Assessing the Genetic Architecture of Epithelial Ovarian Cancer Histological Subtypes

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Abstract

Epithelial ovarian cancer (EOC) is one of the deadliest common cancers. The five most common types of disease are high-grade and low-grade serous, endometrioid, mucinous and clear–cell carcinoma. Each of these subtypes presents distinct molecular pathogeneses and sensitivities to treatments. Recent studies show that certain genetic variants confer susceptibility to all subtypes whilst other variants are subtype-specific. Here we perform an extensive analysis of the genetic architecture of EOC subtypes. To this end, we used data of 10,014 invasive EOC patients and 21,233 controls from the Ovarian Cancer Association Consortium genotyped in the iCOGS array (211,155 SNPs). We estimate the array heritability (attributable to variants tagged on arrays) of each subtype and their genetic correlations. We also look for genetic overlaps with factors such as obesity, smoking behaviours, diabetes, age at menarche, and height. We estimated the array heritabilities of high-grade serous disease (h^2_g= 8.8 ± 1.1%), endometrioid (h^2_g= 3.2 ± 1.6%), clear-cell (h^2_g = 6.7 ± 3.3%) and all EOC (h^2_g= 5.6 ± 0.6%). Known associated loci contributed approximately 40% of the total array heritability for each subtype. The contribution of each chromosome to the total heritability was not proportional to chromosome size. Through bivariate and cross-trait LD score regression, we found evidence of shared genetic backgrounds between the three high-grade subtypes: serous, endometrioid and undifferentiated. Finally, we found significant genetic correlations of all EOC with diabetes and obesity using a polygenic prediction approach.

Introduction

In developed countries, epithelial ovarian cancer (EOC) is the leading gynaecological malignancy with an estimated annual incidence rate of 12 per 100,000 and a poor 5 year survival between 20% and 50% (Chornokur et al. 2015; Sopik et al. 2015; Sung et al. 2014). About 90% of invasive tumours in the ovary are of epithelial origin (Kurman R 2014). These tumours are divided into various histological subtypes that include: serous, mucinous, endometrioid, clear cell, Brenner, other minor types, as well as undifferentiated, mixed and unclassified carcinomas (Prat 2012; Sung et al. 2014). Serous carcinomas can be subdivided into high-grade (90%) and low-grade disease (10%) (Kurman and Shih Ie 2008; Malpica et al. 2004; Shih Ie and Kurman 2004).

Each epithelial ovarian cancer histologic subtype exhibits a distinct etiologic and molecular pathogenesis and sensitivity to treatment (e.g., chemotherapeutic agents) (Anglesio et al. 2013; Della Pepa et al. 2015; Risch et al. 1996; Shih Ie and Kurman 2004; Soslow 2008). It has been suggested that serous carcinomas arise from the epithelial mucosal lining of the fallopian tube fimbriae or from endosalpingiotic deposits on the ovarian or peritoneal surfaces. Clear-cell and endometrioid subtypes may arise from endometriotic lesions (Kurman R 2014; Wiegand et al. 2010), while mucinous tumours do not yet have a clear origin, though metaplastic transformation of the epithelial lining of ovarian inclusion cysts has been suggested. Serous carcinoma is by far the most deadly type of EOC, with 5-year survival of less than 20% for patients suffering from high-grade disease and 50% for those with low-grade disease (Malpica et al. 2004). In contrast, women with mucinous, endometrioid or clear-cell carcinomas tend to have better prognosis, with estimated 5-year survivals of 50%-60% (Malpica et al. 2004; Simons et al. 2015). These differences in survival are due at least in part to the fact that high-grade serous carcinomas are usually detected at advanced stages of disease but the other subtypes at earlier stages (Devouassoux-Shisheboran and Genestie 2015; Malpica et al. 2004; Simons et al. 2015).

Genetic studies have shown that around 20% of patients with high-grade serous cancers carry germ-line and somatic mutations in *BRCA1* or *BRCA2* (Alsop et al. 2012; Berchuck et al. 1998) along with somatic mutations in *TP53* that are present in most tumours (Cancer Genome Atlas Research 2011). Alterations in *KRAS* and *BRAF* but not *TP53* have been associated with low-grade serous carcinomas (Della Pepa et al. 2015; Grisham et al. 2013; Jones et al. 2012). Mucinous carcinomas also frequently have somatic mutations in *KRAS* (Cuatrecasas et al. 1997) in addition to mutations in *HER2* (Anglesio et al. 2013). Endometrioid and clear cell carcinomas often carry somatic mutations in *AR1D1A* and *PIK3CA* (Jones et al. 2010). In addition, genome-wide association studies (GWAS) have found 20 common polymorphisms associated with risk of EOC (Bojesen et al. 2013; Bolton et al. 2010; Goode et al. 2010; Permuth-Wey et al. 2013; Pharoah et al. 2013; Song et al. 2009).

Specific germ-line SNPs are commonly found in the different EOC subtypes. However, these variants explain only a fraction of the cases, thus it is not known whether or not other genetic components are shared among the subtypes. One of our previous studies (Lu et al. 2015) estimated the array heritability (i.e., heritability explained by about 200,000 genotyped SNPs but not all the genome) of all EOC to be 5.6%, and 8.8% for the most common EOC subtype, high-grade serous.

Beside genetic factors predisposing to these diseases, some environmental factors such as smoking (Collaborative Group on Epidemiological Studies of Ovarian et al. 2012; Faber et al. 2013) and obesity (Aune et al. 2015; Collaborative Group on Epidemiological Studies of Ovarian 2012; Olsen et al. 2013) may be associated with increases in risk of some subtypes of EOC. In addition, traits including achieved height (Aune et al. 2015; Wiren et al. 2014) and diabetes mellitus (Gapstur et al. 2012; Lee et al. 2013) have been positively associated to EOC. In contrast, some studies have shown that age at menarche (Gong et al. 2013) is inversely associated with risk of EOC. Evidence suggests that all these traits have heritable components. Genetic variation may explain as much as 80% of the total variance of height (Yang et al. 2010) or even 40% for smoking behaviour (Vink and Boomsma 2011; Vink et al. 2005). It is possible that part of the heritability of EOC may be explained by the heritability of these traits, if they are associated with EOC risk.

