-conferring (T) allele for all three TFs (Supplementary Table 9, Fig. 2).

We also evaluated locus-specific enrichment of tissue specific H3K27ac signals with candidate causal risk SNPs based on the hypothesis that multiple SNPs may be working together to mediate risk (Fig. 3). Variants at chromosome 20q11.21 were enriched in PAX8 binding sites and regions of active chromatin in EOC cell lines and FTSECs; the 9q22.1 risk locus showed strong enrichment of SNPs within active chromatin in primary tumors; and six of the seven risk SNPs at 3p23 were located in CTCF peaks suggesting they may play a role in gene repression. Risk SNPs at the 4p15.33, 21q22.1, 6q26 and 20p11.32 loci also showed enrichment in active and or/poised regulatory elements detected in ovarian cancer relevant cell types.

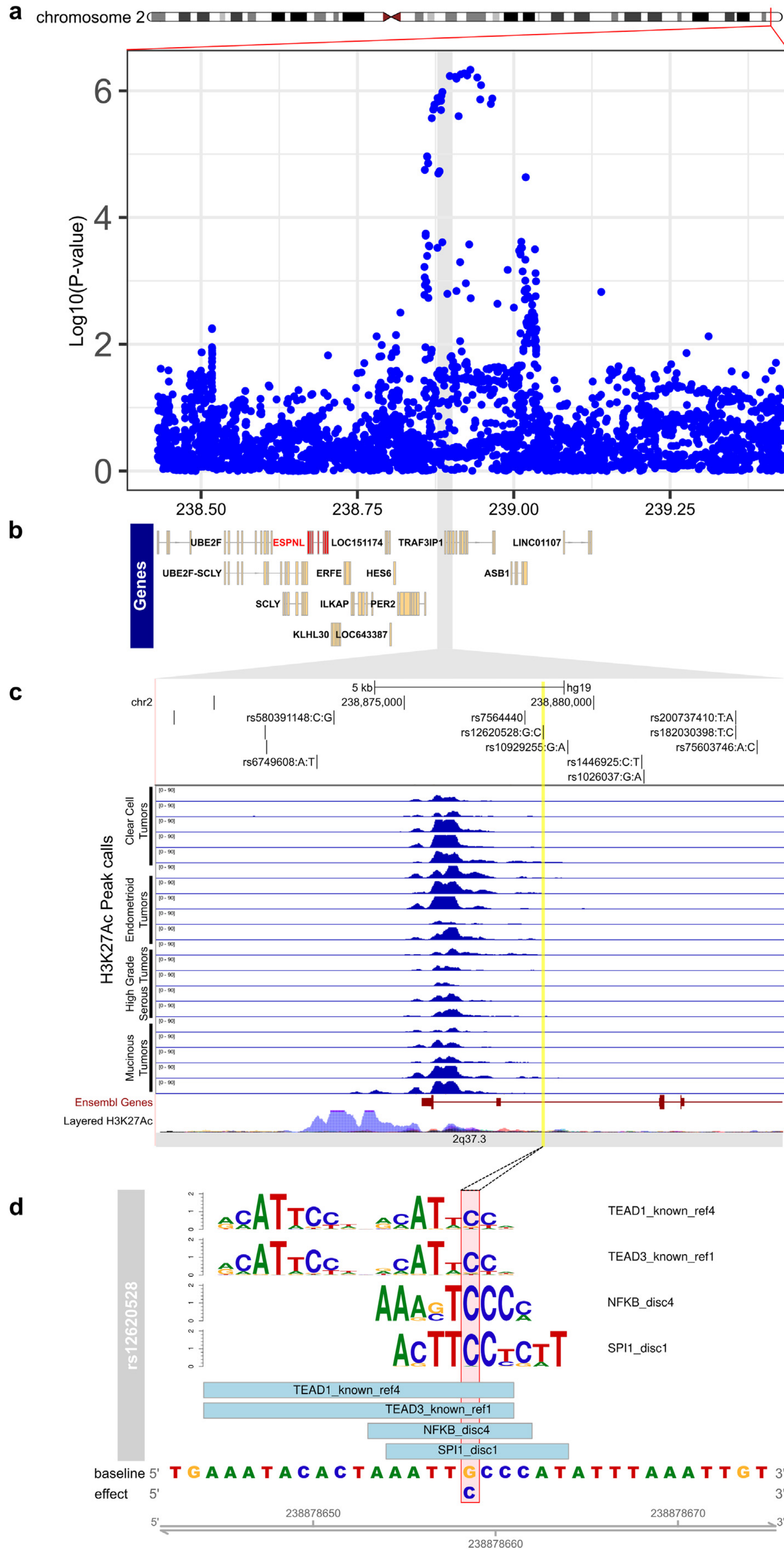
3.3. Expression quantitative trait locus (eQTL) analyses to identify target genes at risk loci

