Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2011 June ; 20(6): 1131–1145. doi:10.1158/1055-9965.EPI-10-1224.

Inherited Variants in Mitochondrial Biogenesis Genes May Influence Epithelial Ovarian Cancer Risk

Jennifer Permuth-Wey[1,2], **Y. Ann Chen**[3], **Ya-Yu Tsai**[1], **Zhihua Chen**[4], **Xiaotao Qu**[4], **Johnathan M. Lancaster**[5], **Heather Stockwell**[2], **Getachew Dagne**[2], **Edwin Iversen**[6], **Harvey Risch**[7], **Jill Barnholtz-Sloan**[8], **Julie M. Cunningham**[9], **Robert A. Vierkant**[10], **Brooke L. Fridley**[10], **Rebecca Sutphen**[11], **John McLaughlin**[12], **Steven A. Narod**[13], **Ellen L. Goode**[10], **Joellen M. Schildkraut**[14], **David Fenstermacher**[4], **Catherine M. Phelan**[1], and **Thomas A. Sellers**[1]

[1]Cancer Epidemiology Program, Division of Population Sciences, Moffitt Cancer Center, Tampa, FL, USA.

[2]Department of Epidemiology and Biostatistics, College of Public Health, University of South Florida, Tampa, FL, USA.

[3]Department of Biostatistics, Moffitt Cancer Center, Tampa, FL, USA.

[4]Department of Biomedical Informatics, Moffitt Cancer Center, Tampa, FL, USA.

[5]Department of Women's Oncology, Moffitt Cancer Center, Tampa, FL, USA.

[6]Department of Statistical Science, Duke University Medical Center, Durham, NC, USA.

[7]Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, USA.

[8]Case Comprehensive Cancer Center, Case School of Medicine, Cleveland, OH, USA.

[9]Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, MN, USA.

[10]Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN, USA.

[11]Pediatrics Epidemiology Center, College of Medicine, University of South Florida, Tampa, FL, USA.

[12]Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada.

[13]Center for Research in Women's Health, Toronto, ON, Canada.

[14]Department of Community and Family Medicine, Duke University Medical Center, Durham, NC, USA.

Abstract

Background—Mitochondria contribute to oxidative stress, a phenomenon implicated in ovarian carcinogenesis. We hypothesized that inherited variants in mitochondrial-related genes influence epithelial ovarian cancer (EOC) susceptibility.

Address correspondence to: Thomas A. Sellers, PhD, Director, Moffitt Research Institute, Executive Vice President/Associate Center Director, Cancer Prevention & Control Division, 12902 Magnolia Drive, MRC-CANCONT, Tampa, FL 33612-9416, P: 813-745-1315, F: 813-745-6525, Thomas.Sellers@Moffitt.org.

Methods—Through a multi-center study of 1,815 Caucasian EOC cases and 1,900 controls, we investigated associations between EOC risk and 128 single nucleotide polymorphisms (SNPs) from 22 genes/regions within the mitochondrial genome (mtDNA) and 2,839 nuclear-encoded SNPs localized to 138 genes involved in mitochondrial biogenesis (BIO, n=35), steroid hormone metabolism (HOR, n=13), and oxidative phosphorylation (OXP, n=90) pathways. Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) between genotype and case status. Overall significance of each gene and pathway was evaluated using Fisher's method to combine SNP-level evidence. At the SNP-level, we investigated whether lifetime ovulation, hormone replacement therapy (HRT), and cigarette smoking were confounders or modifiers of associations.

Results—Inter-individual variation involving BIO was most strongly associated with EOC risk (empirical P=0.050), especially for *NRF1*, *MTERF*, *PPARGC1A*, *ESRRA*, and *CAMK2D*. Several SNP-level associations strengthened after adjustment for non-genetic factors, particularly for *MTERF*. Statistical interactions with cigarette smoking and HRT use were observed with *MTERF* and *CAMK2D* SNPs, respectively. Overall variation within mtDNA, HOR, and OXP was not statistically significant (empirical P>0.10).

Conclusion—We provide novel evidence to suggest that variants in mitochondrial biogenesis genes may influence EOC susceptibility.

Impact—A deeper understanding of the complex mechanisms implicated in mitochondrial biogenesis and oxidative stress may aid in developing strategies to reduce morbidity and mortality from EOC.

Keywords

polymorphisms; oxidative stress; genetic susceptibility; mitochondria; ovarian cancer

INTRODUCTION

Epithelial ovarian cancer (EOC) is the ninth most commonly diagnosed female cancer and the most lethal gynecologic malignancy in the United States (1). Although the etiology remains largely unknown, there are two leading hypotheses. The incessant ovulation hypothesis (2) states that repeated ovulations over-stimulate the ovarian surface epithelium, causing inflammation and increased cellular proliferation during epithelial repair, leading to DNA replication errors and malignant transformation. The gonadotropin hypothesis (3) posits that gonadotropins are responsible for this process. Supporting these hypotheses, epidemiologic studies have shown that factors which interrupt ovulation and/or lower gonadotropin levels, including pregnancy, oral contraceptive use, and lactation, are inversely associated with risk (4). However, factors unrelated to hormones, reproduction, or ovulation may also influence risk, suggesting alternate mechanisms may also contribute to the genesis of EOC (4).