In this work, we investigate three aspects of the genetic architecture of EOC and its subtypes: (i) the total genetic contribution of all array-genotyped SNPs (genome-wide, per chromosome and after accounting for known EOC associated loci); (ii) the genetic correlations between EOC subtypes; and (iii) the genetic correlations between EOC subtypes and risk factors such as obesity and smoking. To this end, we use genotype and risk-factor data from studies participating in the Ovarian Cancer Association Consortium (OCAC). We quantify genetic contributions to disease using genome-wide complex trait analysis (GCTA) (Lee et al. 2011; Yang et al. 2010; Yang et al. 2011a). Then, we evaluate shared genetic backgrounds between EOC subtypes and candidate risk factors using complementary approaches: bivariate linear mixed models (Lee et al. 2012), cross-trait LD score regression (Bulik-Sullivan et al. 2015a) and polygenic risk prediction (International Schizophrenia et al. 2009).

Methods

Data

We used data from the Ovarian Cancer Association Consortium (OCAC). This dataset consists of custom Illumina iCOGS array genotyping of 47,630 cases and controls in 43 OCAC studies. Detailed description of the content of the array can be found elsewhere (Pharoah et al. 2013). In brief, the array consists of 211,155 variants within breast, ovarian and prostate cancer susceptibility loci as well as candidate SNPs, SNPs associated with other cancers and SNPs associated with relevant quantitative traits such as body mass index (BMI) and the onset of menarche.

We applied standard quality control (QC) for the genotype data. First, we selected only samples from European ancestry studies and that were within 6 s.d. from the genotype-derived PC1 and PC2 from the 1000 Genomes European population [Supplementary Figure 1]. We excluded individuals with missing genotypes in 5% or more of the SNPs. Likewise, we removed SNPs with call rates below 99%, minor allele frequencies (MAF) below 1% and SNPs that deviated from Hardy-Weinberg equilibrium at P<0.0001 (Lu et al. 2014). Further, given that our analytic methods are sensitive to relatedness (e.g., results may be biased by common environmental factors in relatives) we removed individuals such that no sample pairs had identity by descent (IBD) > 10% (i.e., less than second cousins), giving more priority to keeping cases than controls. In concordance with one of our previous work (Lu et al. 2015), we focused only on those with invasive EOC tumours. In total, 10,014 EOC cases and 21,233 controls met these criteria and were genotyped for 195,183 SNPs. The number of cases according to histologic subtype are displayed in Table 1. The numbers of initial cases and controls per study are summarized in Supplementary Table 1.

Analysis

We estimated the variance explained by all SNPs in the array (h^2_g) (Lee et al. 2011), the variance after removing known loci, and the variance explained by each chromosome for each of the EOC subtypes. We used GCTA to calculate one genetic relationship matrix (GRM) for all autosomes.

The estimated variance explained was transformed from the observed scale to an unobserved continuous "liability" scale using a probit transformation (Lee et al. 2011) taking into account the disease prevalence. The lifetime risk of the various EOC subtypes were calculated as the lifetime risk of ovarian cancer (~1% according to the Surveillance, Epidemiology and End Results (SEER), http://seer.cancer.gov/statfacts) multiplied by the relative proportion of each subtype according to SEER program DevCan database (http://surveillance.cancer.gov/devcan/canques.html) in all ovarian cancer. Given that around 90% of ovarian cancers are of epithelial origin, we used 0.9% as the prevalence for all EOC. As h^2_g, is derived solely from the SNPs tagged on the genotyping array instead of the whole genome, it provides a lower bound on heritability estimates (Lu et al. 2014). Phenotypes were modeled as a linear function of the sum of the additive effects due to all SNPs associated with trait-associated variants and residual effects. Variance components were estimated using residual maximum likelihood (REML) (Yang et al. 2010). For tests of whether a variance component is zero or not, the test is one-sided and under the null hypothesis that the test statistic follows a 50:50 mixture of a point mass at zero and the χ_1 distribution (Yang et al. 2010; Yang et al. 2011a). One sided p-values were calculated to estimate statistical significance. Likewise, To estimate the proportion of h^2_g that is explained by the known loci (WNT4, RSPO1, SYNPO2, GPX6, ABO, ATAD5, C19orf62, CMYC, TIPARP, BNC2, ARHGAP27, TERT, RAD51B/C/D, BRIP1, BARD1, PALB2, NDN, CHMP4C, MLLT10, HNF1B, *BRCA1*, *BRCA2, KRAS, TP53, HER2, AR1D1A* and *PIK3CA* (Bojesen et al. 2013; Bolton et al. 2010;

Goode et al. 2010; Permuth-Wey et al. 2013; Pharoah et al. 2013; Song et al. 2009)), we re-computed the GRM with the SNPs (6,391 SNPs) close to the known loci SNPs (+/- 1 megabase either side) removed.

Similarly, in order to investigate the genetic contributions within of each of the chromosomes, we computed one GRM per chromosome and performed analyses using REML fitting the 22 genetic variance components in the model as implemented in GCTA with the flag –*mgrm* (multiple GRMs) (Yang et al. 2011b). Given that loading 22 GRMs with the 21,051 controls and the cases of the various histotypes was computationally intractable, we assigned to each case just one control of the same study, yielding smaller GRMs (e.g., for high-grade Serous cancer there were 3,705 cases and 3,705 controls). We then normalized the contribution of each chromosome by the number of independent SNPs (percentage) in the iCOGs array per chromosome. This number of independent SNPs was estimated through LD pruning using the PLINK command –*indep* 50 5 1.2, where 50 is the window size (#SNPs), 5 is the number of SNPs the window can shift, and 1.2 is $1/(1-R^2)$, where R^2 is the multiple correlation coefficient for a SNP regressed on all other SNPs simultaneously (Chang et al. 2015). In order to approximate the s.e. of the variance explained by each chromosome, we performed a jackknifing procedure up to 1000 times, taking 80% of the cases and 80% of the controls each time. Given the complexity of the sample, around 20% of the jackknifing repetitions did not converge within 1000 iterations so the standard errors were computed from just the 800 successful jackknifings.