We performed eQTL analysis using three whole transcriptomic data sets: (1) 404 primary HGSOCs from The Cancer Genome Atlas (TCGA) [32]; (2) 105 primary OSEC samples; and (3) 60 primary FTSEC samples. We regressed out the effects of copy number and methylation on gene expression in tumors [34]. For all three data sets we evaluated associations between the 1283 candidate causal risk SNPs spanning the 29 loci and gene expression for all genes within a 1 Mb window spanning the index SNPs at each locus. We applied a threshold of $P < 3.9 \times 10^{-5}$ for eQTL associations based on correction for testing 1283 SNPs. Where multiple SNPs at the same locus were associated with expression of the same gene due to linkage disequilibrium, we report the strongest associations.

We identified a significant eQTL signal for SNP rs34307842 associated with *ESPNL* expression at the 2q37.3 locus in primary HGSOCs ($P_{\mathrm{eQTL}} = 1.2 \times 10^{-7}$, Fig. 4, Supplementary Table 10). The GT allele of rs34307842 was associated with increased *ESPNL* expression and increased HGSOC risk. We examined the epigenomic landscape at this locus and identified a putatively functional variant, rs12620528, which is in linkage disequilibrium with an eQTL SNP for *ESPNL* (rs10929255, $r^2 = 0.86$ in Chinese and Japanese populations) (Fig. 5). This SNP lies within an intron of *UBE2F*, around 130 kb centromeric to *ESPNL*. SNP rs12620528 coincides with active chromatin detected in primary ovarian tumors and significantly alters binding motifs for TEAD factors, NFKB and SPI1 (Fig. 5 c,d).

Significant eQTLs ($P_{\mathrm{eQTL}} < 3.9 \times 10^{-5}$) were also identified for 9 genes at 3 additional sub-genome-wide significant risk regions in primary FTSECs and for 41 genes spanning 6 sub-genome-wide significant risk regions in OSECs (Supplementary Tables 11 &12). Notably, at 6q21.32 the index risk SNP rs72492309 ($P_{\mathrm{risk}} = 2.2 \times 10^{-6}$, BFDP =

Fig. 5. Functional analysis of the 2q37 risk locus. (a) Regional association plot for all invasive cancers, with genes in the regions indicated in panel (b). (c) ChIP-seq in OC-relevant cell types. The locations of the top 6 candidate causal alleles are indicated. (d) SNP rs6902488 significantly alters binding sites for 4 transcription factors. Position weight matrices are shown for each, the height of each letter indicates the importance of that base in the motif.


