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Genome-wide Association Study of Subtype-Specific Epithelial Ovarian Cancer Risk Alleles Using Pooled DNA

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Abstract

Epithelial ovarian cancer (EOC) is a heterogeneous cancer with both genetic and environmental risk factors. Variants influencing the risk of developing the less-common EOC subtypes have not been fully investigated. We performed a genome-wide association study (GWAS) of EOC according to subtype by pooling genomic DNA from 545 cases and 398 controls of European descent, and testing for allelic associations. We evaluated for replication 188 variants from the GWAS (56 variants for mucinous, 55 for endometrioid and clear cell, 53 for low malignant potential (LMP) serous, and 24 for invasive serous EOC), selected using pre-defined criteria. Genotypes from 13,188 cases and 23,164 controls of European descent were used to perform unconditional logistic regression under the log-additive genetic model; odds ratios (OR) and 95% confidence intervals are reported. Nine variants tagging 6 loci were associated with subtype-specific EOC risk at $P < 0.05$, and had an OR that agreed in direction of effect with the GWAS results. Several of these variants are in or near genes with a biological rationale for conferring EOC risk, including *ZFP36L1* and *RAD51B* for mucinous EOC (rs17106154, OR=1.17, $P=0.029$, $n=1,483$ cases), *GRB10* for endometrioid and clear cell EOC (rs2190503, $P=0.014$, $n=2,903$ cases), and *C22orf26/BPIL2* for LMP serous EOC (rs9609538, OR=0.86, $P=0.0043$, $n=892$ cases). In analyses that included the 75 GWAS samples, the association between rs9609538 (OR=0.84, $P=0.0007$) and LMP serous EOC risk remained statistically significant at $P < 0.0012$ adjusted for multiple testing. Replication in additional samples will be important to verify these results for the less-common EOC subtypes.

Keywords

histological subtype; serous; endometrioid; clear cell; mucinous; BPIL2

Background

Epithelial ovarian cancer (EOC) is a heterogeneous cancer with distinct and clinically relevant subtypes that are characterized by differences in morphology, gene expression profile, and molecular genetic features (Gilks et al. 2008; Kalloger et al. 2010; Kobel et al. 2010). It has become apparent that the main histological subtypes, comprising ~70% serous, 11% endometrioid, 12% clear cell, and 3% mucinous EOC (Kobel et al. 2010), have different genetic (Gayther and Pharoah 2010; Lynch et al. 1991; Lynch et al. 1985; Shulman

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2010) and epidemiologic (Faber et al. 2013) risk factors, precursor lesions (Pearce et al. 2012; Piek et al. 2001), pattern of spread, response to platinum-taxane based treatment, and patient outcome (Vaughan et al. 2011), compelling many to assert that they are different diseases (Gomez-Raposo et al. 2010; Kobel et al. 2010; Kurman and Shih 2010). Gene expression profiling has further classified serous EOC tumours into those of a less-common low grade (3% of all EOC) and more-common high grade (68% of all EOC) type (Kobel et al. 2010; Tothill et al. 2008), a finding supported by differences in the clinical behaviour of these tumours (Matsuno et al. 2013). Much of the excess familial risk observed for EOC remains unexplained and may be improved by investigations that stratify by histological subtype.

Genome-wide association studies (GWAS) have identified several common susceptibility variants for EOC (Bolton et al. 2010; Goode et al. 2010; Permuth-Wey et al. 2013; Pharoah et al. 2013; Song et al. 2009). The majority of these has been most strongly associated with serous EOC, unsurprising given the GWAS design carries forward for genotyping in subsequent stages those single nucleotide polymorphisms (SNPs) with the smallest P-values associated with the most prevalent serous EOC subtype. Fewer genome-wide significant associations have been reported for the less-common EOC subtypes. In our most recent analyses of data from over 40 international studies of EOC within the Ovarian Cancer Association Consortium (OCAC), we reported that common susceptibility variants in the candidate *HNF1B* gene (Shen et al. 2013), and the candidate *TERT* locus (Bojesen et al. 2013) differentially associate with risk by subtype and imply that distinct mechanisms are involved in pathogenesis. Examining risk factors separately by histological subtype, together with assembling studies of large numbers of women with these cancers, is critical to understanding this disease.

To extend the findings of the existing EOC GWAS (Bolton et al. 2010; Goode et al. 2010; Pharoah et al. 2013; Song et al. 2009), we performed a GWAS according to EOC histological subtype using a DNA pooling strategy. The focus was to discover genetic variants associated with the less-common EOC subtypes (endometrioid, clear cell, mucinous, and low-malignant potential [LMP] serous). The DNA pooling strategy is an efficient approach to assess genetic associations and has been successfully performed for various disease subtypes (Pearson et al. 2007; Schrauwen et al. 2009; Skibola et al. 2009). In the first stage, individual DNA samples were physically combined to create subtype-specific case pools, and a control pool, and DNA pools (not individual samples) were assayed using commercially available SNP arrays. Data from arrays were used to estimate SNP allele frequencies or allelotypes for each DNA pool, and not to determine genotypes. Pool allelotypes were then used in allele-based tests to evaluate SNP associations with EOC subtypes. In the second stage, replication of allelic associations was performed by individual genotyping (IG) for a large number of women contributing samples to the OCAC.

