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Identification of novel epithelial ovarian cancer loci in women of African ancestry

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Key words: ovarian cancer, African ancestry, genome wide association study, gene expression, eQTLs

Abbreviations: AABC: African American Breast Cancer Consortium; AAPC: African American Prostate Cancer Consortium; BFDPs: Bayesian false-discovery probabilities; BWHS: Black Women's Health Study; CI: confidence interval; COGS: Collaborative Oncological Gene-Environment Study; EAF: effect allele frequency; effHC: effective heterozygosity count; EOC: epithelial ovarian cancer; eQTL: expression quantitative trait locus; FFPE: formalin-fixed paraffin-embedded; *FST*: follistatin; GAME-ON: The Genetic Associations and Mechanisms in Oncology network; GWAS: genome-wide association studies; HC: heterozygosity count; HGSOC: high-grade serous ovarian carcinomas; MAGEs: melanoma-associated antigen; NCI: National Cancer Institute; NCOCS: North Carolina Ovarian Cancer Study; ncRNA: noncoding RNA; OCAC: Ovarian Cancer Association Consortium; PCOS: polycystic ovary syndrome; QC: quality control; SCCS: Southern Community Cohort Study; SNP: single nucleotide polymorphism; WHI: Women's Health Initiative

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Women of African ancestry have lower incidence of epithelial ovarian cancer (EOC) yet worse survival compared to women of European ancestry. We conducted a genome-wide association study in African ancestry women with 755 EOC cases, including 537 high-grade serous ovarian carcinomas (HGSOC) and 1,235 controls. We identified four novel loci with suggestive evidence of association with EOC ($p < 1 \times 10^{-6}$), including rs4525119 (intronic to *AKR1C3*), rs7643459 (intronic to *LOC101927394*), rs4286604 (12 kb 3′ of *UGT2A2*) and rs142091544 (5 kb 5′ of *WWC1*). For HGSOC, we identified six loci with suggestive evidence of association including rs37792 (132 kb 5′ of follistatin [*FST*]), rs57403204 (81 kb 3′ of *MAGEC1*), rs79079890 (*LOC105376360* intronic), rs66459581 (5 kb 5′ of *PRPSAP1*), rs116046250 (*GABRG3* intronic) and rs192876988 (32 kb 3′ of *GK2*). Among the identified variants, two are near genes known to regulate hormones and diseases of the ovary (*AKR1C3* and *FST*), and two are linked to cancer (*AKR1C3* and *MAGEC1*). In follow-up studies of the 10 identified variants, the *GK2* region SNP, rs192876988, showed an inverse association with EOC in European ancestry women ($p = 0.002$), increased risk of ER positive breast cancer in African ancestry women ($p = 0.027$) and decreased expression of *GK2* in HGSOC tissue from African ancestry women ($p = 0.004$). A European ancestry-derived polygenic risk score showed positive associations with EOC and HGSOC in women of African ancestry suggesting shared genetic architecture. Our investigation presents evidence of variants for EOC shared among European and African ancestry women and identifies novel EOC risk loci in women of African ancestry.

What's new?

Women of African ancestry have lower incidence of epithelial ovarian cancer (EOC) yet worse survival compared to women of European ancestry. To date, genome-wide association studies (GWAS) have identified 30 common, low-penetrant EOC susceptibility alleles. However, most studies were restricted to European ancestry women, and it remains to be determined whether there is any concordance among women of African descent. In this first GWAS conducted in women of African ancestry, the authors report ten novel associated SNPs. The results also suggest there may be some shared genetic architecture between women of European and African ancestry for susceptibility to ovarian cancer.

Introduction

Epithelial ovarian cancer (EOC) is a rare but deadly disease that has a slightly higher incidence in women of European ancestry compared to the women of African ancestry.[1] However, in the United States, the 5-year relative survival is much worse for African American women at 35% compared to 47% for European ancestry women.[1] To date, genome-wide association studies (GWAS) have identified 30 common, low penetrant EOC susceptibility alleles,[2] but due to small sample sizes of other ethnic groups, most published GWAS studies of EOC have been restricted to European ancestry women. There have been no GWAS in women of African ancestry. Although there are 30 confirmed GWAS single nucleotide polymorphisms (SNPs) that have been reported in European ancestry women, it is unknown whether there is any concordance among women of African descent.

The Genetic Associations and Mechanisms in Oncology (GAME-ON) network designed a custom Illumina array, the OncoArray, in order to replicate previous GWAS findings and identify new cancer susceptibility loci.[3] The OncoArray

includes ~533,000 variants (of which 260,660 formed a GWAS backbone) and was used for coordinated genotyping of over 400,000 cancer cases and controls, including EOC case–control studies of the Ovarian Cancer Association Consortium (OCAC) and the multicenter African American Cancer Epidemiology Study (AACES).[4] The present study conducted a GWAS in 755 EOC cases and 1,235 controls of African ancestry from the OCAC and AACES. To increase the sample size, additional genotype data were combined from the OCAC Collaborative Oncological Gene-Environment Study (COGS) and three EOC GWAS[5] to evaluate the concordance of confirmed GWAS SNPs found in women of European ancestry. We present the results of these association analyses together with expression quantitative trait locus (eQTL) analyses for SNPs reaching a suggestive threshold of $p < 1 \times 10^{-6}$. The functional annotation of the EOC susceptibility loci in women of African Ancestry is described.

Materials and Methods

Study samples

All subjects included in this analysis were of African descent and provided written informed consent as well as data and blood samples under ethics committee-approved protocols.

The GAME-ON OncoArray data set comprised 63 OCAC studies and the AACES.[4] The analyses for our study were restricted to 32 studies that contributed samples from individuals of African descent (Supplementary Table S1).