To investigate the genetic correlations between the subtypes, in order to remove potential biases from overlapping control samples from the different studies, we matched each case to 1 control of the same study, and distributed controls in such a way that each EOC subtype had separate sets of controls. For example, all of the controls for mucinous EOC were different from the endometrioid EOC controls.

Genetic correlation (r_g) represents the proportion of the total genetic variance that two traits share. In order to investigate the r_g between EOC subtypes, we used two distinct approaches that can be applied to population-based samples. We first used the GRM in a bivariate mixed-effects linear model implemented in GCTA (Cross-Disorder Group of the Psychiatric Genomics et al. 2013) to compute the genetic correlations between the various EOC subtypes. The estimated genetic correlation is the additive genetic covariance between traits, normalized by the geometric mean of the individual trait genetic variances (producing values from -1 to +1). The additive genetic covariance was estimated by relating trait covariances between unrelated individuals to genetic relationship estimates from marker data. Increased covariance between traits with high genetic relationship values implies a positive genetic correlation between traits. In order to control for any potential effects of population stratification, all the analyses were performed using the first 10 principal components (PCs) of the genotypes as covariates. Estimates are reported as genetic correlation ± standard error.

We also used cross-trait LD score regression (Bulik-Sullivan et al. 2015a), a recently developed approach that is able to estimate genetic correlations using solely GWAS summary statistics and is not affected by sample overlap. We first ran genome-wide association analyses using the same samples as when computing h^2_g per each EOC subtype (i.e., we repeatedly made use of all of the controls for analysis of each subtype) and with the 10 first PCs and study site as covariates. Genomic inflation factors for these GWAS analyses ranged from 0.99 for mucinous cancer to 1.07 for all EOC. We used the LD-scores estimated by Bulik-Sullivan, *et al.*(Bulik-Sullivan et al. 2015a; Bulik-Sullivan et al. 2015b) available at *http://www.broadinstitute.org/~bulik/eur_ldscores/* which are based on the 1000 Genomes European population and estimated within 1-cM windows. We then estimated the genetic correlation using software available at *https://github.com/bulik/ldsc* with the default parameters.

Genetic correlations between EOC subtypes and risk factors

Using cross-trait LD score regression, we estimated genetic correlations between risk factors and EOC histotypes. To this end, we used publicly available GWAS summary results from the latest GWAS meta-analyses of BMI and height from the Genetic Investigation of Anthropometric Traits (GIANT) consortium. These analyses included 339,225 (Locke et al. 2015) and 253,288 (Wood et al. 2014) individuals, respectively. We also estimated genetic correlations using the GIANT extreme anthropometric traits GWAS which used obesity class 1 (BMI>30), class 2 (BMI>35) and class 3 (BMI>40) groups as cases, and individuals with BMI<=25 as controls, in a sample of 263,407 individuals (Berndt et al. 2013). Genetic overlaps with age at menarche was carried out based on the GWAS of the Reproductive Genetics Consortium which involved 182,416 women (Perry et al. 2014). Smoking behaviour genetic predisposition was approximated based on the Tobacco and Genetics Consortium GWAS which involved 74,053 participants (Tobacco and Genetics 2010). Finally, for diabetes, we used the summary results for type 2 diabetes GWAS of the DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) consortium, which involved 34,840 cases and 114,981 controls (Morris et al. 2012).

We also carried out a polygenic risk-prediction approach. This method involves the computation of polygenic risk scores (PGRS) of each of the risk factors and uses these scores to predict disease status (International Schizophrenia et al. 2009). The PGRS describes a predicted phenotypic value based on the genetic component and is computed by aggregating the magnitude of associations of many variants. These associations are estimated using a discovery set of subjects (e.g., for height or BMI) to identify relevant SNPs and estimate the magnitude of association of each, and these magnitudes or the number of "high-risk" alleles in each SNP are then summed to create a score. Subsequently, we examine the association of this score within a target subject set (e.g., EOC cases and controls). If the score association is significant, it implies a genetic correlation between the two traits. In this study, we selected

variants to compute the PGRS based on 11 p-value thresholds (<0.00001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1). Given the nature of the iCOGS array in which many loci have high densities of tagged SNPs, we performed linkage disequilibrium (LD) clumping in order to remove correlated variants ($r^2>0.2$) within 500kb windows for each component of the PGRS. The computations for PGRS and LD clumping were performed with PLINK (Chang et al. 2015). Finally, we standardized each of the PGRS to have mean 0 and variance 1 and examined their associations with the various EOC subtypes through logistic regression, adjusted for the first 10 PCs.

Multiple testing correction

The polygenic risk prediction approach carries a high multiple testing burden, as does consideration of the various histologic groups and risk factors. However, given that we computed 11 PGRS for each trait based on sequential p-value thresholds, our statistics are not independent. In order to estimate the real number of independent hypotheses, we computed the correlation matrix of all the PGRS used in this study and fed this into a Matrix Spectral Decomposition (matSpD) algorithm (Nyholt 2004), to estimate the number of independent variables. This algorithm provides an equivalent number of independent variables in a correlation matrix, by examining the ratio of the observed eigenvalue variance to its theoretical maximum. We estimated the number of independent PGRS to be 35 out of the 88 PGRS. As we examined these 35 independent PGRS in five separate EOC subtypes (high-grade serous, endometrioid, clear cell, mucinous and unknown), our significance threshold for the polygenic risk prediction analyses was 0.05/(35*5)=.00029.