Methods

Discovery stage study population

Participants were from the Ovarian Cancer in Alberta and British Columbia (OVAL-BC) population-based case-control study (abbreviated as “OVA”). Eligible cases had incident,

histologically-confirmed EOC, and were identified from the provincial cancer registries of British Columbia (BC) and Alberta (AB) between 2002 and 2011. Eligible control women were identified from provincial health care enrollment rosters or from a province-wide mammography program (BC after 2005). Participants provided blood or saliva samples for DNA. Of 1,578 cases and 2,222 controls (response 64.9% and 55.6%, respectively) in OVA, 545 cases and 398 controls were recruited in BC before June 30, 2008 and comprised the discovery stage sample. The study was approved by the Research Ethics Boards of the BC Cancer Agency, the University of British Columbia and the University of Calgary. All subjects gave written informed consent.

Discovery stage pool construction and quality control (QC)

Histologic review of cases at our centre adhered to contemporary diagnostic criteria (Gilks et al. 2008). Tumour histology codes were abstracted from pathology reports and four case pools were constructed. These pools contained DNA from: 1) 24 LMP and 60 invasive mucinous cases combined, 2) 72 invasive endometrioid and 42 invasive clear cell cases combined, 3) 75 LMP serous cases, and 4) 272 invasive serous cases (Table 1). We based our decision to combine LMP and invasive mucinous cases on the recent evidence that mucinous tumours develop along a continuum from benign to borderline to invasive (Kurman and Ronnett 2010), and therefore reflect the same disease (i.e. pooling these samples should not introduce genetic heterogeneity). Although the morphologies of the endometrioid and clear cell subtypes differ (Tavassoulou and Devilee 2003), both are associated with endometriosis (Pearce et al. 2012) and share similar somatic alterations, including mutations in *ARID1A* (Jones et al. 2010; Wiegand et al. 2010), *KRAS* (Kuo et al. 2009; Stewart et al. 2012), *PTEN* (Catusus et al. 2004; Obata et al. 1998), and defects in mismatch repair genes (Albarracin et al. 2004; Cai et al. 2004). To explore possible shared heritable risk factors, these two subtypes were combined and analyzed as a single pool. LMP and invasive serous samples were analyzed as separate pools, consistent with these being distinct molecular subtypes (Tothill et al. 2008). Controls in OVA were frequency-matched by age to all cases used in pools (by 10 year age bins), and one control pool was constructed using 398 samples (Table 1). No exclusions were made for family history of cancer or *BRCA1* and *BRCA2* status (unknown), and only women of self-reported European descent were included.

DNA was extracted from peripheral venous blood (90% of subjects) using a modified salting out protocol (Sambrook J 2000), and from saliva (10% of subjects) using OraGene kits (DNA Genotek, PA, USA). Genotyping call rates were compared previously between OVA blood (99.7%) and saliva (98.1%) (unpublished). DNA samples were adjusted to between 50-100ng/uL and then precisely quantified in duplicate by fluorometry using PicoGreen™ (Molecular Probes, Eugene, OR, USA). For each EOC subtype, individual samples of 2-4uL were manually pipetted into a single pool of 200ng of DNA. Pools were assayed on Human660W-Quad v1 (660-Quad) genotyping beadchips (Illumina, San Diego, CA, USA), and imaged at The Centre for Applied Genomics (Toronto, ON, Canada). The red and green channel intensities for each 660-Quad array were extracted and used to estimate the relative allele frequency (RAF) of SNPs for each DNA pool as red intensity/(red intensity + green intensity), following a previously described approach (Pearson et al. 2007). Each DNA pool

was assayed using 12 replicate 660-Quad beadchips. Replicate arrays were used to reduce the error in RAF estimation (Earp et al. 2011). Details of the 660-Quad array QC are described in the online Supplementary Methods.

Discovery stage association analysis

The RAF of SNPs for each EOC DNA pool was compared with the RAF of SNPs for the control DNA pool, and allele-based tests were used to evaluate SNP associations according to subtypes using the SingleMarker test, implemented in the program GENEPOOL (Homer et al. 2008; Pearson et al. 2007). The SingleMarker test is a modified two-tailed Student's *t*-test that divides the difference in RAF of SNPs between cases and controls by the variance components specific to pooling (for example, variance due to pool construction, and variance due to SNP arrays) (Earp et al. 2011; Homer et al. 2008; Pearson et al. 2007).