Genotype data and quality control (QC)

Genotyping was performed at five genotyping centers: University of Cambridge, Center for Inherited Disease Research, National Cancer Institute (NCI), Genome Quebec and Mayo Clinic. OncoArray sample QC for the genotypes received from Cambridge was similar to that carried out for the other projects that used the OncoArray as described in Pharoah *et al.*[3] Samples were excluded if the genotyping call rate was <95%, for high or low heterozygosity, if the individual was not female or had ambiguous sex, or were duplicates. SNP QC was carried out according to the OncoArray QC guidelines.[3] Sample level QC included restriction to female samples, as well as check for call rate >95%, heterozygosity (either too big or too small), removal of ineligible samples and relationship inference to check for unexpected first-degree relatives. SNP level QC included filter on call rate >95% and Hardy–Weinberg Equilibrium p-value $>1 \times 10^{-5}$. After applying these filters for QC, there were 466,142 SNPs remaining for 2,088 samples (832 EOC cases and 1,255 controls).

Genetic ancestry analysis

Intercontinental ancestry was calculated for the OCAC and AACES samples using the software package FastPop[6] (http://sourceforge.net/projects/fastpop/) that was developed specifically for the OncoArray Consortium. Only the African ancestry samples, defined as having >50% African ancestry, were used for the GWAS reported here (755 EOC cases and 1,235 controls). Among the cases, 537 were high-grade serous ovarian carcinoma (HGSOC), 21 low-grade serous, 31 endometrioid, 24 clear cell, 51 mucinous 12 mixed cell, 65 other EOC and 14 with missing histotype. Principal components computed using FastPop[6] were further used to adjust for population structure in our GWAS.

Genome-wide imputation of genotypes

Using the genotyped SNPs that passed QC, haplotypes were phased with SHAPEIT v2[7] followed by imputation to the 1,000 Genomes Phase 3 v5 reference set[8] using Minimac3.[9]

Association analyses in ovarian cancer cases and controls of African descent

Genome-wide association analysis was performed by logistic regression with adjustment for two principal components of ancestry using a score test to account for genotype uncertainty as implemented in SNPTESTv2.5.2.[10] For genotyped SNPs, we included results only for those SNPs with Hardy–Weinberg Equilibrium p-value $>1 \times 10^{-5}$ and heterozygosity count (HC) >30, where HC is defined as $N \times \text{MAF} \times (1\text{-MAF})$ for each SNP, N represents the sample size (either the number of cases or the number of controls), and MAF represents the SNP minor allele frequency. For imputed SNPs, we included those SNPs with imputation R-squared >0.5, and effective heterozygosity count (effHC) >30, where effHC is defined as the imputation R-squared × HC. Note that we applied QC filters separately for cases and controls to select SNPs carried forward for genetic association analysis, such that a minimum HC (or effective HC) of 30 was observed among each of the case and control groups. After applying these filters, there were 12,486,624 and 11,083,029 SNPs remaining in the GWAS of EOC and HGSOC, respectively. We examined quantile–quantile plots for the SNPs remaining after applying filters (Supplementary Fig. S1), and obtained lambdas of 1.01 in both the EOC and HGSOC analyses, indicating that our analyses were free from obvious inflation in the distribution of observed p-values. We calculated Bayesian false-discovery probabilities (BFDPs) for associated SNPs assuming prior probabilities of association 1:1,000 and1:10,000 to facilitate interpretation of the reported SNP associations.[11]

Expression quantitative trait locus (eQTL) analysis for selected GWAS SNPs

We pursued eQTL analysis using gene expression measurements from formalin-fixed paraffin-embedded (FFPE) tissue specimens collected from the facility where the cytoreductive surgery was performed for 260 African ancestry HGSOC cases in the AACES and a case–control study in OCAC, the North Carolina Ovarian Cancer Study (NCOCS). RNA was extracted using the Qiagen AllPrep DNA/RNA FFPE isolation reagents in conjunction with the Qiagen GeneRead kit, and RNA was assayed on Affymetrix Human Transcriptome 2.0 ST GeneChips.

R (version 3.5.2) Bioconductor (version 3.8) was used to quantitate expression levels for targeted genes. We used robust multi-array average from the oligo package (target = "core") to normalize the expression intensities[12] and ComBat (Bioconductor-sva) to remove batch effects.[13] We then mapped probe intensity measurements to gene identifiers[14] before generating box plots of expression distributions by genotype. For each of the 10 SNPs identified in the GWAS of EOC and HGSOC (Table 1), we examined genes and transcripts within the region of identified GWAS SNPs for eQTL evidence using an additive model with adjustment for age and the first two principal components of ancestry. For the selected transcripts, we report all eQTL associations demonstrating nominal statistical significance at $p < 0.05$ for available transcripts falling within the region of identified GWAS SNPs.

Examination of pleiotropy of GWAS SNPs associated with EOC in women of African ancestry with breast and prostate cancer in African ancestry individuals

Because we were unable to identify other GWAS of EOC in women of African ancestry, independent validation of GWAS results was not possible. Therefore, we examined the association of the 10 SNPs identified in the present African ancestry GWAS of EOC or HGSOC at $p < 1 \times 10^{-6}$ (Table 1) with previously completed studies of breast cancer (overall, ER positive and ER negative) and prostate cancer in populations of African descent. Genetic associations in breast cancer were determined from 3,007 cases, of which 987 are ER negative and 1,518 are ER positive, and 2,720 African ancestry controls from the African American Breast Cancer Consortium (AABC), using the Illumina Human 1M-Duo BeadChip.[15] The genotype associations for prostate cancer were from 4,853 cases and 4,678 controls in the African American Prostate Cancer Consortium (AAPC), using the Illumina Infinium 1M-Duo.[16] For the selected SNPs, evidence of association from the studies of breast and prostate cancer is reported at a nominal level ($p < 0.05$) without adjustment for multiple comparisons.