Emerging experimental and epidemiologic evidence suggests EOC development and progression may be caused by oxidative stress (5–9), a phenomenon arising due to overproduction of reactive oxygen species (ROS) in mitochondria (10). Mitochondria are semiautonomous membrane-bound organelles that participate in free radical production, apoptosis, and energy metabolism (11). Aside from the nucleus, the mitochondrion is the only cellular organelle that contains its own genome (mtDNA) and genetic machinery. MtDNA is a maternally-inherited, 16.6 kilobase double-stranded, closed circular molecule, that encodes 37 genes (13 polypeptides, 22 transfer RNAs, and 2 ribosomal RNAs) mainly involved in oxidative phosphorylation (OXP), a process whereby molecular oxygen is oxidized to water, creating adenosine triphosphate (ATP) and the by-product, ROS (11).

Approximately 1500 proteins encoded by nuclear DNA (nDNA) generate new mitochondria (known as mitochondrial biogenesis) and maintain mitochondrial structure and function by regulating processes such as OXP, apoptosis, and mtDNA replication, transcription, and translation (11). Importantly, interplay between the genomes can influence disease processes, and studies implicate nDNA-encoded genes involved in mitochondrial biogenesis as regulators of nuclear-mitochondrial interactions (5, 11, 12).

While much attention has been focused on somatic mtDNA mutations (11, 13), less research has been directed to the influence of germline variants on mitochondrial dysfunction and cancer development. Single nucleotide polymorphisms (SNPs) in mtDNA and/or nDNA may enhance cancer risk through subtle changes in encoded proteins, altered OXP activity, and excess ROS production over time (Figure 1) (10, 13). In particular, variation involving mtDNA (13) and the following three categories or 'pathways' of nDNA may alter cancer risk by promoting oxidative stress. *Mitochondrial biogenesis (BIO)* proteins represent transcription factors and co-activators that regulate mtDNA and nDNA involvement in OXP; because defective mitochondrial biogenesis may promote slower mitochondrial turnover and altered OXP activity, control of this process is integral for maintenance of energy production and prevention of ROS accumulation (12, 14, 15). Similar to their mtDNA-encoded counterparts, nDNA-encoded *(OXP)* proteins, when impaired, may alter OXP activity and contribute to diseases including hereditary cancer syndromes (16). S*teroid hormone metabolism (HOR)* proteins, primarily receptors localized to mitochondria, modulate mitochondrial gene expression and OXP activity (17, 18). SNPs in these pathways may also influence risk by interacting with one another or with non-genetic factors known to contribute to ROS accumulation (Figure 1).

Few studies have examined potential associations between inherited mitochondrial SNPs and/or haplotypes and cancer risk (19–25). Only one study (25) investigated nDNA in addition to mtDNA, and only one small study in China (22) involved ovarian cancer. Thus, there is a need for large-scale studies that comprehensively evaluate inherited mitochondrial variation and EOC risk and incorporate epidemiologic risk factors into the analysis.

METHODS

Study Design and Population

The study protocol was approved by the institutional review board at each center, and all study participants provided written informed consent. Data derive from four case-control studies of EOC: the Mayo Clinic Ovarian Cancer Study (MAY) (Rochester, MN), Duke University's North Carolina Ovarian Cancer Study (NCO) (Durham, NC), the University of Toronto Familial Ovarian Tumor Study (TOR) (Ontario, Canada), and H. Lee Moffitt Cancer Center and Research Institute's Tampa Bay Ovarian Cancer Study (TBO) (Tampa, FL). Study characteristics are summarized in Table 1. All studies recruited incident, pathologically confirmed primary EOC cases, either borderline or invasive, aged 20 and above. Three studies used population-based rapid ascertainment for the cases (NCO, TOR, TBO), and one study was clinic-based (MAY). All controls had at least one ovary intact at the reference date and were frequency-matched to cases on age-group and race.

Demographic data and information on known and suspected EOC risk factors was collected from participants using study-specific questionnaires. Data collected included race/ethnicity, age at diagnosis/interview, menstrual and reproductive history, exogenous hormone use, medical and surgical history, adult height and weight, smoking history, and family history of ovarian and breast cancer in first-degree relatives. To increase etiologic homogeneity, we excluded cases with non-epithelial or borderline tumors, non-whites, known *BRCA1* and *BRCA2* mutation carriers, Jewish women (because of the likelihood of carrying a *BRCA*

mutation), and women with a prior history of ovarian, breast, endometrial, or early-onset colorectal cancer (because of the likelihood of having a mismatch repair defect).