SNP selection for replication stage

One hundred and ninety-eight SNPs from the pool-based GWAS were individually genotyped in samples from OCAC using a custom Illumina Infinium iSelect array as part of the Collaborative Oncological Gene-environment Study (COGS) (Pharoah et al. 2013). These 198 SNPs were selected roughly equally from among the mucinous (61 SNPs), endometrioid/clear cell (59 SNPs), and LMP serous (54 SNPs) GWAS results (Table 2). Fewer invasive serous SNPs (24 SNPs) were selected for replication as the previous EOC GWAS had greater power to detect associations for this subtype (Song et al. 2009). During SNP selection, two categories of SNPs were considered: (1) SNPs in high linkage disequilibrium (LD) $r^2 > 0.8$ (based on HapMap European ancestry data) with at least one other SNP analyzed by the pool-based GWAS (referred to as 'cluster' SNPs), and (2) SNPs from the pool-based GWAS that had no other SNPs in high LD (referred to as 'singleton' SNPs). In the first category, consistency in the strength of P-values between SNPs in high LD was used to prioritize SNPs for selection. If a top-ranked SNP (ranked by smallest P-value) was in high LD with a SNP(s) that failed to also rank highly, then the top-ranked SNP was not chosen for replication. We defined a top-ranked SNP as one within the top ~0.5% ($n=3,300$) of GWAS P-values. We reasoned that multiple highly-ranked SNPs in LD with each other were more likely to reflect true positive association results, and SNPs in high LD should yield very similar results assuming experimental error is not an issue. In the second category, singleton SNPs were chosen based on rank alone (by smallest SingleMarker P-value). We selected 30 clusters (48 SNPs) and 13 singleton SNPs to tag a total of 43 loci for replication in mucinous EOC, 30 clusters (46 SNPs) and 13 singleton SNPs tagged 42 loci for endometrioid/clear cell EOC, 30 clusters (41 SNPs) and 13 singleton SNPs tagged 43 loci for LMP serous EOC, and 15 clusters (24 SNPs) and 0 singletons tagged 15 loci for invasive serous EOC, for a total of 159 cluster SNPs and 39 singleton SNPs chosen for replication (Table 2). Some clusters were composed of 2 - 5 SNPs depending on the number of highly-ranked SNPs in the region, and the potential for biological relevance given proximity to a gene. Genome-wide significance (or other P-value thresholds) were not used as criteria in choosing SNPs for replication because pool-based GWAS often do not achieve these stringent thresholds; but, this strategy has been successful in selecting SNPs that attain stringent P-value thresholds in the replication stage (Pearson et al. 2007; Schrauwen et al. 2009; Skibola et al. 2009).

Replication stage study populations

Forty-three studies participating in OCAC contributed samples and data to the COGS effort. The OCAC studies have been described previously (Pharoah et al. 2013). All studies had data on disease status, age at diagnosis or interview, and histological subtype. Most studies frequency-matched controls to cases on age group and race. Nine studies were case-only and were combined with case-control studies from the same geographical regions. Two Australian studies were also combined, creating 34 case-control sets.

Replication stage genotyping and QC

The COGS genotyping and QC process has been described (Pharoah et al. 2013). Briefly, OCAC samples were genotyped at two centers: McGill University and Genome Quebec Innovation Centre (Montreal, PQ) and the Mayo Clinic Medical Genome Facility (Rochester, MN) and genotype calling and QC were performed centrally at the University of Cambridge (Cambridge, UK). Of 47,630 OCAC samples genotyped, 44,308 passed QC. Concordance was > 99.6% among duplicates. Samples were excluded as follows: 1) a call rate of < 95%; 2) heterozygosity >5 standard deviations from the ancestry-specific mean; 3) ambiguous sex; 4) lowest call rate from a first-degree relative pair; 5) duplicate samples that were non-concordant for genotype or genotypic duplicates not concordant for phenotype. Of the 198 SNPs chosen by the current investigation, 188 (94.9%) passed QC. SNPs were excluded if: (1) the call rate was < 95% with MAF > 5% or < 99% with MAF < 5%; (2) they were monomorphic; (3) P-values of HWE in controls were < 10⁻⁷; (4) there was > 2% discordance in duplicate pairs; or (5) no genotypes were called.

As an additional QC check, SNP-EOC associations detected in the pool-based GWAS data were compared with SNP-EOC associations evaluated using genotyped data for 915 of the 943 discovery samples (97%) with sufficient DNA for genotyping. These samples were genotyped on the custom Illumina Infinium iSelect array as part of the COGS initiative, and evaluated with an allelic χ^2 test (PLINK v1.07), the test most comparable to the SingleMarker test.

Replication stage association analysis

Analyses were further restricted to 36,352 eligible subjects (13,188 cases and 23,164 controls) of European descent. For each EOC subtype, SNP associations were estimated using unconditional logistic regression treating the number of minor alleles as an ordinal variable (log-additive model), and adjusting for population substructure by including the first 5 eigenvalues from principal components analysis (see (Pharoah et al. 2013)). Minor allele frequency for each SNP was calculated using genotypes for control subjects of European descent in OCAC. Separate analyses were carried out for each study within EOC subtype, and odds ratios (ORs) and 95% confidence intervals (CIs) were then combined across studies using fixed-effects meta-analysis. Analyses were performed including and excluding the OVA study (i.e. the source of the discovery stage samples). The I^2 test of heterogeneity was estimated to quantify the proportion of total variation due to heterogeneity across studies, and the heterogeneity of ORs between studies was tested with Cochran's Q statistic. The R statistical package *rmeta* was used to generate forest plots. Statistical analysis was conducted in PLINK (v1.07) (Purcell et al. 2007). Adjustment for

multiple testing was performed using a Bonferroni correction of the Type I error. Because unique SNPs were selected for each EOC subtype, we treated each set of SNPs independently, and treated correlated (cluster) SNPs as one independent test (Table 2).

Results

Descriptive characteristics of the discovery stage cases and controls are shown in Table 1 and of the pool-based GWAS SNP selection in Table 2. Pool-based allelotype associations were highly concordant with their values determined from individual genotypes (online Supplementary Table 1). Of the 188 SNPs successfully genotyped, 89% were significant at $P < 0.05$. This level of concordance is consistent with previous pool-based GWAS (Skibola et al. 2009). Associations between SNPs and each EOC subtype are reported in Table 3 and in the Figures. There was no heterogeneity in ORs between studies for any of the associations.

