A genome-wide association study identifies susceptibility loci for ovarian cancer at 2q31 and 8q24

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A Genome-Wide Association Study Identifies Susceptibility Loci for Ovarian Cancer at 2q31 and 8q24

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Abstract

Ovarian cancer (OC) accounts for more deaths than all other gynecological cancers combined. To identify common low-penetrance OC susceptibility genes, we conducted a genome-wide association study (GWAS) of 507,094 SNPs in 1,768 cases and 2,354 controls, with follow-up of 21,955 SNPs in 4,162 cases and 4,810 controls, leading to the identification of a confirmed susceptibility locus at 9p22 (*BNC2*)[1]. Here, we report on nine additional candidate loci ($p \leq 10^{-4}$), identified after stratifying cases by histology, genotyped in an additional 4,353 cases and 6,021 controls. Two novel susceptibility loci with $p \leq 5 \times 10^{-8}$ were confirmed (8q24, $p = 8.0 \times 10^{-15}$ and 2q31, $p = 3.8 \times 10^{-14}$); two additional loci were also identified that approached genome-wide significance (3q25, $p = 7.1 \times 10^{-8}$ and 17q21, $p = 1.4 \times 10^{-7}$). The associations with serous OC were generally stronger than other subtypes. Analysis of *HOXD1*, *MYC*, *TiPARP*, and *SKAP1* at these loci, and *BNC2* at 9p22, supports a functional role for these genes in OC development.

Invasive epithelial OC is a rare but often lethal disease. Individuals with an OC family history have approximately a two-fold increased risk, even after accounting for mutations in known highly-penetrant susceptibility genes, suggesting that other risk alleles await identification. GWAS have identified common genetic variants influencing risks for a range of cancers, including our recent identification of low-risk OC alleles at 9p22.2[1]. The most significant association conferred a 20% reduction in risk with each copy of the minor allele; this association was stronger for serous subtype, suggesting that disease heterogeneity may have reduced the power of our GWAS[1]. To identify further susceptibility alleles, we have followed-up additional candidate loci from that study after stratifying cases by histological subtype.

Our GWAS was performed for women of European ancestry diagnosed with the major histological subtypes of invasive epithelial OC: serous, mucinous, endometrioid, and clear cell (Table 1, Supplementary Table 1). Phase I included 1,768 cases and 2,354 controls from the UK genotyped using the Illumina Infinium 610K and 550K arrays, respectively. Data were available for 507,094 genotyped SNPs and 1,549,784 imputed SNPs from HapMap. The top-ranked 21,955 SNPs, selected based on analysis of all subtypes combined, were genotyped in an additional 4,162 cases and 4,810 controls in Phase II leading to the identification of a compelling association near the basonuclin 2 (*BNC2*) gene at 9p22.2 ($p=5.4\times10^{-22}$, rs3814113 for serous subtype)[1]. Quantile-quantile (Q-Q) plots of the distribution of test statistics to compare genotype frequencies in cases versus controls in the stage 1 and stage 2 data, suggested there was little evidence of any general inflation of the test statistics (estimated inflation factor $\lambda_{1000} = 1.026$ and 1.0086 respectively based on the bottom 90% of the distribution)[1].

In the current study, combined Phase I and Phase II data from this GWAS were re-analyzed overall and restricting to 'serous only'. This revealed nine loci with a p-value $\leq10^{-4}$ for all subtypes (1p31, 1p36, 2q31, 11p14, and 17q21) and/or for serous subtype (2p22, 3q25, 7p21, and 8q24). Thirty SNPs from these loci were genotyped in an additional 4,353 cases and 6,021 controls in Phase III; thus, data were available for combined analysis of 10,283 cases (including 5,841 serous) and 13,185 controls. In addition, results on an independent 194 cases and 40,933 controls were incorporated using metaanalyses. There was little additional evidence of association for five loci in either the Phase III or combined data, although trends in association were in the same direction for some SNPs (Supplementary Table 2; Supplementary Table 3). For SNPs at two loci (2q31, 8q24), there was strong support for associations in the Phase III data alone ($p<0.001$) and in the combined analyses of all cases or serous cases only ($p\leq5\times10^{-8}$). At two other loci, 3q25 and 17q21, there was good evidence for associations in the combined analysis of serous cases, but these did not quite reach genome wide significance ($p=7.1\times10^{-8}$ and $p=1.4\times10^{-7}$ respectively). No heterogeneity across studies was observed ($p>0.05$); these data are summarized in Table 2, Figure 1 and Supplementary Figure 1).