Results

Genetic contribution of each chromosome and known loci

Fitting a GRM computed after removing known EOC-associated loci in univariate mixed-effect linear models implemented in GCTA (Yang et al. 2010; Yang et al. 2011a), we found that the known loci contributed about 40% of the total heritability of EOC and each of the subtypes **[Table 1]**. The estimated heritability of all EOC dropped from 5.6% to 3.6% once we removed known EOC-associated loci from the GRM. We observed a similar reduction of variance explained by the polygenic component for the EOC subtypes high-grade serous (8.8% to 4.7%), endometrioid (3.2% to 2.0%) and clear cell (6.7% to 4.6%) **[Table 1]**. Interestingly, in contrast to grade 1 and grade 2 (G1/G2) endometrioid where the heritability did not drop substantially (4.4% to 3.7%), grade 3 (G3) endometrioid h^2_g dropped from 4.9% to 0.9%. As shown previously (Lu et al. 2015), the heritability of mucinous cancer was not detectably different from 0. We were unable to perform any analyses for low-grade serous cancer given the small sample size (N_{cases}=350). We also had a set of cases with unknown EOC subtype classification; we expect that a high portion of these are individuals with undifferentiated high-grade serous, endometrioid or mixed serous EOC subtypes. For these, the heritability

dropped from 7.0% to 4.1% after removing known loci.

In order to inspect the contributions of heritability per chromosome, we computed one GRM per chromosome, and fitted the multiple genetic variance components into linear mixed models as above. We found that the chromosomal contributions were not proportional to the number of independent SNPs in each of the chromosomes **[Figure 1]**. For example, the contribution of chromosomes 9, 11, 17 and 19 to high-grade serous EOC were larger than expected the 95% confidence interval (approximated through jackknifing 1000 times) did not overlap with 1. In contrast chromosomes 4, 10, 12, 14, 18 and 20 contributed less than expected.

Genetic correlation between EOC subtypes

We used the GRM as a random effect in a bivariate mixed-effects linear model implemented in GCTA to assess genetic heterogeneity across EOC histologic subtypes. **Table 2** summarizes the genetic correlations between the various EOC subtypes. We found significant genetic overlap between high-grade serous EOC and endometrioid EOC (r_g = 0.63 ± 0.27 ; *P*=.0029). Given that high-grade serous disease is not infrequently misclassified as endometrioid EOC (Gilks et al. 2008), we also estimated the genetic correlations separating (G1/G2) endometrioid disease from (G3). Here we found that the genetic correlation between high-grade serous and G1/G2 endometrioid cancer was lower (r_g = 0.33 ± 0.23; P=.062) than between G3 endometrioid and high-grade serous cancer (r_g = 1.00 ± 0.83; P=.00078), suggesting that potential misclassification may have inflated the genetic correlation estimate when using all endometrioid EOC. Interestingly, we observed an appreciable but non-significant genetic overlap of about r_g = 0.5 between low-grade endometrioid and clear-cell EOC. We also found that the genetic correlations between "unknown/unclassified" EOC and high-grade serous and high-grade endometrioid disease were significant and essentially 1 (r_g = 1.0 ± 0.30; *P*=10^{-7} and r_g = 1.0 ± 0.96 *P*=.0049, respectively). The REML bivariate analyses involving Mucinous did not converge so did not yield any meaningful estimates. Further, removing known associated loci from the analyses affected the genetic correlation between endometrioid EOC (high and low grade) in a way that this was no longer significant [**Table 2**].

Given that splitting the controls during the bivariate analyses to avoid sample overlap could have resulted in decreased power to detect genetic correlations; we complemented the genetic correlation analysis with the cross-trait LD score regression method, which is not biased by overlapping samples. In line with our results above, we found a statistically significant genetic correlation between high-grade serous EOC and endometrioid EOC (r_g =0.67 ± 0.25; *P*=7.4E-03), high-grade serous EOC and unknown EOC (r_g = 0.63 ± 0.25; *P*=.013) and endometrioid EOC and unknown EOC (r_g =1.00 ± 0.30; *P*=5.7E-04) **[Table 3]**.

Genetic overlap of EOC subtypes and associated environmental factors

In order to investigate the genetic overlap between all EOC and age at menarche, BMI,

obesity, smoking, height and diabetes we used the cross-trait LD score regression method as well as a polygenic risk-prediction approach. We did not detect any significant genetic correlations using cross-trait LD score regression **[Table 4]**. However, through the polygenic risk prediction approach, we found significant genetic overlap (at Bonferroni P-value threshold = .00029) of all EOC with obesity and with diabetes **[Table 5]**. The genetic overlap with diabetes appeared mainly in association with mucinous EOC. Overall, the directions of association are consistent with what has been reported in observational studies (Aune et al. 2015; Collaborative Group on Epidemiological Studies of Ovarian 2012; Faber et al. 2013; Olsen et al. 2013), although most of these associations are not significant.

Discussion

In this work, we have investigated the genetic architecture of EOC and its different subtypes. Our univariate analyses show an extent of hidden heritability inherent in the iCOGS array, with known associated loci accounting for about 40% of the total array heritability for most EOC histotypes, except for high-grade endometrioid, where they account for most of h_g^2. Is important to note that to reach these estimates we removed 2Mb per locus, which was done to ensure that no effect of these loci remained; however, this could also have inflated the estimates. We also showed that the hidden heritability is not spread proportionally across the chromosomes, with some contributing very little to the array heritability and others up to 5 times more than expected given their iCOGS SNP compositions. A limitation in our univariate experiments was that it was underpowered to compute meaningful estimates for low-grade serous and mucinous EOC. Although we had a bigger sample size for mucinous EOC than clear cell EOC, the analyses could have been affected by how each individual study deal with mucin-producing peritoneal tumours.

Using bivariate linear mixed-model and cross-trait LD score-regression approaches, we investigated genetic correlations between the various EOC subtypes. The bivariate linear mixed model provides unbiased estimates of genetic correlation and it requires individual genotype data in order to compute the GRM. Cross-trait LD score regression only requires summary results from the discovery set, and in contrast to the bivariate mixed-model approach, it allows sample overlap (in this case, overlapping controls) (Bulik-Sullivan et al. 2015a). Whilst studies have shown shared germ-line risk mutations across the various EOC subtypes, these account for only a small fraction of general heritability (Bojesen et al. 2013; Bolton et al. 2010; Goode et al. 2010; Permuth-Wey et al. 2013; Pharoah et al. 2013; Song et al. 2009). We found a very high genetic correlation between high-grade serous EOC and poorly differentiated (G3, high-grade) endometrioid disease, and with unknown/unclassified EOC, which represents undifferentiated epithelial carcinoma. These correlations seem entirely reasonable, because high-grade endometrioid disease is sometimes misdiagnosed as high-grade serous, or may constitute a version of high-grade serous with slightly different differentiation. Undifferentiated ovarian carcinoma clinically resembles high-grade serous in

response to treatment and in mortality. Low-grade serous, low-grade endometrioid and clear-cell carcinoma (which is relatively low grade) are heritability-distinct from the high-grade diseases and behave that way. Mucinous ovarian cancer seems to be a largely separate disease and has its own set of risk factors (Risch et al. 1996). It does not appear to be related heritably to the other ovarian cancer histotypes.