Biospecimen Collection and Processing

Blood served as the source of genomic DNA and was collected at each study site at the conclusion of the interview (NCO, TBO, and TOR) or in the course of medical care (MAY). Genomic DNA was isolated from whole blood using PureGene DNA isolation reagents (Gentra Systems, Minneapolis, MN), re-suspended in TE buffer, and stored at 4°C. Samples were bar-coded with a unique subject identification number to ensure accurate and reliable sample processing and storage.

Genotyping Method and Quality Control

All samples were genotyped using the Illumina Infinium 610K Array at the Mayo Clinic Genotyping Shared Resource Facility (Rochester, MN) by laboratory personnel blinded to case-control status. Each 96-well plate contained 375 ng DNA of random mixtures of case and control samples, two blind duplicates, and two replicates of a CEPH family trio (mother, father, child) from the Coriell Institute. A quality assurance (QA) panel of 96 SNPs was run on the Illumina Bead Express platform to test sample performance and ensure concordance of replicate samples.

Illumina's Genome Studio™ software was used to perform automated genotype clustering and calling of the potential 550,000 beadtypes. SNPs were excluded from further analysis if a) the call rate was <95%, b) they were monomorphic upon manual clustering, or c) there were unresolved replicate errors. Among 81 pairs of replicate samples, the overall concordance rate was 99.93%.The overall genotype call rate was 99.7%.

We attempted to genotype unique samples from 4,150 eligible subjects. Of these, 394 were excluded because genotypes were generated at fewer than 95% of SNPs (i.e. sample call rate <95%); 81.4% of these exclusions (n=321) were attributed to poor quality DNA from the TOR site. Other reasons for exclusion included sample failure under the QA panel (n=15), ambiguous gender (n=7), unresolved identical genotypes (n=8), self-report as non-Caucasian (n=2), and those predicted by STRUCTURE (26) analysis to have less than 80% European ancestry (n=9). This resulted in a final sample size of 3,715 subjects (1,815 cases and 1,900 controls).

Identification of candidate genes and SNPs

MtDNA content on the 610K array was determined by comparing an annotation file supplied by Illumina with the MitoMap database (27). Of 138 mtDNA SNPs from 22 genes and 8 regulatory regions included on the 610K array, 128 (92%) had call rates greater than 95%, but only 24 SNPs had MAF greater than or equal to 5% (Table 2). Information regarding nDNA proteins was derived from published literature and several databases, including MitoProteome (28), the Human Mitochondrial Protein Database (29), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database. A total of 3064 SNPs in 142 nDNA genes of interest (35 BIO, 14 HOR, and 93 OXP) are on the 610K array and of these, 2839 SNPs (93%) from 138 genes had call rates greater than 95% (Table 2). To evaluate evidence for SNP-level associations at nDNA markers that were not directly genotyped, imputation was carried out using MACH version 1.0.16 using phased data from HapMap release 22 (genome build 36) on individuals with European ancestry (CEU). We imputed 4,445 SNPs that were not genotyped as part of the 610K array that reside within the 138 nDNA-encoded genes.

Statistical Methods

Descriptive statistics were generated using means and standard deviations for continuous variables and frequencies and percents for categorical variables. Distributions of covariates among cases and controls were compared within each study site using t-tests and X^2 tests for continuous and categorical variables, respectively. Genotype frequencies of nDNA SNPs among controls were tested for HWE using X^2 goodness-of-fit tests. Statistical analysis was performed using SAS Version 9.1 (SAS Inc., Cary, NC) and PLINK software (30).

Association testing was performed for SNPs, haplotypes, genes, and pathways. To examine associations between each SNP and EOC risk, unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) between carriage of the minor versus major allele of maternally-inherited mtDNA SNPs and case status, and between genotypes and case status for nDNA SNPs. Log-additive, dominant, and recessive genetic models were fit for each nDNA SNP; the major allele was considered to be the reference allele during modeling. All models were adjusted for the design variables of age (continuous) and study site (indicator variables for NCO, TBO, and TOR). Due to the potential for population stratification, models were also adjusted for a quantitative variable for the first principal component representing European ancestry (26). Wald X^2 tests were used to obtain P values for dominant and recessive SNP effects, and the Cochran Armitage trend test was used to estimate P for trend for log-additive effects. The best-fitting model was represented by the one with the smallest P value. All P values were two-sided, and a nominal $P < 0.05$ was considered the threshold of significance for SNP-level tests.

We permuted case-control status 100,000 times to generate point-wise empirical p-values (EMP1) using PLINK. The EMP1 represents the proportion of permutations in which the minimum simulated P value was less than the observed P value. To predict the potential functional significance of risk-associated and strongly correlated ($r^2>0.80$) SNPs, we used the SNPinfo(31) and FastSNP (32) web-based tools.