At the 8q24 locus, the minor allele of rs10088218 was associated with a decreased risk (Table 2), with the association being more significant for serous subtype (odds ratio, OR, 0.76; 95% confidence intervals, 95%CI, 0.70-0.81; $p=8.0\times10^{-15}$); the additional 8q24 SNPs rs1516982 ($r^2=0.46$) and rs10098821 ($r^2=0.80$) were also highly significant (Supplementary Table 2). The minor allele of rs2072590 at 2q31 was associated with an increased risk which was primarily significant for serous subtype (OR 1.20, 95%CI 1.14-1.25, $p=3.8\times10^{-14}$); more significant results were also seen for serous subtype for minor alleles of rs2665390 at 3q25 (OR 1.24, 95%CI 1.15-1.34, $p=7.1\times10^{-8}$) and rs9303542 at 17q21 (OR 1.14, 95%CI 1.09-1.20, $p=1.4\times10^{-7}$).

We observed significant heterogeneity for associations at the 8q24 and 2q31 loci when cases were stratified into four histological subtypes ($p=2.9\times10^{-4}$ for rs2072590, $p=1.1\times10^{-7}$ for rs10088218) (Table 2). For both loci, the trends in association for endometrioid and mucinous OC were in the same direction as serous cases, but there was no evidence of association at either locus in clear cell OC. To a lesser degree, a difference in risk by subtype was also observed at 3q25 ($p=0.02$ for rs2665390). We also examined SNP associations by age and family history of OC in first-degree relatives. No differences in risk were observed by age (Supplementary Table 4) and, among the four novel loci, no interactions by family history were observed (Supplementary Table 5).

We also tested the most significant risk-associated SNPs at 8q24, 2q31, 3q25 and 17q21 for association with overall survival after a diagnosis of OC, in 'all' case and 'serous only' case

subgroups. For the latter group, we also performed an analysis adjusting for tumor stage and grade. We found borderline evidence for an association with survival at 17q21 in all cases (HR=1.06, 95% CI 1.00-1.13, P=0.04) and in serous only cases (HR=1.08, 95% CI 1.00-1.16, P=0.04). This effect was not greatly attenuated when adjusting for stage and grade amongst serous cases (HR=1.06, 95% CI 0.97-1.15, P=0.15). There was no evidence of association with survival at 8q24, 2q31 and 3q25.

We used Pupasuite (http://pupasuite.bioinfo.cipf.es/) for *in silico* analyses of risk-associated and strongly-correlated SNPs (r^2>0.80) in the four loci but failed to find compelling evidence for any functional role (Supplementary Table 6). Genotyping of additional SNPs identified from HapMap and the 1,000 Genomes Project (http://www.1000genomes.org/page.php) is required to fine-map these loci to identify both causal variants and target genes. Even so, known genomic architecture may provide some insights into functional mechanisms underlying OC susceptibility. For example, common variants at 8q24 have previously been shown to confer susceptibility to multiple cancer phenotypes including prostate, colorectal, breast, and bladder cancers[2-7], and previous functional studies suggest that common variants may be associated with transcriptional regulation of *MYC*[8,9]. Most risk associations at 8q24 are located 5′ of *MYC*; but the three most significant SNPs for OC lie >700 kb 3′ of *MYC* in an apparent gene desert, suggesting either that *MYC* is not the target gene for OC, or possibly that variants in this region are also capable of distant regulation of *MYC* (Figure 1a).

rs2665390 at 3q25 is intronic to the *TiPARP* gene; there are no other candidate genes within 200 kb of this SNP (Figure 1b). *TiPARP* is a poly (ADP-ribose) polymerase (PARP)[10] and is a particularly intriguing candidate gene for OC for two reasons. First, recent reports show that *BRCA1/2* deficient cells survive by using PARP1 as an alternative DNA repair mechanism[11]. This has led to the development of a novel therapy based on synthetic inhibition of PARP1 for breast and ovarian cancer patients carrying *BRCA1* or *BRCA2* mutations[12]. Second, TiPARP is inducible by dioxin[13], raising the hypothesis that this environmental contaminant may influence OC risk among susceptible women.