Concordance of associated SNPs across women of African and European ancestry

We examined whether susceptibility genes for EOC previously identified in European ancestry women[2] were associated with EOC among women of African ancestry as well as whether the loci identified among women of African ancestry in this analysis were associated with EOC among European ancestry women.

Fine mapping of gene regions was performed for (*i*) the loci previously identified as significantly associated with EOC in European ancestry women among African ancestry women and (*ii*) the loci identified as significantly associated with EOC in those of African ancestry in the present analysis among European ancestry women. Plots were generated for each region defined by the position of the most strongly associated SNP +/− 400 kb using the LocusZoom software with the hg19/1000 Genomes Nov 2014 AFR (or EUR depending on

Table 1. SNPs demonstrating genome-wide suggestive evidence of association in the African Ancestry OncoArray Analysis and comparison with results of OCAC studies of women of European ancestry

Subtype	Nearest gene	SNP ID (effect/other allele)	Build 37 Chr:Pos	African ancestry[2]				European ancestry[3]		
				EAF	OR (95% CI)	*p* Value	BFDP[4]	EAF	OR (95% CI)	*p* Value
EOC	*AKR1C3*	rs4525119 (T/C)	10:5091954	0.331	0.70 (0.61–0.81)	4.9×10^{-7}	8%	0.300	1.00 (0.97–1.03)	0.936
	LOC101927394	rs7643459[1] (T/G)	3:8004828	0.362	1.40 (1.22–1.60)	8.4×10^{-7}	5%	0.421	1.00 (0.98–1.03)	0.742
	UGT2A2	rs4286604 (A/G)	4:70442165	0.268	0.69 (0.59–0.80)	8.5×10^{-7}	5%	0.227	1.01 (0.98–1.05)	0.421
	WWC1	rs142091544 (T/C)	5:167714000	0.034	3.22 (2.02–5.13)	9.4×10^{-7}	9%	0.010	0.97 (0.83–1.13)	0.665
HGSOC	*FST*	rs37792 (G/A)	5:52644647	0.342	0.65 (0.55–0.76)	6.0×10^{-8}	<1%	0.308	1.03 (0.99–1.06)	0.110
	MAGEC1	rs57403204 (G/A)	X:141078552	0.064	2.62 (1.83–3.76)	1.7×10^{-7}	1%	0.013	1.03 (0.90–1.18)	0.682
	LOC105376360	rs79079890 (G/T)	10:3684148	0.032	3.20 (2.05–4.99)	3.0×10^{-7}	3%	0.131	1.02 (0.98–1.08)	0.534
	PRPSAP1	rs66459581 (A/AC)	17:74355264	0.234	1.63 (1.35–1.97)	5.1×10^{-7}	2%	0.089	0.97 (0.92–1.03)	0.377
	GABRG3	rs116046250 (G/T)	15:27231950	0.046	2.95 (1.92–4.54)	8.7×10^{-7}	7%	–	–	–
	LOC105377300/GK2	rs192876988 (C/T)	4:80297251	0.046	3.01 (1.94–4.68)	9.2×10^{-7}	8%	0.014	0.75 (0.62–0.90)	0.002

We show the strongest associated SNP for each locus reaching $p < 1 \times 10^{-6}$ for each subtype.
[1]Genotyped SNPs, if not indicated otherwise then imputed.
[2]African ancestry was defined as >50% African ancestry as calculated by FastPop.
[3]European ancestry was defined by self-report.
[4]Bayesian false discovery probability (BFDP) assuming a prior of 1:1,000 among women of African ancestry.
Abbreviations: CI, confidence interval; EAF, effect allele frequency; EOC, epithelial ovarian cancer; HGSOC, high-grade serous ovarian carcinoma; OR, odds ratio; SNP, single nucleotide polymorphism.

the ethnic population) as the reference panel for linkage disequilibrium information. Significance for each region of interest was defined by both a Bonferroni threshold (alpha-level of 0.05/number of SNPs tested in that region) and a more conservative, suggestive threshold (alpha-level of 0.05/[number of SNPs tested in that region/3]). To further examine the global genetic architecture in the two populations, we calculated a polygenic risk score using 24 SNPs from published GWAS of ovarian cancer in European ancestry women, excluding SNPs associated only with mucinous tumors.[3,17]

Data availability

The majority of the GWAS data set used during the current study are available at the database of Genotypes and Phenotypes (dbGaP) under accession number phs001882.v1.p1 (OncoArray – FOCI data). Other portions are not publicly available due to privacy or ethical restrictions, but will be made available upon reasonable request.

Results

Genome-wide association of EOC and HGSOC in African ancestry women

Genetic association analyses were performed using genotype data from 755 invasive EOC cases (537 HGSOC) and 1,235 controls of African ancestry from OCAC and AACES. The numbers of participants by study for OCAC are shown in Supplementary Table S1. The Manhattan plots from the GWAS in African ancestry women for both overall EOC and HGSOC are shown in Supplementary Figure S2. We did not observe any genetic markers that were statistically significantly associated with EOC or HGSOC risk at the standard genome-wide significance level of $p < 5 \times 10^{-8}$.