To evaluate whether established or suspected EOC risk factors (lifetime ovulation, hormones, cigarette smoking) postulated to contribute to oxidative stress may confound the most promising SNP-EOC risk associations, we conducted analyses among subjects with complete data for variables of interest (N=2662; 1217 cases, 1445 controls, representing 72% of 3715 total subjects). Similar to other studies that estimated lifetime ovulation (33), a composite variable for lifetime ovulatory cycles (LOC) was estimated by considering self-reported ovulatory and anovulatory periods using the following modified formula:

$$\text{LOC}=[[\text{index age}-\text{age at first menstrual period}]-[(\text{number of months pregnant}+\text{number of months of oral contraceptive use})/12\ \text{mont}$$

where 13 cycles/year is assumed based on an average cycle length of 28 days. For post-menopausal women, index age was defined as age at menopause. For pre- or peri-menopausal women, age at interview/ diagnosis served as the index age. The LOC composite variable was subsequently categorized based on tertiles of the distribution among controls. Other factors that may influence LOC, such as breastfeeding, missed or irregular periods, or spontaneous or elective abortions, were not considered because pertinent data were not ascertained by or available from all participating sites. For each SNP investigated, the model included age (continuous), study site (indicator variable), LOC category (low, medium, high), duration of unopposed estrogen or combined estrogen-progestin hormone replacement therapy (HRT) use (continuous), and pack-years of cigarettes smoked (continuous).

To explore SNP-environment interactions, modifying effects of the following non-genetic variables were considered: LOC category (high and medium tertile vs. lowest tertile), HRT use (ever versus never), smoking history (ever versus never), and menopausal status (peri/pre versus post). Multiplicative interactions were evaluated by fitting logistic regression models with the corresponding SNP under a dominant effect, non-genetic variable, and interaction term. *P* values for testing the interaction effects were obtained using Wald X^2 tests. For interactions with $P < 0.05$, stratified analyses were conducted.

For each gene having multiple SNPs associated with risk, we estimated pair-wise linkage disequilibrium (LD) using Haploview v4.1 (34). For regions with high LD ($r^2 > 0.80$), we tested for associations between haplotypes and case-control status using the Haplo.stats program and R software v2.10.0 according to methods proposed by Schaid et al. (35). Estimates of ORs, 95% CIs, and *P*-values were obtained, with adjustment for age and study site under the specified genetic model. Rarer haplotypes (frequencies <10%) were combined into a single category to minimize sparse cell counts. LD plots displaying the SNP correlation structure in selected regions are displayed in Supplementary Figure 1.

To complement single SNP analysis, we used Fisher's method (FM) (36) to combine association evidence from a group of SNPs within a gene for gene-level analysis. This method has been shown to have high statistical power in detecting associations (37). Briefly, the test statistic combines multiple results in the following form, $F = -2\sum_{i=1}^{m} \ln p_i$, where p_i is the SNP-level *p*-value (adjusted for age and study site) for the i^{th} SNP within each gene. To enhance power to detect associations, only common SNPs (MAF ≥ 5%) were included in this analysis. Due to the correlation structure of SNP data, the statistical significance for each gene was assessed using 10,000 permutations with case/control labels permuted. The same statistical test was performed for the pathway-level analysis; association tests for each of the 4 pathways (mtDNA, BIO, OXP, HOR) were conducted. In a similar fashion, the statistical significance for each pathway was assessed using 10,000 permutations. Similar to methods employed by Goode et al. (38), the results were interpreted hierarchically at the pathway, gene and SNP levels. Pathway- and gene-level results were not corrected for multiple testing due to the exploratory nature of this analysis.

RESULTS

Subject characteristics

The distributions of selected characteristics of the 1815 EOC cases and 1900 controls are summarized by study site and case-control status in Table 3. Despite frequency-matching on age-group, cases were older (60.0 ± 11.6 years) than controls (56.8 ± 12.0 years) ($P<0.0001$). As compared to controls, cases tended to have lower education levels ($P<0.0001$), a higher number of LOC ($P<0.0001$), were less likely to have used oral contraceptives (OC) ($P=0.002$), had longer duration of HRT use ($P<0.0001$), and a higher number of pack-years of cigarettes smoked ($P<0.0001$).

Association Testing Results

Results from association testing for mtDNA and the 3 nDNA pathways are presented in a tiered approach, beginning with evaluation at the pathway-level, followed by gene- and SNP-level results. At the pathway and gene levels, we focused on results significant at empirical $P<0.10$. At the SNP level, we focused on results significant at $P<0.05$ and $EMP1<0.05$, and comment on *in silico* findings and imputated genotype results where appropriate. Adjustment for the first principal component representing European ancestry

did not change parameter estimates and CIs appreciably, so results from simple models are presented. We report on haplotype effects with global $P<0.05$.