We also considered whether the heritability of EOC and its subtypes could be explained (at least partly) via factors such as obesity, height, diabetes, smoking and age at menarche. As these factors have genetic components, it is plausible that the heritability of EOC could reflect the heritability of a causal factor. Using cross-trait LD score regression, we had insufficient power to detect genetic correlations, as this approach is greatly affected by small numbers of SNPs and by small sample sizes. However, through a polygenic risk prediction approach – which, although it does not directly quantify genetic overlap, is powerful for detecting genetic correlations between traits when the discovery and target sets are well powered (Dudbridge 2013), we found a significant positive genetic overlap between diabetes, obesity and all EOC. This genetic overlap appeared to be concentrated within mucinous disease and may not reflect other EOC histotypes. Genetic correlation in this analysis is estimated based on a large number of SNPs, so it is possible that the correlations seen between diabetes and obesity and EOC may be mediated by an upstream phenotype (e.g. hormonal changes). Genetic overlap analyses between EOC and the other risk factors did not reveal any other significant associations. Potential reasons for this include small sample sizes for some of the EOC subtypes, and incomplete mapping of relevant variants of the risk factors (i.e., variants in the iCOGS array explain only a limited amount of variance of the risk factors).

Is important to note that our results were derived from SNPs tagged in the iCOGS array. Hence the numbers of SNPs included in the analyses (195,183 SNPs) are smaller than in a typical GWAS array. Additional analyses could be performed on imputed genotypes from the iCOGS data; however, the iCOGS array is not designed to tag the whole genome, so imputation would likely still be limited to the existing tagged regions. Nevertheless, this array, which included several SNPs associated with other cancer types as well as with relevant quantitative traits such as BMI and the onset of menarche (Pharoah et al. 2013), allowed us to establish reasonably accurate estimates where the target sample sizes were well powered (e.g., high-grade serous, endometrioid, unknown/undifferentiated, and all EOC).

In summary, our results show that the major important EOC subtypes are genetically very homogeneous, and likely arise from a combination of known risk factors plus genetic contributions (beyond the known genetic predisposition mutations). This commonality highlights that high-grade disease could be considered a single clinical entity, with perhaps only minor variation between the serous, endometrioid and undifferentiated types. Low-grade histotypes, as well as mucinous ovarian cancer, likely represent more distinct pathologic variation. We also found that a great proportion of heritability is "missing". Our

analyses will be complemented once data of individuals genotyped in the OncoArray, which integrates a GWAS backbone, becomes available.

Acknowledgements

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Table 1. Array heritabilities (h^2_g) and standard errors (s.e.) for invasive EOC according to histological subtype. Results for all iCOGS SNPs and after removing known associated loci. Disease prevalence of EOC subtypes is calculated as the lifetime risk of ovarian cancer multiplied by the relative proportion of the corresponding EOC subtype. See Methods section . Bolded estimates are statistically significantly different from 0.

Subtype	Cases	Controls	Lifetime risk	All SNPs			Removing Known Loci*		
				h^2_g	s.e.	P-value	h^2_g	s.e.	P-value
High-grade Serous	4098	21233	0.0055	**0.088**	**0.010**	**2.2E-16**	**0.047**	**0.009**	**1.83E-09**
Clear cell	620	21233	0.0005	**0.067**	**0.033**	**0.017**	0.046	0.029	0.058
Endometrioid (all)	1342	21233	0.001	**0.032**	**0.016**	**0.016**	0.020	0.014	0.077
Endometrioid G1/G2	906	21233	0.001	**0.044**	**0.024**	**0.025**	**0.037**	**0.021**	**0.037**
Endometrioid G3	436	21233	0.001	0.049	0.046	0.127	0.009	0.041	0.417
Mucinous	658	21233	0.0005	0.000	0.028	0.5	0.000	0.025	0.5
Unknown	2934	21233	0.009	**0.070**	**0.015**	**1.1E-10**	**0.041**	**0.012**	**1.1E-04**
All	10014	21233	0.009	**0.056**	**0.006**	**2.2E-16**	**0.036**	**0.005**	**2.2E-16**

*Loci removed: WNT4, RSPO1, SYNPO2, GPX6, ABO, ATAD5, C19orf62, CMYC, TIPARP, BNC2, ARHGAP27, TERT, RAD51B/C/D, BRIP1, BARD1, PALB2, NDN, CHMP4C, MLLT10, HNF1B, *BRCA1, BRCA2, KRAS, TP53, HER2, AR1D1A* and *PIK3CA.*

Table 2. Genetic correlations and (standard error) between major EOC subtypes as estimated from iCOGS array. Lower triangular matrix shows the genetic correlation using all the SNPs in the iCOGS array, while the upper triangular matrix shows the genetic correlation after removing known associated loci. For these calculations, each case was matched to one control in a way that none of the subtypes share any controls. Analyses for mucinous and low-grade serous EOC subtypes were underpowered to yield reliable estimates.