Mucinous subtype

Fifty-six SNPs representing 43 loci were tested for association with 1,483 LMP and invasive mucinous EOC cases and 21,530 controls. Five SNPs were associated with mucinous EOC risk at $P < 0.05$ with an OR that agreed in direction of effect with the OVA discovery samples (Table 3). Replication results including the OVA study are also presented in Table 3 with the corresponding Forest plots shown in Figure 1. SNP rs11108890 on chromosome 12 had the lowest P-value and was associated with increasing mucinous EOC risk (OR= 1.33, 95% CI: 1.11–1.61, $P = 0.0026$). With the exception of SNPs rs970651 and rs7981902 on chromosome 13 ($r^2 = 0.76$), which tagged a cluster of 8 highly ranked SNPs, these 5 SNPs are not in LD with other SNPs chosen for replication by the pool-based GWAS. These SNPs were statistically significant at the Bonferroni adjusted critical value of 0.0012. In exploratory analyses, we examined the associations of these 5 SNPs in the other EOC subtypes (see online Supplementary Table 2). rs11108890 was associated with endometrioid (but not clear cell) EOC risk (OR=1.23, 95% CI: 1.02-1.49, $P = 0.03$). No other associations at $P < 0.05$ were evident.

Endometrioid/Clear Cell subtype

Fifty-five SNPs representing 42 loci were tested for association with 2,903 invasive endometrioid/clear cell EOC cases (these subtypes were combined to be consistent with the DNA pool constructed in the discovery stage) and 21,528 controls. Three SNPs (rs2190503, rs6593140, rs2329554) were associated with endometrioid/clear cell EOC risk at $P < 0.05$ with an OR that agreed in direction of effect with the OVA discovery samples (Table 3). rs2190503 and rs6593140 are in complete LD, and rs2329554 is in moderate LD with them ($r^2 = 0.6$). These SNPs were selected to tag a cluster of 13 highly-ranked SNPs on chromosome 7. SNP rs2190503 had the lowest P-value ($P = 0.014$) and was associated with increased risk (OR=1.11, 95% CI: 1.02-1.21); but, these SNPs were not significant at the Bonferroni adjusted critical value of 0.0012. In exploratory analyses, we examined the associations of these 3 SNPs in the other EOC subtypes and found them to also be associated with all subtype and invasive serous EOC risk (see online Supplementary Table 2). When endometrioid and clear cell EOC were stratified, effect estimates were consistent with those reported in the endometrioid/clear cell combined analysis for all three SNPs,

though the associations were marginally or not statistically significant. This result likely relates to power limitations given the small number of endometrioid and clear cell cases.

LMP serous subtype

Fifty-three SNPs representing 43 loci were tested for association with 892 LMP serous EOC and 21,528 controls. SNP rs9609538 was associated with risk at $P < 0.05$ with an OR that agreed in direction of effect with the OVA discovery samples (OR= 0.86, 95% CI: 0.77-0.95, $p=0.0043$; Table 3). In analyses that included the discovery stage samples (2.6% of total samples), the association between rs9609538 and LMP serous EOC was statistically significant at the Bonferroni adjusted critical value of 0.0012 (OR=0.84, 95% CI: 0.76-0.93, $P=0.0007$). rs9609538 was the only SNP chosen to tag a cluster of 4 highly-ranked SNPs on chromosome 22. rs9609538 was not associated with risk in any of the other EOC subtypes (see online Supplementary Table 2).

Invasive serous subtype

Twenty-four SNPs representing 15 loci were tested for association with 6,881 invasive serous EOC and 21,530 controls. None of these SNPs was associated with risk (data not shown).

Discussion

Our objective was to discover risk alleles for the less-common EOC subtypes by performing a pool-based GWAS, followed by replication of associations using genotypes from 13,188 cases and 23,164 controls from OCAC. Nine SNPs tagging 6 loci were found to be associated with risk at $P < 0.05$ with ORs that agreed in direction of effect with the discovery stage samples. Only one of these, rs9609538, remained statistically significant with LMP serous EOC following correction for multiple testing in analyses that included the 75 discovery samples.

SNP rs9609538 was associated with decreased risk for LMP serous EOC and, in exploratory analyses, was not associated with any other subtype. This SNP lies on chromosome 22 within a 1Mb region of 11 genes (*YWHAH*, *LOC402057*, *SLC5A1*, *LOC150297*, *RFPL2*, *SLC5A4*, *RFPL3*, *C22orf28*, *BPIL2*, *FBXO7*, and *SYN3*). The minor allele of rs9609538 is predicted to alter transcription factor (TF) binding site activity (multiple TFs including AIRE, AP-4, and CDP CR3) and miRNA binding site activity (hsa-miR-516a-5p and hsa-miR-548d-3p) based on FuncPred algorithms (Xu and Taylor 2009). SNP rs9609538 is positioned between *C22orf28* (~500 bp upstream) and *BPIL2* (5bp downstream). *BPIL2* is reported to be a rarely expressed lipid transfer/lipoplysaccharide binding protein, involved in recognizing the outer membrane of Gram-negative bacteria (Mulero et al. 2002). It was reported to be abnormally highly-expressed in the inflamed skin of psoriasis patients, and implicated in the inflammation and/or immune response (Mulero et al. 2002). The relevance of inflammation processes to risk of LMP tumours was also recently suggested by the association of *TNFSF10* (or *TRAIL*) with this EOC subtype (Charbonneau et al., *in submission*). *C22orf28* encodes a tRNA-splicing ligase protein. Although *BPIL2* seems a plausible candidate gene, fine mapping of the association in a larger sample following by

functional assays are needed to determine the gene targeted by this association, followed by further work to determine how it exerts its effects.