Mitochondrial DNA (mtDNA)—Pathway analysis revealed that inherited variation in mtDNA (17 genes/regulatory regions, 24 SNPs analyzed) was not significantly associated with EOC risk (empirical $P=0.510$), therefore, gene- and SNP- level results are interpreted with caution. At the gene-level, only *MT-CO1* (*COX1*; cytochrome c oxidase 1) was associated with risk (empirical $P=0.006$) (Supplementary Table 1). At the SNP-level, a synonymous SNP in *MT-CO1*, T6777C (OR: 0.68, 95%CI: 0.51–0.92, $P=0.006$) appeared to decrease risk (Table 4), consistent with haplotype analysis (OR: 0.81, 95%CI: 0.70–0.94, $P=0.006$) (Supplementary Figure 1A). Association results for all evaluated mtDNA genes and SNPs are in Supplementary Tables 1 and 2, respectively.

Mitochondrial biogenesis (BIO)—Of the three nDNA pathways studied, only the 25 genes (1051 SNPs) involved in BIO had a pathway-wide association with EOC risk (empirical $P= 0.050$). At the gene-level, *NRF1* (nuclear respiratory factor 1) had the strongest result (empirical $P=0.041$). Four other genes also demonstrated gene-wide associations, including *MTERF* (mitochondrial transcription termination factor), *PPARGC1A* (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha), *ESRRA* (estrogen-related receptor alpha), and *CAMK2D* (calcium/calmodulin-dependent protein kinase D), with empirical *P*-values of 0.044, 0.060, 0.066, and 0.067, respectively (Supplementary Table 1).

Association results for BIO SNPs significantly associated with EOC risk are displayed in Table 4. For *NRF1*, the strongest association with EOC risk was observed under a recessive model with rs10245560 (OR (95% CI) =0.80 (0.68–0.95), $P=0.010$) (Supplementary Figure 1B). *MTERF* includes several sets of correlated risk-associated SNPs (Supplementary Figure 1C), with ORs ranging from 0.89–2.90. Of all BIO genes, *PPARGC1A* contains the most risk-associated SNPs, the majority of which flank the 5' or 3' UTR, including several correlated SNP-pairs (Supplementary Figure 1D). *ESRRA* contains rs11600990, a SNP associated with decreased risk under a dominant model (OR (95% CI) =0.83 (0.72–0.97), $P=0.016$)) that was predicted by SNPinfo to reside in a putative transcription factor binding site (TFBS). Finally, we identified several intronic SNPs in *CAMK2D*, including rs2040742, a predicted intronic enhancer by FastSNP associated with decreased risk (Supplementary Figure 1E). After adjustment for lifetime ovulation, HRT use, and pack-years smoked, similar magnitudes of association were observed for the majority of BIO SNPs depicted in Table 4, although associations seemed to strengthen for several SNPs in *MTERF*, *PPARGC1A*, and *CAMK2D*.

Imputed genotypes reinforced SNP-level associations for several BIO genes. For example, rs10954252 and rs9929 are imputed SNPs within *NRF1* and *CAMK2D*, respectively, that were most strongly associated with EOC risk (OR (95% CI)= 1.14 (1.04–1.25), $P=0.006$) for rs10954252 and (OR (95% CI=1.36 (1.12–1.66), P=0.002) for rs9929)) (data not shown). Imputation also revealed previously undetected signals for *MAPK1* (mitogen activated protein kinase 1) and *TFB1M* (transcription factor B1, mitochondrial), with the strongest signal identified for *TFB1M* rs721101 (OR (95% CI=0.86 (0.77–0.96), $P=0.006$) (data not shown).

Steroid hormone metabolism (HOR)—Inter-individual variation within HOR pathway (9 genes, 532 SNPs analyzed) was not significantly associated with EOC risk (empirical $P=0.35$), so gene- and SNP-level results are interpreted cautiously. *ESR2* (estrogen receptor 2) is the only HOR gene that was associated with EOC risk (empirical $P=0.026$) (Supplementary Table 1). Four intronic *ESR2* SNPs were individually associated with EOC

risk (Table 4), and a haplotype block including rs1256062, rs1256061, and rs12435857 was associated with a slightly increased risk under an additive model (OR=1.22, 95% CI: 1.01–1.49, global *P*=0.029) (Supplementary Figure 1F). Imputation reinforced evidence of associations within *ESR2*, and identified 17 new signals for *NR3C1/GR* (glucocorticoid receptor) (data not shown).

Oxidative phosphorylation (OXP)—Overall, the OXP pathway (73 genes, 1043 SNPs) was not statistically significantly associated with EOC risk (empirical *P*=0.68) (Supplementary Table 1). Individually, seven OXP genes demonstrated statistically significant gene-level empirical *P*-values, and *TUFM* (Tu translation elongation factor) had the strongest gene-level result (empirical *P*=0.014, Supplementary Table 1). At the SNP level, *TUFM* rs9972768 resides in a putative TFBS and is associated with an 11% increased risk with each copy of the minor allele (Table 4).