The 2q31 locus contains a family of homeobox (HOX) genes involved in regulating embryogenesis and organogenesis (Figure 1c). Altered expression of HOX genes has been reported in many cancers[14,15]. The OC risk-associated SNP rs2072590 lies in non-coding DNA downstream of *HOXD3* and upstream of *HOXD1*, and it tags SNPs in the *HOXD3* 3′ UTR. Both genes have been implicated in neoplastic development[16,17].

Finally, associated and correlated SNPs at 17q21 are intronic to *SKAP1*, which has strong homology to the *SRC* oncogene at the C-terminal end (Figure 1d). *SKAP1* regulates mitotic progression, specifically at the transition of metaphase to anaphase[18]. In T-cells, constitutive expression of *SKAP1* suppresses activation of *RAS* and *RAF1*, both of which have been implicated in the early stage development of OC[19].

We evaluated risk-associated SNPs and candidate genes from these four novel loci, as well as from *BNC2*[1], for evidence of a functional role in OC development by examining genotype associated gene expression for *BNC2* (9p22), *MYC* (8q24), *TiPARP* (3q25), *HOXD1* and *HOXD3* (both at 2q31), and *SKAP1* (17q21). We found no evidence of genotype associated gene expression in an analysis of 48 normal primary human ovarian surface epithelial (POE) cell cultures (Supplementary Table 7) although power was limited due to the relatively small numbers. We also compared the expression of each of the five candidate genes between 48 POE and 24 OC cell lines and found highly-significant differences in gene expression between normal and cancer cells for *BNC2*, *TiPARP*, *HOXD1*, and *SKAP1* (Figure 2;

Supplementary Figure 2). These data suggest that *BNC2* and *TiPARP* have a loss-of-function role, and that *HOXD1* and *SKAP1* have a gain-of-function role in OC development.

Gene expression was also examined in an *in vitro* model of OC initiation and progression established through oncogenic expression of *MYC* and mutant *KRAS* ^{G12V} in POE cells (Figure 2; Supplementary Table 9). We found that *BNC2* and *TiPARP* expression decreases, and *SKAP1* expression increases, with neoplastic development, consistent with the expression of these genes in POE *versus* OC cells. We also investigated gene expression for 216 primary serous OC samples analysed by The Cancer Genome Atlas Project (http://cancergenome.nih.gov). These data support frequent loss of *BNC2* and *TiPARP* and gain of *HOXD1* expression in OC development (Supplementary Figure 3).

In summary, we report two confirmed novel common low-penetrance OC susceptibility loci, and a further two candidate susceptibility loci, adding to a growing list that includes *BNC2* and the 19p13 locus presented in an accompanying report by Bolton and colleagues. All six susceptibility loci suggest possible functional relevance of candidate genes that could plausibly be involved in OC development and aid in our understanding of disease aetiology. Strikingly, these data also suggest that common germline variation influences the clinico-pathological development of disease, as previously reported for the highly-penetrant (*BRCA1* and *BRCA2*) germline variants[20].

Methods

Phase I and II

As described previously1, Phase I self-reported white European participants were from four collections of invasive epithelial OC cases and two collections of controls22,23 (Supplementary Table 1). Logistic regression and linear trend tests examined SNP associations (including imputed genotypes) using imputed genotype weights and ethnicity-related principal components. Phase II SNPs were selected based on Phase I ranked test statistics (all, serous) weighted by imputation status and accuracy (r^2)[24] and design score, and participants were of European ancestry from 12 studies. Logistic regression adjusted for a HapMap-based ancestry score[25] and an ancestry-informative-marker-based principal component.