Although the other 8 SNPs were not significantly associated with subtype-specific EOC risk following adjustments for multiple testing, several loci tagged by these SNPs are in or near genes that have a plausible biological rationale for influencing ovarian cancer pathogenesis. These include rs17106154, which lies within a ~150kb LD region of *ZFP36L1* (also known as *BRF1*, *TIS11B*, and *Berg36*). *ZFP36L1* is highly expressed in the ovary (Hacker et al. 2010) and was identified as a VEGF mRNA-destabilizing protein (Planel et al. 2010). That *ZFP36L1* is altered in 7% of adenoid cystic carcinomas (Ho et al. 2013) and only 1% of invasive serous EOCs in The Cancer Genome Atlas is consistent with our finding that the SNP is associated with mucinous (a cystic tumor) but not invasive serous EOC. Three non-coding SNPs (rs2190503, rs6593140, rs2329554) tagging one locus upstream/intronic to *GRB10* were associated with risk of endometrioid/clear cell EOC. *GRB10* functions in the feedback inhibition of the PI3K/AKT and RAS/MAPK pathways (Hsu et al. 2011; Yu et al. 2011), and genes in these pathway are frequently mutated in endometrioid and clear cell tumours, and occasionally in serous tumours (Gilks 2010). *GRB10* may be a tumour suppressor gene that acts in parallel with *PTEN* to ensure proper levels of activation of the PI3K/AKT pathway (Hsu et al. 2011; Yu et al. 2011). No SNPs were found to be associated with invasive serous risk in the replication samples. However, a previously reported GWAS SNP for invasive serous EOC with a moderately large OR (rs10088218, OR=0.76) ranked highly in our pool-based data (ranked 3389 and in perfect LD with SNPs that ranked 295 and 445), but did not meet our stringent criteria for selection in replication.

There are several limitations to our study. First, the discovery stage sample size was small, reflecting the low incidence of the less-common EOC subtypes. We, therefore, combined endometrioid and clear cell samples for analysis and primarily investigated associations shared between these subtypes. Thus, associations unique to one subtype could not be evaluated. Second, despite rapid progress in recent years, robust histological subtyping remains a challenge for studies of EOC (Gilks et al. 2008; Gilks and Prat 2009; Han et al. 2008; Kobel et al. 2010; Kobel et al. 2009). For example, many tumours that have previously been designated high-grade endometrioid are likely to be high-grade serous EOC (Kobel et al. 2009), and metastatic carcinoma from other organ sites is still difficult to correctly identify from primary mucinous EOC (Kelemen and Kobel 2011). Samples used in the pool-based stage of this study were reviewed using contemporary diagnostic criteria (Gilks et al. 2008); however, many of the OCAC replication studies including samples in our previous GWAS (Song et al. 2009) were not centrally-reviewed, and subtype misclassification may have introduced genetic heterogeneity and reduced statistical power. Third, the number of SNPs (maximum 200) chosen for replication was low. This was a factor restricted by cost and assay design across OCAC investigators and the three other consortia participating in the COGS initiative. The low coverage of genotyped SNPs per locus was also insufficient to allow imputation. Thus, additional genotyping of loci of interest will be needed to narrow down the regions of association.

This study also has several strengths. The DNA pooling design approach has successfully been applied in the GWAS context, including cancer GWAS (Brown et al. 2008; Skibola et

al. 2009). The lack of identified risk alleles for EOC subtypes other than invasive serous prompted the current study, and we report a promising candidate for further interrogation for LMP serous EOC. Finally, the large number of EOC samples in OCAC - the largest assembled to date – and specifically of the less-common EOC subtypes, together with the coordinated genotyping and QC success rates achieved for over 200,000 samples and SNPs across 4 consortia, is a major strength of this study.