SNP-environment interaction analysis

Due to the global significance of the BIO pathway, exploratory models were fitted to test interactions between the 39 risk-associated BIO SNPs (Table 4) and a set of *a priori* determined non-genetic variables linked to oxidative stress: lifetime ovulation, HRT use, cigarette smoking, and menopausal status. This analysis was limited to a dominant genetic effect, for a total of 156 tests (39 SNPs × 4 non-genetic factors). There were 8 interactions significant at $P<0.05$, which is the same as expected based on chance alone, and they involved SNPs from *MTERF*, *PPARGC1A*, and *CAMK2D*. Most noteworthy, stratified analyses showed *MTERF* rs10488506 was a risk factor among ever smokers (OR (95% CI) =1.44 (1.09–1.91), *P*=0.011) but not never smokers (OR (95% CI) =0.98 (0.77–1.24), *P*=0.87). *PPARGC1A* rs1509241 was a risk factor among pre/peri-menopausal women (OR (95% CI) =0.71 (0.56–0.90), *P*=0.004) but not post-menopausal women (OR (95% CI) =0.99 (0.83–1.19), *P*=0.95). HRT use appeared to modify the association between *CAMK2D* rs13107662 and EOC risk, with an inverse association observed among ever users (OR (95% CI) =0.72 (0.58–0.89), *P*=0.003) but no association among never users (OR (95% CI) =0.98 (0.82–1.16), *P*=0.80).

DISCUSSION

Multiple lines of evidence, including animal, *in vivo*, and epidemiologic studies, point to a role for oxidative stress in the etiology and/or progression of EOC (5–9). This report explored the hypothesis that inherited variants in mitochondrial-related genes may influence EOC risk, possibly by contributing to oxidative stress, using a large, homogeneous study population of 1,815 incident pathologically-confirmed Caucasian EOC cases and 1,900 controls. The analysis focused on 128 mtDNA-encoded SNPs from 22 genes and 8 regulatory regions and 2839 nDNA-encoded SNPs from 138 genes having functions related to mitochondrial biogenesis, HOR, and oxidative phosphorylation. We considered various genetic models, haplotypes, gene and pathway-level analyses, *in silico* findings, and evaluation of potential confounders and effect modifiers. Recognizing the large number of statistical comparisons, the data suggest that inherited variants in key genes involved in mitochondrial biogenesis may represent contributors to EOC susceptibility and therefore merit efforts to replicate in additional study populations.

BIO is important due to the DNA damage endured by these organelles over a lifetime which can affect aging, neuromuscular diseases, ovarian insufficiency, and risk of cancer (39–42). This process is influenced by environmental stimuli (i.e. temperature, nutrients, hormones, exercise) that change the energetic and physiological conditions of the cell and in turn increase intracellular calcium concentrations which stimulate kinases to activate

mitochondrial gene transcription (14). Since BIO is repressed in cancer cells (40) and re-activation of BIO can induce apoptosis in cancer cells (39), knowledge gained regarding genetic factors that influence BIO in EOC may have implications for biomarker development and therapeutics.

At the transcriptional level, two classes of nDNA-encoded regulatory proteins direct nuclear-mitochondrial interactions and promote BIO, and our study highlighted genes from each class (14, 15, 41). *MTERF* regulates OXP by acting as an mtDNA transcript terminator, initiator, and controller of mtDNA replication (43). We observed what appeared to be independent association signals from different *MTERF* gene regions, many of which strengthened after accounting for confounding by and/or interaction with HRT use and smoking history. To our knowledge, this is the first report that has linked *MTERF* to cancer development. In response to oxidative stress, *NRF1* activates transcription of antioxidant and detoxification genes (44). Our findings of *NRF1* gene- and SNP-level associations (and a high proportion of associated *NRF2* SNPs) appear promising in light of accumulating data regarding the role for nuclear respiratory factors, especially *NRF2*, in chemoprevention and therapeutics (45). *PPARGC1A* modulates the function of most mitochondrial proteins (12) and serves as a coactivator for *ESRRA*, an inducer of BIO and regulator of OXP and oxidative stress defenses (12, 46). In ovarian tumors, a *PPARGC1A-ESRRA* pathway regulates expression of *VEGF* (vascular endothelial growth factor), a positive regulator of angiogenesis (47). Over-expression of *PPARGC1A* and *ESRRA* has been associated with poor EOC prognosis, suggesting these genes may be useful therapeutic targets (48, 49). *CAMK2D* is a serine/threonine kinase involved in calcium signaling (15); these kinases are dysregulated in many malignancies and participate in cell growth, apoptosis, and angiogenesis (50).