Using a suggestive threshold of $p < 1 \times 10^{-6}$, we identified four distinct loci for association with EOC and six distinct loci for HGSOC (Table 1). The four loci associated with EOC included 10p15.1 (lead SNP rs4525119, intronic to *AKR1C3*, $p = 4.9 \times 10^{-7}$, effect allele frequency [EAF] = 0.33), 3p25.3 (lead SNP rs7643459, intronic to *LOC101927394*, $p = 8.4 \times 10^{-7}$, EAF = 0.36), 4q13.3 (lead SNP rs4286604, 12 kb 3′ of *UGT2A2*, $p = 8.5 \times 10^{-7}$, EAF = 0.27) and 5q34 (lead SNP rs142091544, 5 kb 5′ of *WWC1*, $p = 9.4 \times 10^{-7}$, EAF = 0.03). Of these four loci, none reached the threshold of $p < 1 \times 10^{-6}$ for HGSOC, although a p-value of 1.4×10^{-6}, just below this threshold, was found for rs764359 (odds ratio [OR] = 1.45; 95% confidence interval [CI] = 1.25–1.68). The six loci associated with HGSOC included 5q11.2 (lead SNP rs37792, 132 kb 5′ of *FST* [follistatin], $p = 6.0 \times 10^{-8}$, EAF = 0.34), Xq27.2 (lead SNP rs57403204, 81 kb 3′ of *MAGEC1*, $p = 1.7 \times 10^{-7}$, EAF = 0.06), 10p15.1 (lead SNP rs79079890, *LOC105376360* intronic, $p = 3.0 \times 10^{-7}$, EAF = 0.03), 17p25.1 (lead SNP rs66459581, 5 kb 5′ of *PRPSAP1*, $p = 5.1 \times 10^{-7}$, EAF = 0.23), 15p12 (lead SNP rs116046250, *GABRG3* intronic, $p = 8.7 \times 10^{-7}$, EAF = 0.05) and 4q21.21 (lead SNP rs192876988, 32 kb 3′ of *GK2*, $p = 9.2 \times 10^{-7}$, EAF = 0.05). The regional association plots for these 10 SNPs are shown in Supplementary Figures S3 (EOC) and S4 (HGSOC). For the four loci associated with EOC overall, the BFDP ranged from 5% to 8% assuming a prior of 1:1,000 (Table 1) For the six loci associated with HGSOC, the BFDP ranged from <1% to 8% assuming a prior of 1:1,000 (Table 1). Assuming a prior probability of 1:10,000, we identified one locus for HGSOC with a BFDP < 5% (*FST* rs37792, BFDP = 4%; Supplementary Table S2).

Expression quantitative trait locus (eQTL) analysis for GWAS SNPs

Results of eQTL analyses on 260 HGSOC tissue samples from women of African ancestry for each of the 10 EOC- and HGSOC-associated regions of interest are in Figure 1. We identified the set of genes lying within a ±100 kb region of the most strongly associated SNP for each locus to pursue for the eQTL analysis. For one SNP, rs37792, there were no genes or transcripts identified within a ±100 kb region, so we expanded consideration to a ±500 kb region that included *FST* and three other genes (Supplementary Table S3). Among the gene and transcript targets selected for follow-up, expression data were

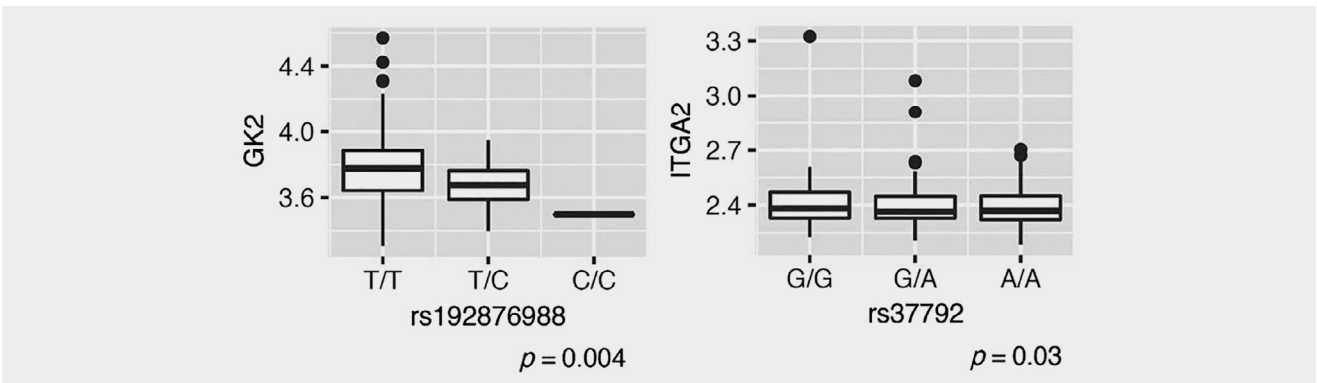

Figure 1. Leading eQTL analysis results in 260 ovarian tissues from AACES and NCOCS participants for SNPs in GK2 and ITGA2. These boxplots represent the distribution of measured expression *vs*. genotype (rounded to the nearest whole number for imputed dosage variables). *p*-Values are reported from additive models with covariate adjustment for age and two principal components of ancestry.

available for 21 genes and transcripts falling within the regions of seven GWAS SNPs. We note that we did not have expression data available for the noncoding transcripts identified within the regions of two SNPs (rs7643459 and rs79079890), so these SNPs and transcripts could not be carried forward for eQTL analysis. Among the SNPs and transcripts examined in eQTL analyses, we identified a significant association for rs192876988, where carriers of allele C showed decreased expression of *GK2* ($p = 0.004$, Fig. 1 and Supplementary Fig. S5). We also identified a nominally significant association for rs37792 ($p = 0.03$).