In conclusion, our pool-based GWAS of EOC risk according to subtype identified 9 SNPs tagging 6 loci with suggestive associations in the mucinous (5 SNPs), endometrioid/clear cell (3 SNPs), and LMP serous (1 SNP) subtypes. Several tagged loci harbor genes that have a plausible biological rationale for conferring EOC risk. Further evaluation in additional samples will be important to verify these results for the less-common EOC subtypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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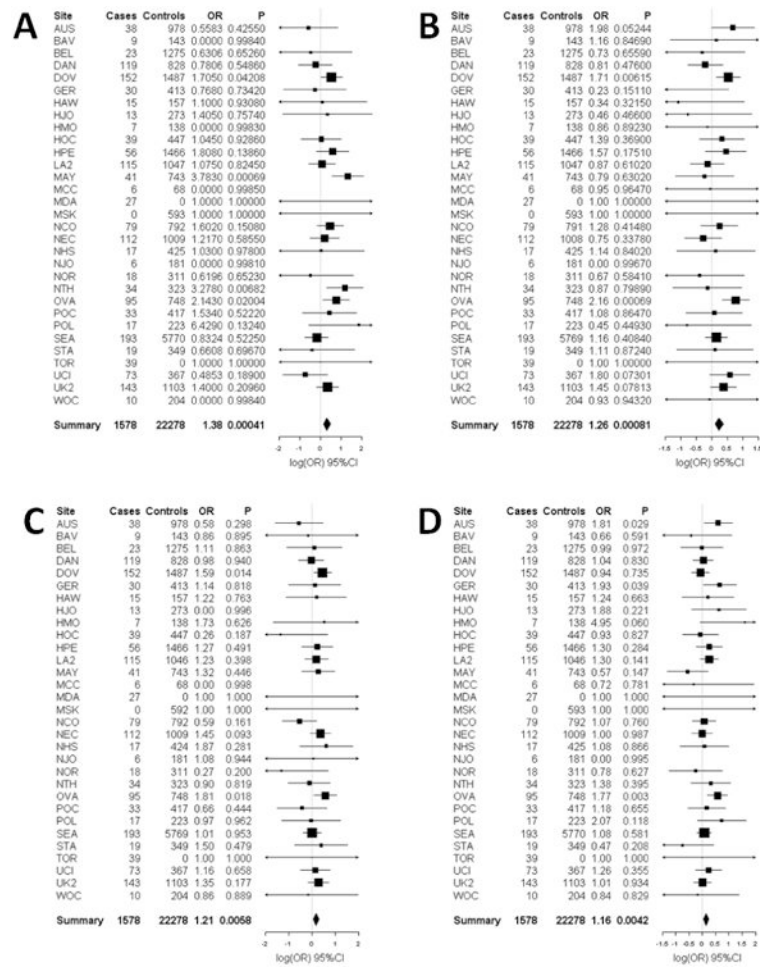
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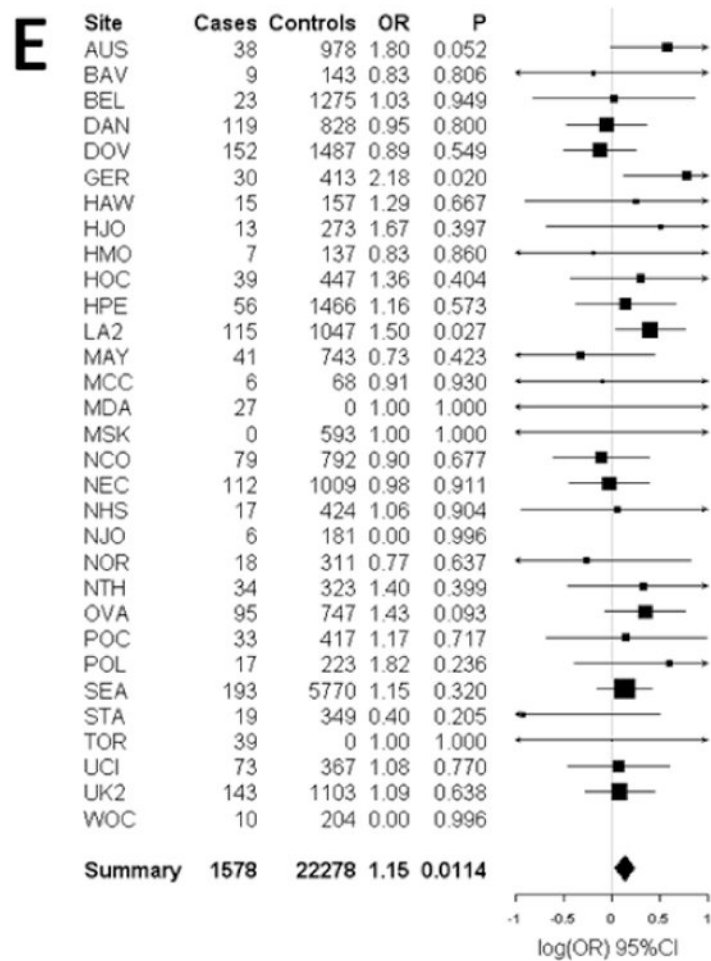


Figure 1.

Forest plots of the study specific and summary odds ratios and 95% confidence intervals for the association between mucinous ovarian cancer risk and five SNPs nominated by our pool-based GWAS: (A) rs11108890 (B) rs933518 (C) rs17106154 (D) rs970651, and (E) rs7981902. Study-specific odds ratios, 95% confidence intervals, and P-values are based on logistic regression assuming an additive genetic mode, adjusting for the first 5 eigenvalues from principal components analysis. Summary odds ratios and 95% confidence intervals are from fixed effects meta-analysis and include the OVA study site. Forest plots were generated using the *rmeta* library implemented in the R project; association analyses were performed using PLINK