Phase III

Sixteen studies contributed invasive epithelial OC cases and controls of European ancestry (Supplementary Table 1). Nine loci (30 SNPs, Supplementary Table 9) were selected based on test statistic rankings from combined Phase I and Phase II analyses ($p < 10^{-5}$ in all or serous cases). Eleven studies used Taqman (two SNPs) and Sequenom MassARRAY (remaining SNPs). Data for four studies were available from a genome wide scan using the same Illumina Infinium 610K array that was used in Phase 1 excluding samples with call rate <95%, >1% discordance, <80% European ancestry, or ambiguous gender. For one study, Illumina Infinium 317K data were used with imputation based on HapMap CEU data following 100 iterations in MACH version 1.0.16, excluding SNPs with r^2 <0.30. For one study, cases and ~2,900 controls were genotyped on a Centaurus (Nanogen) platform (excluding SNPs with >1.5% HapMap mismatch), and additional control data used the Illumina Infinium 317K and HumanCNV370-duo Bead Arrays; per-SNP call rate was >97%, and concordance was >98.5%. Logistic regression modeled the number of observed or imputed minor alleles; no confounding by age was observed. Combined analyses adjusted for study and tested heterogeneity with Cochran's *Q* statistic and I^2 values. Effect modification and differences in risk by subtype were tested with interaction terms and

polytomous regression, respectively. Summary-level data was available for the ICE study; thus meta-analytic techniques fitted fixed and random effects[26,27].

Genotyping Quality Control Measures—The Ovarian Cancer Association Consortium (OCAC) has established robust genotyping quality control (QC) guidelines to ensure accurate genotyping, particularly across multiple studies. Data included must pass the following quality control criteria: (1) > 3% sample duplicate samples included in each study; (2) samples from cases and controls mixed on 384 well plates; (3) samples that consistently fail (e.g. for >20% of all SNPs) are removed; (4) genotype data for a SNP are removed if call rates > 90% for each individual 384-well plate, but if > 25% plates from any site are excluded for this reason then all the data from that site are excluded; (5) the overall call rate for a SNP for each study > 95%, which is calculated after ineligible samples and plates are excluded; (6) genotyping concordance rates for the duplicates >98%; (7) Hardy Weinberg Equilibrium (HWE) in White European controls must be P≥0.005. If HWE P = 0.05-0.005 the genotyping clustering quality is reviewed by members of the OCAC genotyping committee (PDP; GC-T, SJR, HS) before inclusion. In addition, as part of overall QC, genotyping consistency across labs is evaluated by genotyping a panel of CEPH-Utah trios including 90 individual DNA samples, 5 duplicate samples and 1 negative control (http://ccr.coriell.org/Sections/Search/Panel_Detail.aspx?PgId=202&Ref=HAPMAPPT01). Genotyping concordance between centres has to be > 98% in order for the genotype data to be included. Genotyping QC for Phase 1 and Phase 2 of the GWAS have been described previously[1].

Genotyping QC in the current study: In Phase 1, 507,094 out of 540,573 SNPs (94%) passed QC. In both Phase 1 and Phase 2 duplicate concordance rates were 99.9%. For phase 3 the data from the five studies using GWAS data met the following overall quality control criteria; (1) Caucasian and greater than 80% European Ancestry; (2) Sample call rate ≥95%: (3) SNP call rate ≥95%; (3) 100% concordance of duplicates (81 replicate pairs) (4) HWE for each SNP $P \geq 10^{-4}$. The Sequenom and Taqman data met the OCAC criteria described above. The results from Phase 3 genotype QC for each of the 30 SNPs are summarized in supplementary table 10.

Survival Analysis

Among the study participants, 7,222 cases had survival time information available and 2,791 died within five years after diagnosis. The effect of the 8q24, 2q31, 3q25 and 17q21 loci on time to all-cause mortality after EOC diagnosis was assessed using Cox regression stratified by study and modeling the per-allele effect as log-additive. Because the EOC cases showed a variable time from diagnosis to study entry, we allowed for left truncation with time at risk starting on date of diagnosis and time under observation beginning at the time of study entry. The analysis was right censored at 5 years after diagnosis in order to reduce the number of non-EOC related deaths.