Breast and prostate cancer associations for selected SNPs identified in the GWAS of EOC and HGSOC

As evidence for pleiotropy has been observed in Europeans,[2] we evaluated pleiotropy with ovarian cancer-associated SNPs among African Americans diagnosed with breast and prostate cancer in the AABC and AAPC, respectively. For selected SNPs from the GWAS of EOC and HGSOC in African ancestry women (Table 1), we examined evidence of association with breast and prostate cancer in individuals of African ancestry. The EOC-associated *LOC101927394* region SNP rs7643459 allele T demonstrated nominal evidence of association with increased risk of ER negative breast cancer ($p = 0.029$) with an OR of 1.13 (95% CI = 1.01, 1.26) (Supplementary Table S4) showing consistent direction with that reported for EOC. The same SNP rs7643459 allele T also showed nominal association with prostate cancer in African Americans ($p = 0.034$; Supplementary Table S5). Within the region of *UGT2A2*, SNP rs4286604 allele A was associated with increased risk of prostate cancer ($p = 0.025$). We note that the A allele for this SNP was identified as having a protective association for EOC (Table 1), indicating a discordant direction of association comparing the relationship with EOC *vs*. prostate cancer. SNP rs142091544 allele T within the *WWC1* region, associated with EOC, demonstrated evidence of association with ER negative breast cancer (OR = 1.55, 95% CI = 1.19, 2.02; $p = 0.001$) indicating a consistent direction compared to the association with EOC. The *LOC105377300*/*GK2* region SNP rs192876988 allele C demonstrated nominal association with increased risk of ER positive breast cancer (OR = 1.32, 95% CI = 1.03, 1.69; $p = 0.027$; Supplementary Table S4), showing a consistent direction of effect with that reported for HGSOC (Table 1).

Concordance of associated SNPs across women of African and European ancestry

One of the 10 SNPs (*LOC105377300*/*GK2* region SNP rs192876988) identified to be associated in women of African ancestry was found to be significantly associated ($p = 0.002$) with HGSOC at the Bonferroni threshold among European ancestry women, although the direction of the association was discordant with that among African ancestry women (Table 1). Of the 30 previously identified GWAS SNPs detected in European ancestry women, four SNPs were significantly associated with EOC among African ancestry women ($p < 0.05$): 19p13.11 (rs4808075, $p = 0.013$), 5p15.33 (rs7705526, $p = 0.014$), 17q21.32 (rs1879586, $p = 0.018$) and 17q12 (rs7405776, $p = 0.026$) (Table 2). Combining the 24 published European ancestry GWAS SNP associations (omitting mucinous associated SNPs due to the small number of cases in the data set), the association of the resulting polygenic risk score with EOC was 1.20 per standard deviation in polygenic risk score (95% CI = 1.09, 1.31; $p = 4.46 \times 10^{-9}$) and 1.26 per standard deviation in polygenic risk score (95% CI: 1.13, 1.39; $p = 3.02 \times 10^{-11}$) for HGSOC, demonstrating a positive association of this European ancestry-derived risk score with EOC risk in women of African ancestry. These are weaker in comparison to the recently reported polygenic risk score for East Asian women of 1.76 per standard deviation for HGSOC ($p = 8.6 \times 10^{-6}$).[18]

The results from fine mapping of the gene regions of the 30 previously identified SNPs[3] associated with EOC and HGSOC in European ancestry women among the sample of African ancestry women identified one risk region in African ancestry women that was significantly associated with EOC after Bonferroni correction, 18q11.2 ($p = 1.84 \times 10^{-5}$) (Table 3 and Supplementary Table S6). The lead SNP in that region (chr18:21555816, rs1258109, 8 kb 5′ of *LOC105372023*) is located ~150 kb from the *LAMA3* region variant previously reported in European ancestry (chr18:21405553, rs8098244). Notably, rs8098244 demonstrates differences in MAF across ethnic groups with MAFs of 0.28 and 0.03 in the 1,000 Genomes European *vs*. African ancestry populations (source: HaploReg v4.1), respectively, corresponding to markedly reduced power to detect associations with this variant in African ancestry women. Four loci were associated with EOC at a suggestive threshold: 9p22.2 (chr9:16978052, rs373094273, $p = 2.67 \times 10^{-5}$, 36 kb 5′ of *LOC105375983*), 8q21.13 (chr8:82866267, rs1839897, $p = 1.44 \times 10^{-5}$,104 kb 3′ of *LOC105375928*), 10q24.33 (chr10:105375295, rs138417137, $P = 3.40 \times 10^{-5}$, *SH3PXD2A* intronic) and 3q22.3 (chr3:138839642, rs75623154, $p = 3.34 \times 10^{-5}$, *BPESC1* intronic). In examination of association with HGSOC, we identified one Bonferroni-significant association at 8q21.13 (chr8:82866267, rs1839897, $p = 3.98 \times 10^{-6}$, 104 kb 3′ of *LOC105375928*) located ~200 kb from the previously reported *CHMP4C* region variant (chr8:82668818, rs76837345). Additionally, a locus in region 12q24.31 reached the suggestive threshold (chr12:121113096, rs111546208, *CABP1* intronic, $p = 2.51 \times 10^{-5}$) for association with HGSOC among African ancestry women.

Of the 10 SNPs newly identified in GWAS of African ancestry women, one, the *GK2* region SNP rs192876988, showed evidence a protective association ($p = 0.002$) in the OCAC European ancestry GWAS that included up to 23,543 EOC cases and 29,444 controls (Table 1). Fine mapping of these gene regions in European ancestry women provided no evidence of another SNP within the region associated with EOC or HGSOC at the Bonferroni significance threshold; however, a SNP in the 4p13 region reached statistical significance at the suggestive threshold, $p = 1.14 \times 10^{-5}$ (Supplementary Table S7). The lead SNP in this region was rs2292092 (chr4:70592790), a variant in the 3′ UTR of the *SULT1B1* gene.