Subtype	High-grade Serous	Endometri oid (all)	Endometri oid G1/G2	Endometri oid G3	Clear Cell	Unknown
High-grade Serous	-	0.48 (0.35) P=0.072	0.24 (0.30) P=0.21	1.0 (2.66) P=0.5	0.29 (0.42) P=0.24	**1.0 (0.510) P=5.1E-04**
Endometrioid (all)	**0.63 (0.27) P=0.0029**	-	-	-	0.73 (0.64) P=0.088	0.50 (0.47) P=0.12
Endometrioid G1/G2	0.33 (0.23) P=0.062	-	-	0.36 (1.25) P=0.30*	0.42 (0.53) P=0.20	0.37 (0.41) P=0.18
Endometrioid G3	**1.0 (0.83) P=7.8E-04**	-	0.42 (0.56) P=0.2*	-	1.00 (1.68) P=0.5	1.0 (4.44) P=0.5
Clear Cell	0.28 (0.33) P=0.18	0.69 (0.56) P=0.074	0.52 (0.54) P=0.14	0.99 (0.87) P=0.073	-	0.09 (0.55) P=0.43
Unknown	**1.0 (0.30) P=1.0E-07**	**0.68 (0.33) P=0.0082**	0.42 (0.29) P=0.057	**1.0 (0.96) P=0.0049**	0.15 (0.39) P=3.5E-01	-

Bolded estimates are significantly different from 0.

* Significance (P-value) where the null hypothesis rG=1.

Table 3. Cross-trait LD score regression between EOC subtypes. Estimates and (standard errors) are reported. Analyses for mucinous and low-grade serous EOC subtypes were underpowered to yield reliable estimates.

	HG Serous	Endometrioid	Endometrioid G1/G2	Endometrioid G3	Clear Cell	Unknown
HG Serous	-	0.82 (0.49) P=0.095	0.35 (0.41) P=0.41	1.0 (1.17) P=0.20	-	0.46 (0.46) P=0.31
Endometrioid	**0.67 (0.25) P=0.0074**	-	-	-	-	**1.0 (0.41) P=0.01**
Endometrioid G1/G2	0.35 (0.25) P=0.15	-	-	0.49 (0.70) P=0.47*	-	**0.85 (0.40) P=0.035**
Endometrioid G3	1.0 (0.79) P=0.15	-	0.53 (0.67) P=0.48*	-	-	1.0 (0.73) P=0.15
Clear Cell	0.53 (0.57) P=0.35	0.91 (0.80) P=0.26	0.71 (0.59) P=0.23	1.00 (1.06) P=0.29	-	-
Unknown	**0.63 (0.25) P=1.3E-02**	**1.0 (0.30) P=5.7E-04**	**0.77 (0.33) P=0.02**	1.00 (0.79) P=0.14	0.38 (0.53) P=0.47	-

Bolded estimates are significantly different from 0.

Table 4. Genetic correlation between risk factors and EOC subtypes using cross-trait LD score regression. Estimates and (standard errors) are reported. Analyses for mucinous and low-grade serous EOC subtypes were underpowered to yield reliable estimates.

	All	HG Serous	Endometrioid	Clear Cell	Unknown
BMI	0.045 (0.07) P=0.52	-0.04 (0.08) P=0.63	0.18 (0.11) P=0.10	-0.01 (0.16) P=0.96	0.07 (0.08) P=0.38
Smoking	-0.34 (0.29) P=0.23	-0.43 (0.33) P=0.20	-0.37 (0.43) P=0.39	-0.44 (0.66) P=0.51	-0.17 (0.31) P=0.58
Height	0.081 (0.062) P=0.19	0.13 (0.09) P=0.15	0.03 (0.09) P=0.69	0.24 (0.17) P=0.17	0.00 (0.08) P=0.98
Menarche	-0.07 (0.08) P=0.38	-0.23 (0.13) P=0.06	-0.04 (0.12) P=0.75	0.32 (0.36) P=0.36	0.05 (0.09) P=0.59
Obesity* >30 BMI	0.05 (0.09) P=0.58	-0.02 (0.09) P=0.86	0.26 (0.17) P=0.13	-0.18 (0.26) P=0.50	0.12 (0.11) P=0.27
Obesity* >35 BMI	0.019 (0.087) P=0.83	-0.03 (0.11) P=0.80	0.02 (0.18) P=0.90	-0.23 (0.37) P=0.54	0.17 (0.12) P=0.17
Obesity* >40 BMI	-0.02 (0.15) P=0.88	-0.02 (0.17) P=0.92	-0.06 (0.30) P=0.84	NA	0.03 (0.19) P=0.89
Diabetes	0.04 (0.12) P=0.75	-0.04 (0.14) P=0.74	0.04 (0.19) P=0.84	-0.29 (0.38) P=0.45	0.21 (0.14) P=0.15

*Reference group was individuals with BMI <=25

Table 5. Odds Ratios corresponding to 1 standard deviation increase in the PGRS and significance estimates (P-values) from the polygenic risk prediction approach between "environmental factors"

PGRS and EOC subtypes. The displayed numbers correspond to the best association p-value out of the 11 different PGRS which were derived using different p-value thresholds. In this part we used the total set of controls with each of the EOC subtypes.

	HG Serous	Mucinous	Clear Cell	Endometrioid	Unknown	ALL
Menarche	0.99 (0.54)	1.09 (0.036)	1.05 (0.2)	1.04 (0.12)	1.04 (0.086)	1.02 (0.17)
BMI	1.04 (0.028)	1.05 (0.26)	1.06 (0.17)	1.07 (0.011)	1.04 (0.068)	1.04 (0.003)
Smoking	1.03 (0.11)	0.93 (0.067)	0.92 (0.049)	1.04 (0.18)	0.95 (0.0071)	0.97 (0.019)
Height	1.03 (0.14)	1.1 (0.015)	1.1 (0.025)	1.04 (0.17)	0.96 (0.06)	1.03 (0.022)
Diabetes	1.04 (0.021)	**1.18 (1.1e-05)**	1.08 (0.067)	1.07 (0.011)	1.04 (0.034)	**1.05 (4.1e-04)**
Obesity >30BMI	1.05 (0.0051)	1.06 (0.15)	1.06 (0.14)	1.04 (0.19)	1.04 (0.032)	**1.05 (2.6e-04)**
Obesity >35BMI	1.03 (0.08)	1.05 (0.21)	0.9 (0.012)	1.02 (0.42)	1.05 (0.028)	1.04 (0.0053)
Obesity >40BMI	1.03 (0.15)	1.06 (0.14)	0.87 (0.0015)	0.96 (0.13)	1.03 (0.19)	0.98 (0.21)

Bolded estimates are statistically significant (Bonferroni P-value threshold 2.9×10^{-4}).