Gene Expression Analysis in POE and OC Cell Lines

Normal POE cell lines were established from brushings of histologically-confirmed disease-free ovaries from total hysterectomies at University College London Hospital, UK; short-term cultures were established as previously described[28]. The non-neoplastic status and epithelial (non-fibroblastic) nature of cells was confirmed by staining for CA125, CK7, FVIII, and FSP. RNA was extracted from POE and OC cell lines (Supplementary Table 11) using RNeasy Mini Kits (QIAgen). Reverse transcribed (RT) RNA was analysed for expression by semi-quantitative real-time PCR using the Applied Biosystems 7900HT genetic analyzer. Gene expression was normalized against the endogenous controls glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin. Real time expression

data were analysed using the comparative Delta-Delta Ct method. Expression values of all cell lines were generated relative to either the lowest or highest expression of a POE cell line, normalized against GAPDH and β-actin. Differences in the relative expression of each candidate gene between OC and POE cell lines were assessed using a nonparametric Wilcoxon Rank sum test. For allele-specific expression analysis, expression was calculated relative to the average expression of common homozygotes for each SNP normalized against the expression of the endogenous control genes; linear regression and Wilcoxon Rank sum tests assessed difference in expression across genotypes.

An *in vitro* Genetic Model of Neoplastic Transformation in Normal Ovarian Epithelial Cells

Methods used for the immortalization of ovarian epithelial cells and over-expression of *MYC* (IOE^{cmyc}) are described elsewhere29. All cell lines were grown in NOSE-CM[28]. Using FuGene6 (Roche), iOE^{cmyc} cell lines were transfected with pcDNA3.1.neo.$KRAS^{G12V}$ (Addgene) to create cell lines stably expressing a mutant form of *KRAS* (*$KRAS^{G12V}$*). *$KRAS^{G12V}$* expression was confirmed by RFLP-PCR. Mutant allele expression was confirmed by RFLP-PCR. Anchorage-independent growth assays were performed as previously described[30]. To test invasiveness, 0.125×10^6 cells were resuspended in serum-free medium and added to rehydrated invasion membranes (Millipore) for 24 hours. 10% serum (Invitrogen) was added to the lower chamber as a chemo-attractant. Invaded cells were lysed, stained with a fluorimetric dye, and analysed on a Varioskan platereader (Thermo). To culture cells in 3D, tissue culture plastics were coated twice with 1.5% polyHEMA dissolved in 95% ethanol and cultured for 14 days. For immunohistochemistry, spheroids were fixed in neutral buffered formalin (VWR), processed into paraffin and stained for MIB1 or WT1 using standard techniques. For microarray analyses, RNA was extracted from spheroids using the QIAgen RNeasy kit (Qiagen), and experiments used the Illumina HT-12 BeadChip platform (Illumina).

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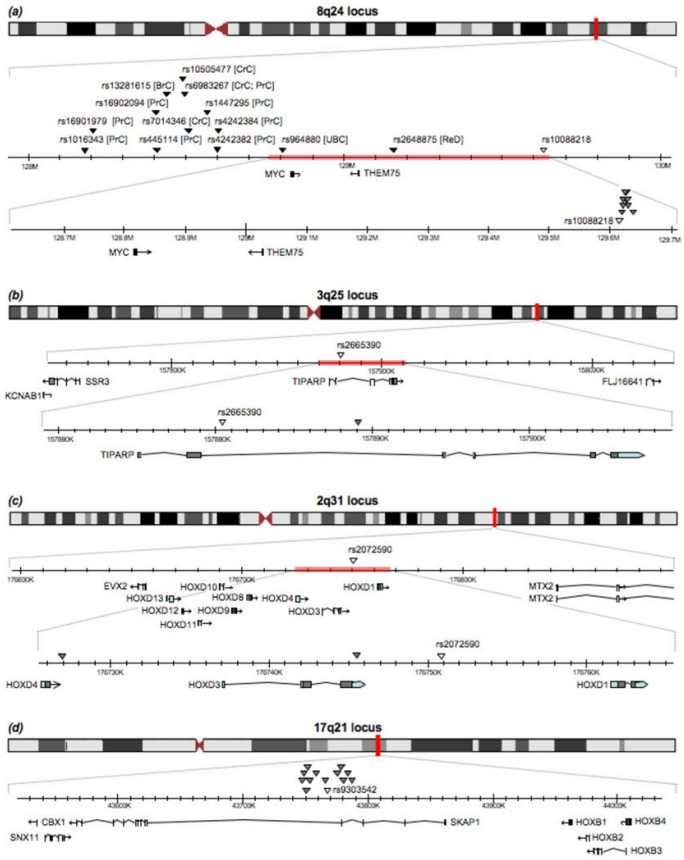

Figure 1.