Given that the HOR, OXP, and mtDNA pathways were not associated with EOC risk in our study, caution should be taken when interpreting gene- and SNP-level results. Consistent with our finding of gene- and SNP-level associations between *ESR2* and EOC risk, studies support a role for *ESR2* in mitochondrial dysfunction, oxidative stress, and ovarian carcinogenesis (18, 51). Based on data suggesting cross-talk between *ESR2* and *ESRRA* (52), the possibility remains that stimulation of mitochondrial proteins by steroid hormones may cause altered BIO and function, leading to hormone-related cancers. All nDNA-encoded subunits of the OXP pathway were encompassed on the 610K array; collectively this pathway may not have been associated with EOC risk because expression of these enzymes is most critical in tissues or organs with high energy demands (i.e. muscle, brain, heart). However, given the role for OXP enzymes in ovaries and female reproductive function, additional research may be warranted in this area (53). Most mtDNA variants are rare (MAF< 1%), possibly due to negative selection of germline changes during evolution (54), limiting power to detect SNP-level and higher associations. Although our coverage of mtDNA genes and SNPs is greater than other studies (19, 23, 25), the array did not encompass one polypeptide (*ATPase 8*) and 14 of 22 tRNAs. Further research is necessary to clarify the role of mtDNA-encoded genes in EOC.

The current study did not evaluate other types of nDNA-encoded SNPs that may impact oxidative stress, such as those that influence antioxidant defenses *(i.e. MPO, GSTM1)*, DNA repair (i.e. *MGMT, XRCC1*), carcinogen metabolism (i.e., *CYP1A1*, *GST*), and inflammation (i.e. *TNF-α, IL-6*), because they have been previously examined (55, 56). Mitochondrial variants involved in intrinsic apoptosis *(i.e. caspases, APAF1)* may also alter EOC risk and should be explored. Another limitation pertains to our sub-analysis in which we evaluated whether lifetime ovulation, HRT, and cigarette smoking may confound or modify observed SNP-EOC risk associations. Because we generated crude estimates of lifetime ovulation that did not consider anovulatory periods due to breastfeeding, menstrual

irregularities, or incomplete pregnancies, this measurement lacks precision, and observed associations may be attenuated. Furthermore, we lacked data on HRT formulation and could not consider effects of unopposed estrogen therapy separately from combined estrogen-progestin HRT. Although sub-analyses were based on only 72% of our study sample, their profile did not appear to systematically differ from the entire study population (data not shown), and comparison of parameter estimates for individual SNPs using simple and full models generally showed magnitudes of association similar in direction and strength (Table 4).

In summary, this study reports on the largest number of inherited mitochondrial polymorphisms, genes, and pathways to date for associations with EOC risk, and is the first to suggest that SNPs in BIO genes may represent novel EOC susceptibility loci. Based on internal consistency and biologic plausibility, functional validation of the most promising SNPs identified and characterized as part of this research is warranted. Furthermore, due to the heterogeneous nature of EOC (4), examination of histology-specific effects in larger sample sizes is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all of the women who participated in this research along with all of the researchers, clinicians, and staff who have contributed to the participating studies.

FUNDING

This work was supported by the National Cancer Institute (R01 CA114343 to T.A.S, R01 CA122443 to E.L.G, R01 CA 76016 to J.M.S, R01 CA 106414 to R.S., and P30 CA15083 to the Mayo Clinic Genotyping Shared Resource).

REFERENCES

1. American Cancer Society. Cancer Facts and Figures 2010. Atlanta: American Cancer Society; 2010.
2. Fathalla M. Incessant ovulation- a factor in ovarian neoplasia? Lancet Oncol. 1971; 2:163.
3. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst. 1983; 71:717–721. [PubMed: 6578367]
4. Permuth-Wey J, Sellers TA. Epidemiology of ovarian cancer. Methods Mol Biol. 2009; 472:413–437. [PubMed: 19107446]
5. Desouki MM, Kulawiec M, Bansal S, Das GM, Singh KK. Cross talk between mitochondria and superoxide generating NADPH oxidase in breast and ovarian tumors. Cancer Biol Ther. 2005; 4:1367–1373. [PubMed: 16294028]
6. Karihtala P, Soini Y, Vaskivuo L, Bloigu R, Puistola U. DNA adduct 8-hydroxydeoxyguanosine, a novel putative marker of prognostic significance in ovarian carcinoma. Int J Gynecol Cancer. 2009; 19:1047–1051. [PubMed: 19820366]
7. Murdoch, WJ. Ovulatory Factor in Ovarian Carcinogenesis. New York: Springer; 2008.
8. Roy D, Cai Q, Felty Q, Narayan S. Estrogen-induced generation of reactive oxygen and nitrogen species, gene damage, and estrogen-dependent cancers. J Toxicol Environ Health B Crit Rev. 2007; 10:235–257.
9. Senthil K, Aranganathan S, Nalini N. Evidence of oxidative stress in the circulation of ovarian cancer patients. Clin Chim Acta. 2004; 339:27–32. [PubMed: 14687890]
10. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol. 2010; 38:96–109. [PubMed: 20019356]
11. Modica-Napolitano JS, Kulawiec M, Singh KK. Mitochondria and human cancer. Curr Mol Med. 2007; 7:121–131. [PubMed: 17311537]