*Reference group was individuals with BMI <=25

Figure 1. Contribution to the heritability by chromosome versus expected. Black vertical lines show the 95% confidence intervals approximated through jackknifing up to 1000 times. These are only shown for those instances that do not overlap with 1 to facilitate visualization. The same graph with all confidence intervals is included as supplementary figure 2.
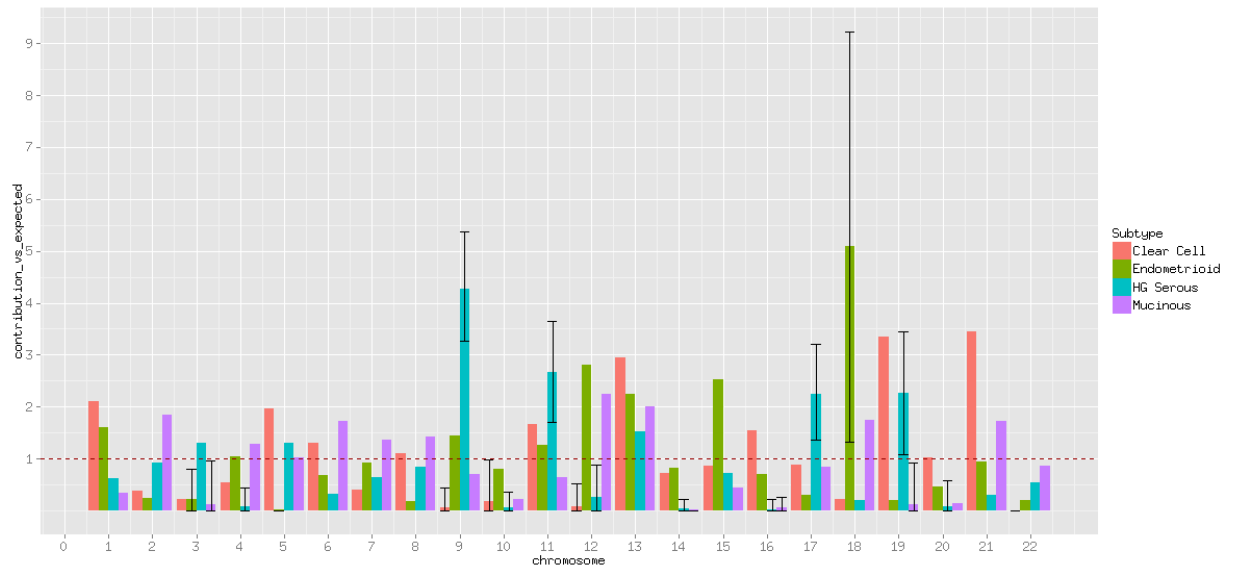
Supplementary Material: Assessing the Genetic Architecture of Epithelial Ovarian Cancer Histological Subtypes.
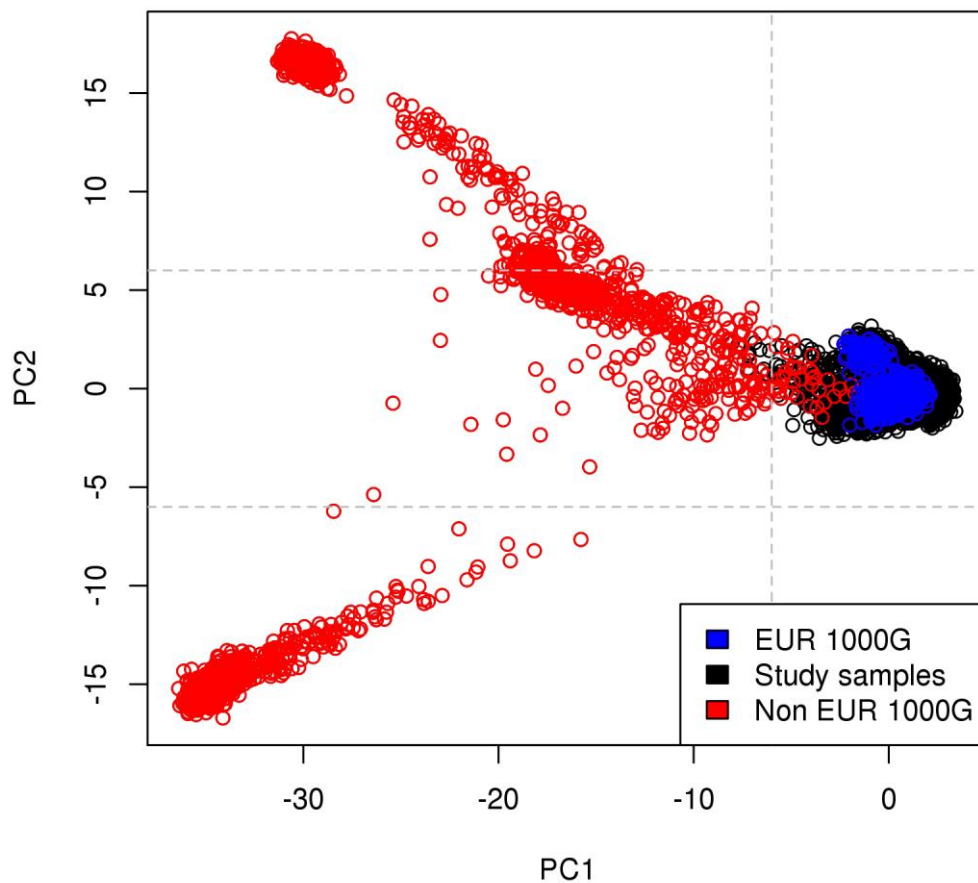
Supplementary Figure 1. Genotype principal component analysis of OCAC samples and 1000 Genomes. X and Y axes display the number of standard deviations from 1000 Genomes EUR populations. Dotted lines enclose the samples used in this study.