Genomic architecture of the four novel ovarian cancer susceptibility regions identified from the EOC-GWAS. Key: Clear triangles- the location of SNP with the most statistically significant association at each locus; Grey triangles- the location of SNPs correlated with the associated SNP with r^2>0.8 **(a)** The 8q24.21 locus: the most significant SNP (rs10088218) lies >700kb distal to *CMYC* in an otherwise 'gene desert'. Lower resolution map: rs10088218 is located with respect to 13 other SNPs (black triangles) significantly associated with susceptibility to prostate cancer (PrC), colorectal cancer (CrC), breast cancer (BrC), urinary bladder cancer (UBC) and renal disease (ReD). Higher magnification map: rs10088218 is located with respect to 10 other HapMap SNPs correlated with rs10088218 **(b)** The most significant SNP at 3q25.31 (rs2665390) lies within TiPARP, the only gene within a ~200kb region spanning this SNP. Higher resolution map: rs2665390 location with respect to the only other highly correlated HapMap SNP, which is also in TiPARP. **(c)** 2q31.1 contains the HOXD gene family. The most significantly associated SNP (rs2072590) lies in a non-coding region ~5kb distal to *HOXD3* and ~10kb proximal to *HOXD1*. Higher resolution map: The location of rs2072590 with respect to two correlated SNPs, one 3′ of *HOXD3*, the other distal to *HOXD4*. **(d)** 17q21.31 contains rs9303542, which is located in the intron of *SKAP1*, which lies distal to the HOXB family of transcription factors. This SNP is correlated with several other SNPs all located in *SKAP1*.