Table 2. Association of SNPs previously identified in European Ancestry GWAS of EOC among women of African ancestry

Locus	SNP ID	Build 37 Chr:Pos	Nearest gene	Phenotype	European ancestry[1]		African ancestry[2]			Power[3] With/without Bonferroni correction
					OR (95% CI)	*p* Value	MAF	OR (95% CI)	*p* Value	
Confirmed SNPs in European ancestry OncoArray meta-analysis										
1p34.3	rs58722170	1:38096421	*RSPO1*	Serous	1.10 (1.07–1.13)	1.4E−09	0.203	0.99 (0.85–1.17)	0.976	0.025/0.222
2q14.1	rs752590	2:113972945	*PAX8*	Mucinous	1.30 (1.21–1.39)	2.2E−12	0.408	1.00 (0.88–1.15)	0.949	0.799/0.978
2q31.1	rs711830	2:177037311	*HOXD3*	Mucinous	1.27 (1.20–1.35)	1.1E−14	0.114	1.10 (0.90–1.34)	0.370	0.240/0.684
2q31.1	rs6755777	2:177043226	*HAGLR*	Serous	1.12 (1.09–1.15)	2.7E−15	0.131	1.06 (0.88–1.28)	0.548	0.026/0.223
3q25.31	rs62274041	3:156435640	*TIPARP*	HGSC	1.57 (1.48–1.66)	2.1E−57	–	–	–	–
5p15.33	rs10069690	5:1279790	*TERT*	Serous	1.13 (1.09–1.17)	1.5E−12	0.401	0.94 (0.83–1.08)	0.406	0.097/0.454
5p15.33	rs7705526	5:1285974	*TERT*	Serous borderline	1.38 (1.29–1.48)	5.5E−19	0.189	1.23 (1.04–1.45)	0.014	0.818/0.982
8q21.13	rs76837345	8:82668818	*CHMP4C*	HGSC	1.20 (1.13–1.28)	9.0E−10	–	–	–	–
8q24.21	rs1400482	8:129541931	*LINC00824*	Serous	1.23 (1.19–1.28)	7.4E−26	–	–	–	–
9p22.2	rs10962692	9:16915874	*BNC2*	HGSC	1.36 (1.30–1.42)	1.4E−47	0.032	0.97 (0.67–1.41)	0.875	0.087/0.431
9q34.2	rs8176685	9:136138765	*ABO*	HGSC	1.15 (1.10–1.19)	5.2E−12	–	–	–	–
10p12.31	rs144962376	10:21878831	*MLLT10*	Serous	1.10 (1.06–1.13)	6.6E−09	–	–	–	–
17q12	rs7405776	17:36093022	*HNF1B*	Serous	1.10 (1.07–1.14)	1.9E−10	0.482	1.16 (1.02–1.32)	0.026	0.046/0.308
17q12	rs11651755	17:36099840	*HNF1B*	Clear cell	0.79 (0.73–0.86)	6.8E−09	0.329	1.11 (0.97–1.28)	0.121	0.566/0.911
17q21.31	rs7207826	17:46500673	*SKAP1*	Serous	1.14 (1.10–1.18)	1.2E−14	0.485	1.05 (0.92–1.19)	0.467	0.127/0.518
17q21.32	rs1879586	17:43567337	*PLEKHM1*	HGSC	1.15 (1.10–1.19)	2.5E−12	0.042	1.49 (1.07–2.07)	0.018	0.012/0.144
19p13.11	rs4808075	19:17390291	*BABAM1*	HGSC	1.20 (1.16–1.24)	3.3E−24	0.237	1.21 (1.04–1.41)	0.013	0.238/0.681
19q11.21	rs688187	19:39732752	*IFNL3*	Mucinous	1.43 (1.33–1.53)	1.2E−22	0.384	0.97 (0.85–1.12)	0.699	0.988/0.999
Newly identified SNPs in European ancestry OncoArray meta-analysis										
2q13	rs2165109	2:111818658	*ACOXL*	HGSC	1.09 (1.05–1.12)	2.0E−08	0.209	0.99 (0.84–1.16)	0.890	0.020/0.192
3q22.3	rs112071820	3:138849110	*BPESC1*	Mucinous	1.29 (1.20–1.37)	1.5E−13	0.328	1.14 (0.99–1.31)	0.072	0.722/0.962
3q28	rs9870207	3:190525516	*GMNC*	Serous borderline, LGSC	1.19 (1.12–1.27)	4.5E−08	0.364	1.08 (0.94–1.24)	0.305	0.290/0.736
4q32.2	rs13113999	4:167187046	*TLL1*	Serous borderline	1.23 (1.14–1.32)	4.7E−08	0.092	0.92 (0.71–1.20)	0.551	0.109/0.482
5q12.3	rs555025179	5:66121089	*MAST4*	Endometrioid	1.18 (1.11–1.26)	4.5E−08	0.415	0.96 (0.82–1.11)	0.565	0.264/0.709
8q21.11	rs150293538	8:77320354	*LINC01111*	Serous borderline, LGSC	2.19 (1.65–2.90)	2.0E−09	0.005	–	–	0.142/0.545
8q24.21	rs9886651	8:128817883	*PVT1*	HGSC	1.08 (1.05–1.11)	1.9E−09	0.150	1.10 (0.92–1.33)	0.295	0.011/0.137
9q31.1	rs320203	9:104943226	*LOC105376188*	Mucinous	1.29 (1.18–1.41)	1.7E−08	0.220	0.92 (0.79–1.07)	0.289	0.579/0.917
10q24.33	rs7902587	10:105694301	*LOC102724351*	Serous borderline, LGSC	1.29 (1.18–1.41)	4.0E−08	0.172	1.01 (0.85–1.21)	0.881	0.470/0.866
12q24.31	rs7953249	12:121403724	*HNF1A-AS1*	HGSC	1.08 (1.06–1.11)	4.5E−10	0.341	1.04 (0.91–1.19)	0.523	0.022/0.203
18q11.2	rs8098244	18:21405553	*LAMA3*	Serous borderline, LGSC	1.19 (1.12–1.27)	3.9E−08	0.051	1.19 (0.87–1.63)	0.275	0.027/0.231
22q12.1	rs6005807	22:28934313	*TTC28/LOC101929594*	HGSC	1.17 (1.10–1.23)	1.2E−08	0.125	1.12 (0.92–1.36)	0.257	0.066/0.374

[1]African ancestry was defined as >50% African ancestry as calculated by FastPop.
[2]European ancestry was defined by self-report.
[3]Power calculations assume a disease prevalence rate of 0.01.
Abbreviations: Chr, chromosome; CI, confidence interval; HGSC, high-grade serous carcinoma; LGSC, low-grade serous carcinoma; MAF, minor allele frequency; OR, odds ratio; Pos, position; SNP, single nucleotide polymorphism.