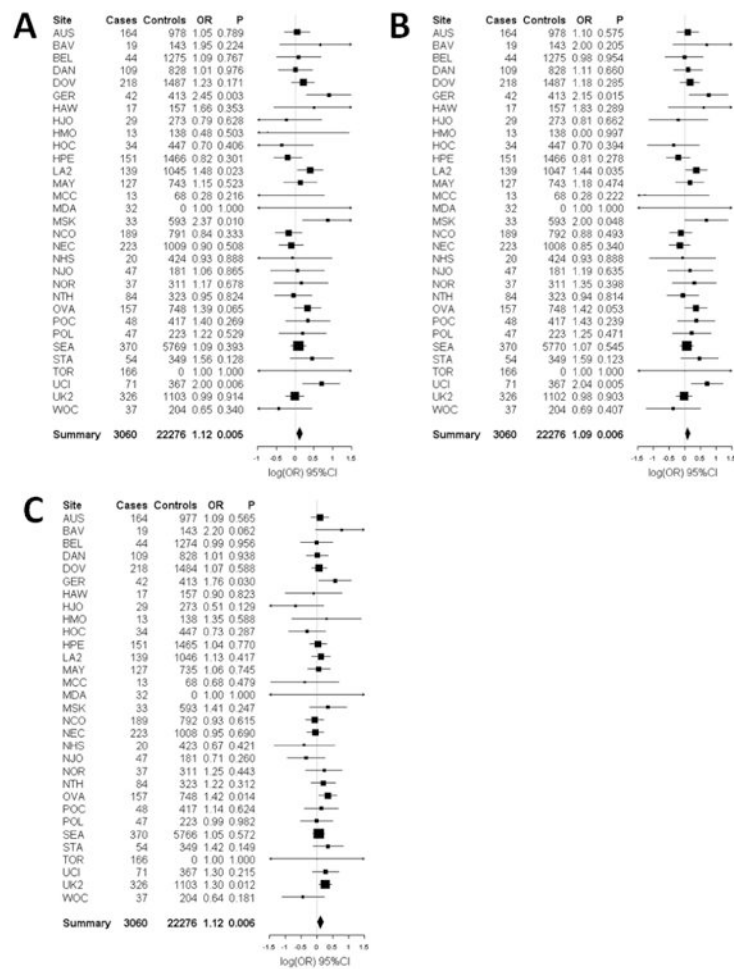
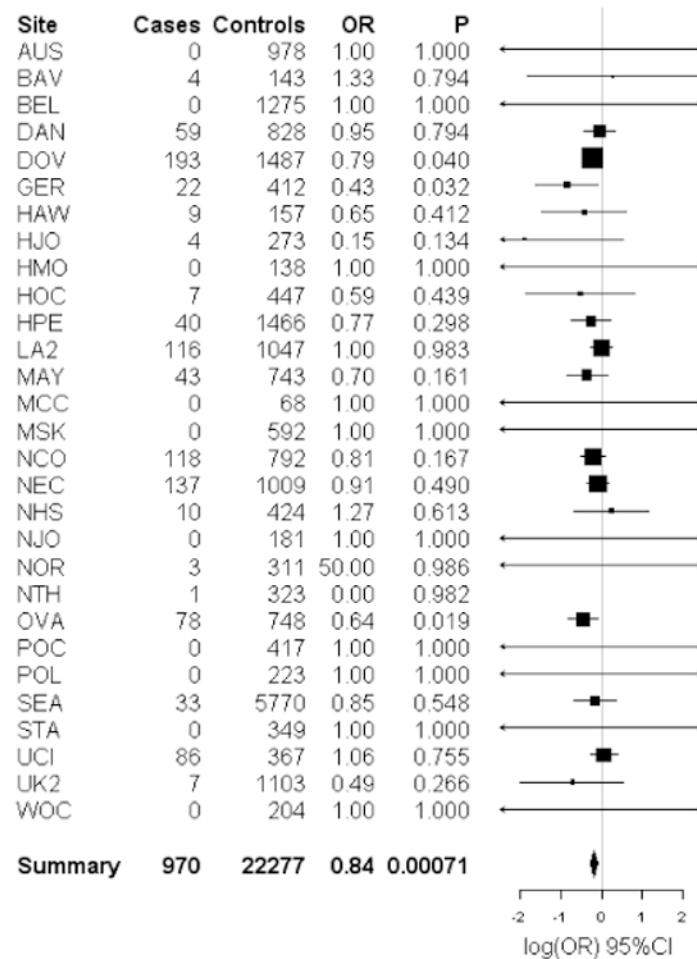


Figure 2. Forest plots of the study specific and summary odds ratios and 95% confidence intervals for the association between endometrioid/clear cell ovarian cancer risk and three SNPs nominated by our pool-based GWAS: (A) rs2190503 (B) rs6593140 and (C) rs2329554. Study-specific odds ratios, 95% confidence intervals, and P-values are based on logistic regression assuming an additive genetic mode, adjusting for the first 5 eigenvalues from principal components analysis. Summary odds ratios and 95% confidence intervals are from fixed effects meta-analysis and include the OVA study site. Forest plots were generated using the *rmeta* library implemented in the R project; association analyses were performed using PLINK

**Figure 3.**

Forest plot of the study specific and summary odds ratios and 95% confidence intervals for the association between low malignant potential serous ovarian cancer risk and one SNP nominated by our pool-based GWAS, rs9609538. Study-specific odds ratios, 95% confidence intervals, and P-values are based on logistic regression assuming an additive genetic model, adjusting for the first 5 eigenvalues from principal components analysis. Summary odds ratios and 95% confidence intervals are from fixed effects meta-analysis and include the OVA study site. Forest plots were generated using the *rmeta* library implemented in the R project; association analyses were performed using PLINK. The MDA and TOR study sites are not plotted; neither study had any LMP serous ovarian cancer cases or controls

Table 1

Characteristics of samples in the discovery stage case-control DNA pools.