a
chromosome 2
Log10(P-value)
6
4
2
0
238.50
238.75
239.00
239.25
b
Genes
UBE2F
ESPNL
LOC151174
TRAF3IP1
LINC01107
UBE2F-SCLY
ERFE
HES6
ASB1
SCLY
ILKAP
PER2
KLHL30
LOC643387
c
5 kb
hg19
chr2
238,875,000
238,880,000
rs580391148:C:G
rs7564440
rs200737410:T:A
rs12620528:G:C
rs182030398:T:C
rs10929255:G:A
rs75603746:A:C
rs6749608:A:T
rs1446925:C:T
rs1026037:G:A
H3K27Ac Peak calls
Clear Cell Tumors
Endometrioid Tumors
High Grade Serous Tumors
Mucinous Tumors
Ensembl Genes
Layered H3K27Ac
2q37.3
d
rs12620528
TEAD1_known_ref4
TEAD3_known_ref1
NFKB_disc4
SPI1_disc1
baseline 5' T G A A A T A C A C T A A A T T G C C C A T A T T T A A A T T G T 3'
effect 5' C 3'
238878650
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26%) was associated with expression of the *HLA-DRB1*, *HLA-DRB6*, and *HLA-DQB1* genes in both FTSECs and OSECs ($P_{eQTL} < 3.9 \times 10^{-5}$). Other significant associations were either cell-type specific or were not replicated between OSECs and FTSECs (Supplementary Tables 11 and 12).

4. Discussion

This study reports the identification of a novel genome-wide significant locus at chromosome 6p25.2 associated with risk of serous and high-grade serous ovarian cancer in East Asian women. We also identified several candidate EOC risk regions that did not reach statistical thresholds of genome-wide significance but where the Bayesian false discovery probabilities (BFDPs) provided additional evidence that the risk associations may be real. There are several likely reasons why we did not identify additional EOC risk regions at genome-wide levels of significance ($P < 5 \times 10^{-8}$). Firstly, this study included 2981 and 257 subjects diagnosed with invasive and borderline EOC, respectively, and so was not strongly powered to identify risk alleles with this level of statistical stringency. Disease heterogeneity will also have affected our ability to identify risk loci; studies in European subjects have shown that common variant risk regions differ for different ovarian cancer histotypes [8,15,19]. Our power to detect associations for specific histotypes in East Asian women was extremely limited given that this study included few cases of rare histologies.

This study would also have been limited by the content of the genotyping array (OncoArray) – the GWAS backbone was primarily designed for European populations and the custom content was largely based on meta-analyses performed in European subjects. Several studies for other phenotypes have shown that the spectrum of disease risk can vary substantially across different populations. Because data from large-scale genotyping in East Asian populations were not included in the design of the OncoArray, there will be bias against risk alleles that are common in these populations but rare in Europeans. The current study supports other findings that show both similarities and differences in risk variants between East Asian and European populations. None of the most significant EOC risk regions identified in this study were associated with risk in European populations; although of the 30 confirmed EOC risk loci so far reported in Europeans, around two-thirds showed evidence of association in our East Asian study. This includes variants at 8q24, which are associated with the risk of multiple cancer types, including ovarian, breast, prostate and colorectal [17,41]. Variants at 8q24 have been previously implicated in candidate SNP studies for ovarian cancer risk in Asian women [42]; and were nominally significant in both this study (BFDP = 12%) and the previous Han Chinese GWAS (Supplementary Table 5). This study did not replicate findings from a three-staged GWAS analysis of a homogenous population of around 2500 Han Chinese women with ovarian cancer, and ~4000 controls, even though we included women of Han Chinese other Chinese ancestry in our analyses. It may be that the previously reported loci did not replicate as they are only associated with EOC risk in Han Chinese and not across other Asian ethnic groups [9].

Similar to most other GWASs, the majority of risk associated SNPs identified in this study, including all candidate causal SNPs at the 6p25.2 locus, were non-coding, suggesting their functional impact is mediated through non-coding elements that regulate gene expression. We leveraged epigenomic data profiled in primary ovarian tumors and cell types representing the precursors of EOC (OSECs and FTSECs) to identify putative functional targets of risk SNPs. At 6p25.2 a risk SNP rs6902488 is located in a region of active chromatin in OSECs, with the alternative allele at this SNP predicted to strongly enhance the binding of three proteins with a known role in EOC biology. STAT3 is a member of the STAT family of transcription factors and has been posited as a therapeutic target for EOC, particularly in chemoresistant cells [43]. P300 is a transcriptional co-activator known to bind to enhancers in many cell types and works in concert with other transcription factors to orchestrate downstream transcriptional changes. Finally, ELF1 is an ETS domain transcription factor and closely related to ELF4, which has previously been implicated as a transforming factor in EOC [44]. Further functional experiments will be required to evaluate which of these factors play a role in mediating risk at this locus.