Table 3. Summary of statistically significant or suggestive results for fine mapping in African ancestry women of loci previously identified in GWAS of European ancestry women

					EOC			HGSC[1]		
Locus	SNP ID	Build 37 Chr:Pos	Nearest gene	Phenotype	Number of SNPs Plotted	Minimum SNP position in region	Minimum SNP *p* value	Number of SNPs plotted	Minimum SNP position in region	Minimum SNP *p* value
Confirmed SNPs in European ancestry OncoArray meta-analysis										
8q21.13	rs76837345	8:82668818	*CHMP4C*	HGSC	4,045	chr8:82866267	1.44E−05[3]	3,523	chr8:82866267	3.98E−06[2]
9p22.2	rs10962692	9:16915874	*BNC2*	HGSC	5,248	chr9:16978052	2.67E−05[3]	4,746	chr9:16986321	5.57E−05
Newly identified SNPs in European ancestry OncoArray meta-analysis										
3q22.3	rs112071820	3:138849110	*BPESC1*	Mucinous	2,922	chr3:138839642	3.34E−05[3]	–	–	–
10q24.33	rs7902587	10:105694301	*LOC102724351*	Serous borderline, LGSC	3,192	chr10:105375295	3.40E−05[3]	2,852	chr10:105300054	1.03E−03
12q24.31	rs7953249	12:121403724	*HNF1A-AS1*	HGSC	3,680	chr12:121113096	6.90E−05	3,272	chr12:121113096	2.51E−05[3]
18q11.2	rs8098244	18:21405553	*LAMA3*	Serous borderline, LGSC	2,685	chr18:21555816	1.84E−05[2]	2,431	chr18:21555816	6.19E−05

[1]Fine mapping among HGSC was completed only for those SNPs associated with serous ovarian cancer.
[2]Significant at the Bonferroni threshold (0.05/number of SNPs plotted).
[3]Significant at the suggestive threshold (0.05/[number of SNPs plotted/3]).
Abbreviations: Chr, chromosome; EOC, epithelial ovarian cancer; HGSC, high-grade serous ovarian cancer; LGSC, low-grade serous ovarian cancer; Pos, position; SNP, single nucleotide polymorphism.

Discussion

Here, we report on the first GWAS of EOC and HGSOC in women of African ancestry. Due to the limited number of EOC cases of African ancestry available for our study, we applied a suggestive threshold of $p < 1 \times 10^{-6}$ for the current investigation. At this suggestive level of statistical significance, we identified four loci associated with EOC in women of African descent and six distinct and novel loci associated with HGSOC in women of African descent. Although one SNP was observed to be associated with HGSOC among European ancestry women, the direction of the association was not concordant with that of African ancestry women. Below, we review the functional relevance of these genes to ovarian cancer and other cancers.

The variant with the smallest *p*-value associated with EOC in women of African descent (rs4525119) is in an intron of *AKR1C3*, a gene which encodes an enzyme of the aldo-keto reductase superfamily.[19] *AKR1C3* plays a role in androgen biosynthesis[20] and has been linked to benign gynecologic conditions, endometriosis and polycystic ovary syndrome (PCOS),[21–24] which are risk factors for ovarian cancer. Consistent with a possible relationship with a predisposition to endometriosis, an OR of 1.78 (95% CI = 1.09–2.90) for the association between a history of endometriosis and invasive EOC risk among African Americans was recently reported in the AACES.[25] Another locus associated with EOC is near the *WWC1* gene, which encodes the WW domain-containing protein 1 (WWC1), also known as KIBRA, and is likely a regulator of the tumor suppressive Hippo signaling pathway.[26] While *WWC1* has been primarily linked to episodic memory and Alzheimer's disease,[27–30] a recent candidate gene study[31] observed an association between *WWC1* variants and risk of estrogen-receptor positive breast cancer in women of African ancestry. Likewise, WWC1/KIBRA has been linked to breast cancer outcomes, including recurrence-free survival and metastasis.[32,33] In the current study, we found an association with ER negative breast cancer for the SNP nearest to the *WWC1* gene. To our knowledge, the other two loci associated with EOC in women of African descent at the suggestive threshold, *LOC101927394* and *UGT2A2*, have not been reported in association with cancer or other diseases. However, when we assessed whether the rs7643459 allele T in *LOC101927394* was associated with cancer in individuals of African descent using data from the AABC and AAPC consortium, we demonstrated a nominal association with risk of ER negative breast cancer and prostate cancer in African ancestry individuals.