DNA pool	ICD-O-3 code ^a	Pool Size, N	Age ^b (standard deviation)	Minimum Age	Maximum Age
Mucinous	84721, 84803, 84703, 90151	84	53 ± 13.2	23	78
Endometrioid/Clear cell	83803, 83103	114	56 ± 10.5	25	79
LMP Serous	84421, 84621	75	53 ± 12.8	21	80
Invasive Serous	84603, 84413, 84613	272	62 ± 10.1	36	80
Control pool		398	57 ± 10.5	31	80

^aInternational Classification of Diseases for Oncology, 3rd Edition

^bMedian age at diagnosis or control selection, years

Table 2
Number of pool-based GWAS SNPs selected for replication by EOC subtype

Subtype	SNP selection method ^a	# SNPs chosen	# SNPs successfully genotyped	# Independent tests of association
Mucinous	Cluster	48	43	43
	Singleton	13	13	
Endometrioid/Clear cell	Cluster	46	43	42
	Singleton	13	12	
LMP Serous	Cluster	41	40	43
	Singleton	13	13	
Invasive Serous	Cluster	24	24	15
	Singleton	0	0	
Total		198	188	--

^aCluster refers to SNPs chosen based on P-value and their being in linkage disequilibrium (LD) with other GWAS SNPs having a small P-value. Singleton refers to SNPs not in LD with other GWAS SNPs, and chosen based on P-value alone. See Methods for details.

Table 3
Associations between SNPs and subtype-specific ovarian cancer risk in discovery and replication stages

Subtype(s)	SNP (Maj>Min)	Location	MAF	Sample description	# Cases	# Controls	OR (95% CI) ^d	P	P _{het}	
	rs11108890 C>A	Chr12:96137530	0.04	Discovery	78	392	3.05 (1.32-7.06)	0.0091		
				Replication	1483	21530	1.33 (1.11-1.61)	0.0026	0.42	
					Rep+OVA	1578	22278	1.38 (1.16-1.66)	0.0004	0.38
					Discovery	78	392	2.29 (1.32-4.00)	0.0034	
		rs933518 G>A	Chr16: 53079622	0.08	Replication	1483	21530	1.20 (1.04-1.38)	0.0141	0.81
					Rep+OVA	1578	22278	1.26 (1.11-1.45)	0.0008	0.41
					Discovery	78	392	1.78 (0.95-3.37)	0.0738	
					Replication	1483	21530	1.17 (1.02-1.35)	0.0287	0.96
Mucinous		rs17106154 A>G	Chr14: 68230927	0.07	Rep+OVA	1578	22278	1.21 (1.06-1.39)	0.0058	0.83
					Discovery	78	392	2.54 (1.54-4.19)	0.0003	
					Replication	1483	21530	1.12 (1.01-1.24)	0.0334	0.81
					Rep+OVA	1578	22278	1.16 (1.05-1.28)	0.0042	0.44
		rs7981902 A>G	Chr13: 47368792	0.13	Discovery	78	392	2.26 (1.32-3.86)	0.0029	
					Replication	1483	21530	1.13 (1.01-1.27)	0.0308	0.94
					Rep+OVA	1578	22278	1.15 (1.03-1.28)	0.0114	0.84
					Discovery	114	392	1.85 (1.21-2.85)	0.0049	
Endometrioid/Clear Cell		rs2190503 G>A	Chr7: 50710111	0.13	Replication	2903	21528	1.11 (1.02-1.21)	0.0137	0.12
					Rep+OVA	3060	22276	1.12 (1.04-1.22)	0.0050	0.11
					Discovery	114	392	2.03 (1.30-3.17)	0.0018	
					Replication	2903	21528	1.11 (1.02-1.21)	0.0195	0.28
		rs6593140 A>G	Chr7: 50765627	0.12	Rep+OVA	3060	22276	1.09 (1.03-1.17)	0.0060	0.46
					Discovery	114	392	1.91 (1.33-2.74)	0.0004	
					Replication	2903	21528	1.08 (1.01-1.16)	0.0195	0.59
					Rep+OVA	3060	22276	1.12 (1.03-1.22)	0.0060	0.25
					Discovery	114	392	1.91 (1.33-2.74)	0.0004	
					Replication	2903	21528	1.08 (1.01-1.16)	0.0195	0.59
				Rep+OVA	3060	22276	1.12 (1.03-1.22)	0.0060	0.25	

Subtype(s)	SNP (Maj>Min)	Location	MAF	Sample description	# Cases	# Controls	OR (95% CI) ^a	P	P _{net}
LMP Serous	rs9609538 A>G	Chr22: 31139832	0.24	Discovery	68	392	0.48 (0.30-0.77)	0.0023	
				Replication	892	21529	0.86 (0.77-0.95)	0.0043	1.00
				Rep+OVA	970	22277	0.84 (0.76-0.93)	0.0007	0.99

Abbreviations used: SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval. Within subtype, table is ordered by replication sample P-value. SNP location given for human genome build NCBI36.3. MAF is based on all European controls in OCAC and genotyped as part of COGS. Discovery samples are OVA samples used in the pool-based GWAS and subsequently genotyped by COGS (28 samples with insufficient DNA were not genotyped; hence, the number of cases and controls in pools may be smaller than in Table 1). Replication samples are from OCAC studies participating in COGS, excluding all OVA samples. Rep+OVA includes OCAC studies participating in COGS and all OVA samples.

^aDiscovery sample OR and P-value are from logistic regression assuming an additive genetic model, implemented in PLINK (v1.07). Replication sample OR and P-value are from fixed effects meta-analysis carried out using the *rmeta* library implemented in the R project, excluding the OVA site. Rep+OVA sample OR and P-value are from fixed effects meta-analysis carried out using the *rmeta* library implemented in the R project, including the OVA site. P_{het} is the P-value for Cochran's-Q measure of between study heterogeneity, generated in *rmeta*. All values are based on individual genotyping data.