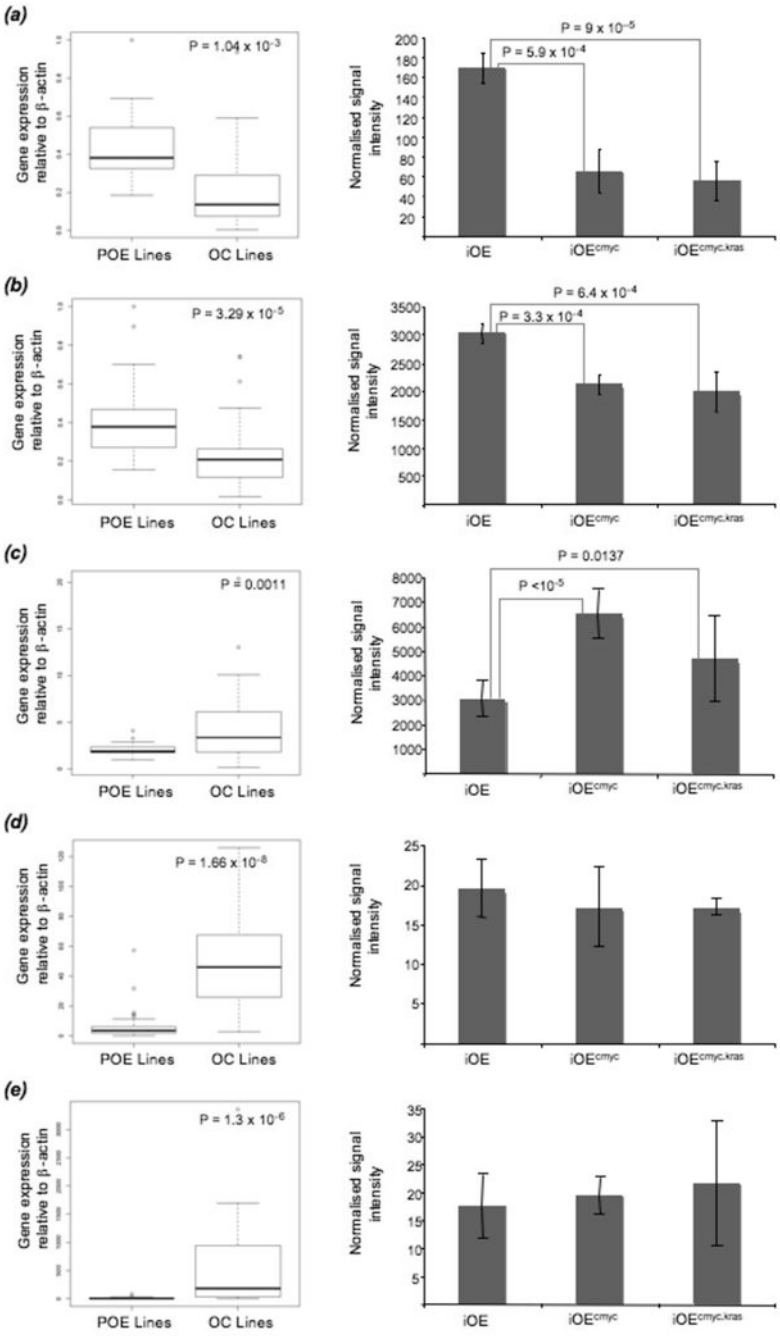

Figure 2.

Gene expression analysis for five candidate EOC susceptibility genes: **(a)** *BNC2* (9p22); **(b)** *TiPARP* (3q25); **(c)** *CMYC* expression (8q24); **(d)** *HOXD1* (2q31), and **(e)** *SKAP1* (17q21). Two different types of analysis are shown: the first panel of each pair shows real time RT-PCR analysis of each gene comparing gene expression between 48 POE and 24 OC cell lines. Gene expression is shown was normalised against b-actin. Expression was also normalized against GAPDH, which gave similar results (supplementary figure 2). Error bars sow standard deviation. The second panel of each pair shows expression microarray data from an *in vitro* stepwise model of neoplastic transformation POE cells. Briefly, gene expression in immortalised POE (iOE) cells, verses iOE^{cmyc} cells overexpressing *CMYC*,

verses iOE$^{cmyc.kras}$ cells overexpressing *CMYC* and mutant *KRAS*. **(a)** *BNC2* expression is significantly lower in OC cell lines verses normal POE cells for both endogenous controls. In the model of neoplastic transformation, *BNC2* expression decreases significantly with each additional oncogenic event, as the cells acquire a more neoplastic phenotype. **(b)** *TiPARP* shows significantly reduced expression in OC cells compared to POE cells for both endogenous controls; *TiPARP* expression also decreases sequentially in the neoplastic transformation model. **(c)** *CMYC* expression is significantly increased in OC compared to POE cells for both endogenous controls and increases in the transforming cell line model. **(d)** Expression of *HOXD3* and *HOXD1* was evaluated. *HOXD3* expression was too low to draw reliable conclusions. *HOXD1* increased in OC compared POE cells, suggesting activation in OC development. **(e)** *SKAP1* expression was significantly greater in OC compared to POE cells. A trend towards increased expression was also observed in progressively transforming iOE cells.

Table 1

Overview of GWAS Study Design

Phase	N Studies	Study population	Genotyping method	N SNPs genotyped	N SNPs passed QC	N SNPs including imputed	N Cases	N Controls
Phase I	6	UK	Illumina 610K/Illumina 550K	620,901	507,094	2,056,878	1,768	2,354
Phase II	12	Europe, USA, Australia	Illumina iSelect	23,590	21,955	21,955	4,162	4,810
Phase III	16	Europe, USA, Canada, Australia	iPlex/Taqman/Illumina 610K/Illumina 317K	30	30	30	4,353	6,021
Meta-Analysis	1	Iceland	Centaurus, Illumina 317K, Illumina HumanCNV370-duo	11	11	11	194	40,933
						Total	**10,477**	**54,118**