The variant with the smallest *p*-value for HGSOC was observed for a SNP upstream of *FST* (rs37792). The *FST* gene encodes a gonadal protein that inhibits the release of follicle-stimulating hormone,[34] and is consistent with the suspected hormonal etiology of ovarian cancer.[35] Polymorphisms of *FST* have been linked to PCOS[36] or markers for PCOS,[37] a risk factor for ovarian cancer.[38] With potential importance to cancer risk, progression and survival, the second most significant HGSOC-associated gene, *MAGEC1*, is a member of the melanoma-associated antigen (MAGEs) gene family and encodes

tumor-specific antigens that can be recognized by autologous cytolytic T lymphocytes.[39] Due to these properties, the MAGE gene family has garnered attention as possible target for cancer immunotherapy.[40] *MAGEC1* expression has been linked to an improved ovarian cancer progression-free survival.[41] Recently, a missense variant in *MAGEC3* was reported to have an X-linked pattern of inheritance in ovarian cancer families.[42]

Several of the SNPs associated with EOC and HGSOC were long noncoding RNA (ncRNA) genes, *LOC101927394*, *LOC105376360* and *LOC105377300* (*GK2*). Little is known about these specific ncRNAs, but ncRNAs are increasingly reported by GWAS studies and are thought to play important roles in gene regulation.[43] SNPs in long ncRNAs have been shown to contribute to the development of ovarian cancer, where a variant within the exonic region of a long ncRNA gene (rs17427875, *HOXA11-AS*) was marginally associated with reduced risk of serous ovarian cancer.[44] We also demonstrated that *LOC105377300*/*GK2* region SNP rs192876988 allele C was associated with an increased risk of ER positive breast cancer in African ancestry women from AABC, and inversely associated with HGSOC in European ancestry women from OCAC. The rs192876988 allele C also showed association with reduced expression of *GK2* in HGSOC tissue samples from women of African ancestry. *GK2* encodes glycerol kinase 2, a key enzyme in the regulation of glycerol uptake and metabolism, and has been associated with glycerol kinase deficiency.[45] It remains unclear whether the association between rs192876988 and *GK2* expression is mediated by the nearby ncRNA.

A few SNPs were identified through fine mapping of loci previously reported in European ancestry-based GWAS of ovarian cancer[3] that may be of importance to ovarian cancer risk among African ancestry women. Four of these SNPs were near or in long ncRNA genes (*LOC105372023*, *LOC105375983*, *LOC105375928* and *BPESC1*), while two SNPs lie in protein coding sequences for *SH3PXD2A* and *CABP1*. The *SH3PXD2A* gene encodes an adaptor protein involved in formation of invadopodia and degradation of the extracellular matrix, which both contribute to tumor invasion.[46] The *CABP1* gene encodes a calcium binding protein that is highly expressed in the brain and retina, and is important in calcium mediated cellular signal transduction.[47] Through the fine mapping of gene regions among European ancestry women, we identified one SNP in the 3′ UTR region of the *SULT1B1* gene. The *SULT1B1* gene encodes a sulfotranferase enzyme that catalyzes the sulfate conjugation of estradiol, thyroid hormones and drugs.[48] Overall, although we identified limited statistical significance in examining the specific genetic variants previously reported in GWAS of European ancestry individuals, our fine mapping effort underscores the possibility of shared genes, pathways and biological mechanisms underlying risk of ovarian cancer in European and African ancestry women.

The OCAC and AACES provided a unique opportunity to evaluate genetic associations in African ancestry women with EOC as no individual study alone has enrolled enough subjects. That said, even with data pooled from 32 individual studies, the sample size was underpowered for detection of genome-wide significant associations. As shown in Table 2, the power to detect associations of SNPs confirmed among European ancestry in those of African ancestry was limited for most SNPs and ranged from 0.015/0.16 to 0.819/0.982 (based on power calculations with/without consideration for multiple comparisons).

There are very few existing studies that were not included in our analysis that have enrolled women of African descent with ovarian cancer. However, the Black Women's Health Study (BWHS), the Women's Health Initiative (WHI) and the Southern Community Cohort Study (SCCS) have EOC cases diagnosed in women of African descent that were not included in our analyses. Since none of these three studies has participated in OCAC or GAME-ON, genotype data generated from the OncoArray project were not available. Thus far, neither the SCCS nor the BWHS have genotyped ovarian cancers in their cohorts. Although the WHI has conducted genome-wide genotyping, a different genetic platform (Affymetrix 6.0 array) was used. When we attempted to add a small number of cases and many African ancestry controls from WHI, there were systematic differences in allele frequencies observed across the two platforms that precluded merging WHI samples with our OCAC and AACES samples without introducing false positives.[49] Due to lack of available GWAS efforts for ovarian cancer in African ancestry women, we were unable to pursue formal replication of our selected GWAS SNPs. Although we successfully identified some signals of association for our identified SNPs in examination of independent samples of African ancestry from case–control studies of breast and prostate cancers, we emphasize that these efforts only allowed us to identify SNPs with shared effects across cancer types, without the ability to confirm any SNPs that have mechanisms specific to ovarian cancer. These observations underscore the need for new genotyping initiatives and new data collection that target minority populations with ovarian cancer. Our study included a GWAS backbone in the OncoArray that was designed for women of European ancestry, and therefore has reduced power for GWAS analysis in women of African ancestry.

This GWAS is the first to report genome-wide associations for ovarian cancer in African ancestry women. Our findings provide suggestions of genetic association for ovarian cancer in African ancestry women. Only 1 of the 10 SNPs associated with ovarian cancer in African ancestry women was found to be associated in European ancestry women, although the direction of the association was not consistent across ethnic groups, perhaps reflecting differences in linkage disequilibrium across groups. Our data show that the suggestive SNP associations for ovarian cancer among women of African ancestry are not generally replicated among women of European ancestry, which have been similarly observed for other cancers and disease states, such as breast cancer.[50] Our results demonstrate that some ovarian cancer GWAS variants identified in women of European ancestry may be associated with ovarian cancer in women of African ancestry.

This finding is further underscored by our report of statistically significant association of the polygenic risk score derived from published European GWAS hits with risk of EOC in women of African ancestry. These findings suggest there may be some shared genetic architecture of EOC between women of European and African ancestry in susceptibility to ovarian cancer. Additional genetic studies leveraging larger sample sizes will be needed to refine genetic risk prediction and elucidate the underlying biology of EOC in African ancestry women.

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