Study Name	Country	Code	Controls	Serous	Mucinous	Endometrioid	Clear Cell	HG Serous	Other	All invasive
Australian Cancer Study	Australia	ACS	175	104	7	22	9	89	32	166
Australian Ovarian Cancer Sutidy	Australia	AOC	802	448	35	84	43	409	118	714
Bavarian Ovarian Cancer Cases and Controls	Germany	BAV	142	56	8	13	6	42	10	93
Belgium Ovarian Cancer Study	Belgium	BEL	1348	194	23	22	23	182	17	274
Diseases of the Ovary and their Evaluation	USA	DOV	1119	293	18	84	29	235	136	515
Diseases of the Ovary and their Evaluation	USA	DVE	368	233	8	64	36	200	78	389
Germany Ovarian Cancer Study	Germany	GER	413	95	21	21	6	68	59	189
Hawaii Ovarian Cancer Study	USA	HAW	156	38	3	12	5	36	2	60
Hannover-Jena Ovarian Cancer Study	Germany	HJO	273	140	9	26	4	107	116	266
Hannover-Minsk Ovarian Cancer Study	Germany	HMO	138	50	7	12	1	1	121	142
Helsinki Ovarian Cancer Study	Finland	HOC	447	113	45	28	13	0	135	221
Hormones and Ovarian Cancer Prediction	USA	HOP	1464	377	30	84	42	333	145	654
Danish Malignant Ovarian Tumor Study	Denmark	MAL	828	272	42	54	33	183	53	440
Mayo Clinic Ovarian Cancer Case Control Study	USA	MAY	10	9	0	1	0	9	0	10
Melbourne Collaborative Cohort Study	Australia	MCC	65	34	7	7	6	19	21	63
MD Anderson Ovarian Cancer Study	USA	MDA	384	190	27	28	4	135	179	373
Memorial Sloan Kettering Cancer Center	USA	MSK	593	382	0	20	18	343	73	467
North Carolina Ovarian Cancer Study	USA	NCO	172	147	18	35	24	132	50	269
New England Case-Control Study	USA	NEC	979	371	41	140	33	331	60	634
Nurses' Health Study I and II	USA	NHS	425	68	7	14	6	0	100	127
New Jersey Ovarian Cancer Study	USA	NJO	180	100	7	27	20	80	27	169
University of Bergen, Haukeland University Hospital, Norway	Norway	NOR	370	135	15	27	11	85	87	237
Nijmegen Ovarian Cancer Study	Netherlands	NTH	323	116	33	64	20	64	52	255
Ovarian Cancer in Alberta and British Columbia	Canada	OVA	748	344	26	103	57	0	445	631
Polish Ovarian Cancer Study	Poland	POC	417	199	33	39	9	0	341	422
Polish Ovarian cancer Case Control Study (NCI)	Poland	POL	186	21	4	10	2	15	11	42
UK Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) Ovarian Cancer Study	UK	SEA	1196	162	38	24	28	104	71	271
UK Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) Ovarian Cancer Study	UK	SEB	4826	11	5	5	0	4	15	29
Southampton Ovarian Cancer Study	UK	SOC	0	102	33	62	11	72	79	267
Family Registry for Ovarian Cancer AND Genetic Epidemiology of Ovarian Cancer	USA	STA	313	154	16	32	20	135	35	251
Familial Ovarian Tumor Study	Canada	TOR	74	8	1	7	2	0	11	21

UC Irvine Ovarian Cancer Study	USA	UCI	367	166	19	48	23	143	32	277
UK Ovarian Cancer Population Study	UK	UKO	1103	117	24	32	25	93	51	236
Los Angeles County Case-Control Studies of Ovarian Cancer	USA	USC	1047	447	44	79	35	341	161	689
Warsaw Ovarian Cancer Study	Poland	WOC	203	132	8	20	17	131	25	202
Total*			21654	5828	662	1350	621	4121	2948	10065

Supplementary Table 1. Description of individual OCAC studies and case-control sample size. *Numbers differ from Table 1 in main manuscript, as these ones reflect the total number before Identity by descent (IBD) <0.10 filtering.
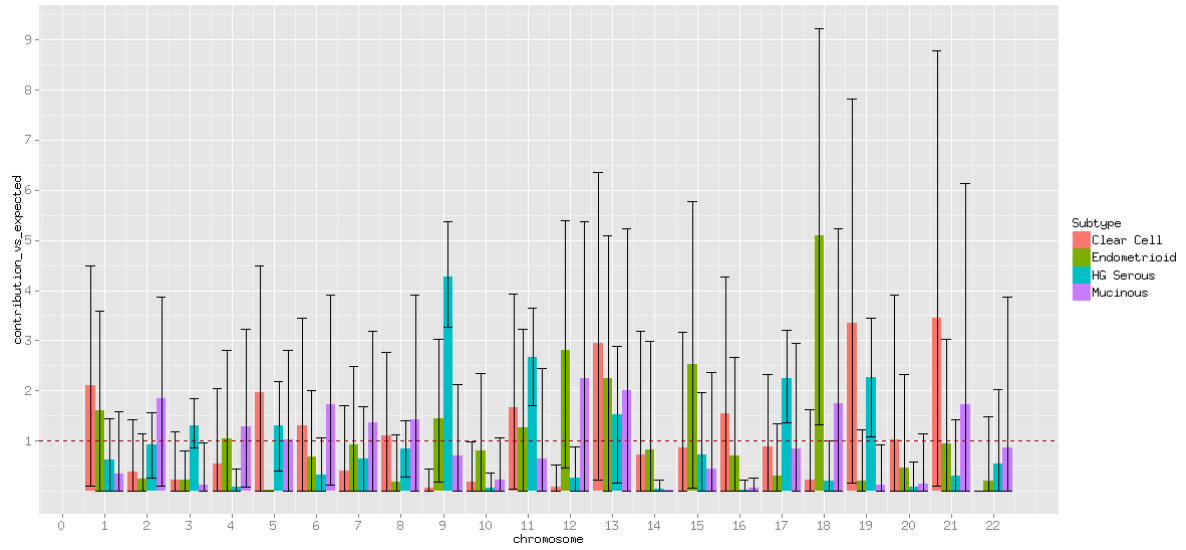
Supplementary Figure 2. Contribution to the heritability by chromosome versus expected. Confidence intervals [0.05,0.95] were approximated through jackknifing up to 1000 times