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Table 2

SNP Associations at Four Novel Loci

Locus	SNP	Set	Cases	Controls	OR (95% CI)	p	p-het
2q31	rs2072590 G>T	Phase I	1,768	2,354	1.16 (1.05,1.27)	2.1×10^{-3}	0.17
		Phase II	4,162	4,810	1.12 (1.05,1.19)	4.4×10^{-4}	
		Phase III	4,342	6,001	**1.19 (1.12,1.26)**	**1.9×10^{-8}**	
		Combined	10,406	16,340	**1.16 (1.12,1.21)**	**4.5×10^{-14}**	
		Serous	5,925	16,340	**1.20 (1.14,1.25)**	**3.8×10^{-14}**	**2.9×10^{-4}**
		Endometrioid	1,595	16,340	1.13 (1.04,1.22)	2.4×10^{-3}	
		Mucinous	796	16,340	1.30 (1.17,1.44)	7.3×10^{-7}	
		Clear Cell	731	16,340	0.97 (0.87,1.09)	0.59	
3q25	rs2665390 T>C	Phase I	1,768	2,354	1.15 (0.98,1.34)	0.08	0.24
		Phase II	4,162	4,810	1.23 (1.11,1.36)	8.2×10^{-5}	
		Phase III	4,285	5,953	1.19 (1.07,1.34)	1.8×10^{-3}	
		Combined	10,406	17,369	**1.19 (1.11,1.27)**	**3.2×10^{-7}**	
		Serous	5,896	17,369	**1.24 (1.15,1.34)**	**7.1×10^{-8}**	**0.02**
		Endometrioid	1,595	17,369	1.23 (1.08,1.40)	1.9×10^{-3}	
		Mucinous	789	17,369	1.02 (0.84,1.23)	0.85	
		Clear Cell	726	17,369	1.12 (0.92,1.35)	0.26	
8q24	rs10088218 G>A	Phase I	1,768	2,354	0.85 (0.74,0.97)	0.02	0.34
		Phase II	4,162	4,810	0.89 (0.81,0.97)	0.01	
		Phase III	4,339	6,007	**0.79 (0.73,0.87)**	**2.8×10^{-7}**	
		Combined	10,462	16,362	**0.84 (0.80,0.89)**	**3.2×10^{-9}**	
		Serous	5,917	16,362	**0.76 (0.70,0.81)**	**8.0×10^{-15}**	**1.1×10^{-7}**
		Endometrioid	1,607	16,362	0.95 (0.85,1.06)	0.36	
		Mucinous	796	16,362	0.90 (0.77,1.05)	0.18	
		Clear Cell	731	16,362	1.21 (1.05,1.40)	0.01	
17q21	rs9303542 A>G	Phase I	1,768	2,354	1.20 (1.08,1.32)	5.3×10^{-4}	0.27

Locus	SNP	Set	Cases	Controls	OR (95% CI)	p	p-het
		Phase II	4,158	4,806	1.14 (1.06,1.21)	1.5×10^{-4}	
		Phase III	4,316	5,931	1.05 (0.99,1.12)	0.13	
		Combined	10,242	13,091	1.11 (1.06,1.16)	1.4×10^{-6}	
		Serous	5,814	13,091	**1.14 (1.09,1.20)**	**1.4×10^{-7}**	0.11
		Endometrioid	1,573	13,091	1.09 (1.00,1.18)	0.05	
		Mucinous	766	13,091	1.03 (0.91,1.15)	0.66	
		Clear Cell	715	13,091	1.05 (0.90,1.15)	0.77	

OR, per-allele odds ratio; 95% CI, confidence interval; Phase I and Phase II adjusted for study site and first principal component; note that rs2665390 and rs10088218 were selected for Phase III based on combined results across phases in serous cases (data not shown); Phase III adjusted for study site; ICE data are included for combined analysis of rs2072590, rs2665390, and rs10088218 using meta-analytic techniques; no consistent patterns of heterogeneity were found when including ICE data and so only fixed effects meta-analysis results are presented; p-het represents heterogeneity test across all studies for combined analyses of all phases and, for subtype analyses, across subtypes excluding the ICE study; endometrioid, mucinous, and clear cell analyses are not adjusted for study; bold indicates association $p<10^{-7}$ and heterogeneity $p<0.05$.