It is unlikely that our epigenomic analyses have captured all possible functional noncoding elements. For example, data representing disease specific transcription factors (e.g. WT1) are not available for the major EOC histotypes and their precursor tissues. This may explain why so few of the candidate causal risk variants we identified at 6p25.2 and other risk loci intersect putative functional biofeatures. Future investigations will require more comprehensive analysis of the non-coding architecture of disease relevant tissues to identify the likely causal risk SNPs and target gene(s).

We identified 28 genomic regions where the Bayesian false discovery probability (BFDP) score provided additional evidence that these loci confer susceptibility to EOC even though the risk associations for these regions failed to reach genome-wide significance. Of particular interest is a locus at chromosome 2q37.3 which is associated with overall EOC risk. A BFDP calculation indicated this is likely to be a true association (for the index SNP rs74917072, $P = 4.65 \times 10^{-7}$ and BFDP = 7%). We identified candidate causal risk SNPs at this locus with significant eQTL associations (top $P = 1.2 \times 10^{-7}$) for the Espin-Like (*ESPNL*) gene using expression data from primary HGSOCs. *ESPNL* is a little studied gene with homology to Espin (*ESPN*). Both *ESPNL* and *ESPN* are involved in actin bundling in hair cell stereocilia in the inner ear (40) and there is some evidence that both are expressed in the normal fallopian tube in the Human Protein Atlas (proteinatlas.org). *ESPNL* has not previously been implicated in cancer, but we speculate that it may play a role in EOC cell motility in response to chemical stimuli.

We also explored eQTLs in novel RNA-seq data generated in OSECs and FTSECs. In both cell types we identified significant eQTL associations after Bonferroni-correction between candidate causal risk alleles and Major Histocompatibility Complex, Class II, DR Beta 1 (*HLA-DRB1*), DR Beta 6 Pseudogene (*HLA-DRB6*), and DQ Beta 1 (*HLA-DQB1*) gene expression at a sub-genome-wide significant risk locus at 6q21.32. The index EOC risk SNP (rs72492309) at this locus is 154 kb away from rs2647012, a genome-wide significant index SNP associated with follicular lymphoma risk in Europeans that has previously been shown to have *cis*-regulatory effects on *HLA-DQB1*, *HLA-DRB1*, and *HLA-DRB6* expression in lymphoblastoid cell lines [45]. SNP rs72492309 is also 91 kb from rs9272143, a SNP known to be associated with both cervical cancer risk ($P = 2.8 \times 10^{-17}$) and *HLA-DRB1* expression ($P = 4 \times 10^{-7}$) [46]. SNPs at this locus are associated at genome-wide significance with inflammatory bowel disease [47], blood pressure [48], autism [49], and Alzheimer's disease [50]. Class II *HLA* genes are predominantly expressed by antigen presenting cells in the immune system, but can be expressed by other cell types, albeit at lower levels. HLA proteins are localized to the surface of cells where they present cellular peptides to the immune system to enable identification and clearance of invading pathogens. Clustered somatic mutations in *HLA* genes in certain cancers point to a role for HLA deregulation during tumorigenesis, and may contribute to immune escape during neoplastic transformation. Mild immune deregulation could feasibly affect cancer susceptibility by modulating tumor suppressive immune pathways.

In summary, this study reports novel EOC susceptibility regions identified in East Asian populations, and a risk spectrum for common variants that includes some population-specific regions. Much larger genetic association studies, based on genetic variance catalogued in East Asian populations are warranted to characterize the full spectrum of risk variation in EOC cases in these populations. Such studies are also needed to define risks associated with different EOC histotypes that may partly explain variations in the clinical presentation of disease in Asian compared to non-Asian populations.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.02.023.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

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All authors read and approved the final manuscript.

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