12. Hock MB, Kralli A. Transcriptional control of mitochondrial biogenesis and function. Annu Rev Physiol. 2009; 71:177–203. [PubMed: 19575678]
13. Singh KK, Kulawiec M. Mitochondrial DNA polymorphism and risk of cancer. Methods Mol Biol. 2009; 471:291–303. [PubMed: 19109786]
14. Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol Rev. 2008; 88:611–638. [PubMed: 18391175]
15. Diaz F, Moraes CT. Mitochondrial biogenesis and turnover. Cell Calcium. 2008; 44:24–35. [PubMed: 18395251]
16. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer. 2003; 3:193–202. [PubMed: 12612654]
17. Gavrilova-Jordan LP, Price TM. Actions of steroids in mitochondria. Semin Reprod Med. 2007; 25:154–164. [PubMed: 17447205]
18. Klinge CM. Estrogenic control of mitochondrial function and biogenesis. J Cell Biochem. 2008
19. Bai RK, Leal SM, Covarrubias D, Liu A, Wong LJ. Mitochondrial genetic background modifies breast cancer risk. Cancer Res. 2007; 67:4687–4694. [PubMed: 17510395]
20. Booker LM, Habermacher GM, Jessie BC, Sun QC, Baumann AK, Amin M, et al. North American white mitochondrial haplogroups in prostate and renal cancer. J Urol. 2006; 175:468–472. discussion 72-3. [PubMed: 16406974]
21. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res. 2005; 65:8028–8033. [PubMed: 16140977]
22. Liu VW, Wang Y, Yang HJ, Tsang PC, Ng TY, Wong LC, et al. Mitochondrial DNA variant 16189T>C is associated with susceptibility to endometrial cancer. Hum Mutat. 2003; 22:173–174. [PubMed: 12872259]
23. Wang L, Bamlet WR, de Andrade M, Boardman LA, Cunningham JM, Thibodeau SN, et al. Mitochondrial genetic polymorphisms and pancreatic cancer risk. Cancer Epidemiol Biomarkers Prev. 2007; 16:1455–1459. [PubMed: 17627010]
24. Xu L, Hu Y, Chen B, Tang W, Han X, Yu H, et al. Mitochondrial polymorphisms as risk factors for endometrial cancer in southwest China. Int J Gynecol Cancer. 2006; 16:1661–1667. [PubMed: 16884381]
25. Wang L, McDonnell SK, Hebbring SJ, Cunningham JM, St Sauver J, Cerhan JR, et al. Polymorphisms in mitochondrial genes and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2008; 17:3558–3566. [PubMed: 19064571]
26. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006; 38:904–909. [PubMed: 16862161]
27. Brandon MC, Lott MT, Nguyen KC, Spolim S, Navathe SB, Baldi P, et al. MITOMAP: a human mitochondrial genome database--2004 update. Nucleic Acids Res. 2005; 33:D611–D613. [PubMed: 15608272]
28. Cotter D, Guda P, Fahy E, Subramaniam S. MitoProteome: mitochondrial protein sequence database and annotation system. Nucleic Acids Res. 2004; 32:D463–D467. [PubMed: 14681458]
29. Human Mitochondrial Protein Database. Available at: http://bioinfo.nist.gov/hmpd/Description.html
30. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. Available at: http://pngu.mgh.harvard.edu/~purcell/plink/. [PubMed: 17701901]
31. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res. 2009; 37:W600–W605. Available at: http://manticore.niehs.nih.gov/. [PubMed: 19417063]
32. Yuan HY, Chiou JJ, Tseng WH, Liu CH, Liu CK, Lin YJ, et al. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. Nucleic Acids Res. 2006; 34:W635–W641. Available at: http://fastsnp.ibms.sinica.edu/tw/. [PubMed: 16845089]

33. Tung KH, Wilkens LR, Wu AH, McDuffie K, Nomura AM, Kolonel LN, et al. Effect of anovulation factors on pre- and postmenopausal ovarian cancer risk: revisiting the incessant ovulation hypothesis. Am J Epidemiol. 2005; 161:321–329. [PubMed: 15692075]
34. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
35. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet. 2002; 70:425–434. [PubMed: 11791212]
36. Fisher, R. Statistical Methods for Research Workers. London: Oliver and Boyd; 1932.
37. Fridley BL, Jenkins G, Biernacka J. Self-Contained Gene-Set Analysis of Expression Data: An Evaluation of Existing and Novel Methods. PLoS ONE. 2010; 5:e12693. [PubMed: 20862301]
38. Goode EL, Maurer MJ, Sellers TA, Phelan CM, Kalli KR, Fridley BL, et al. Inherited determinants of ovarian cancer survival. Clin Cancer Res. 2010; 16:995–1007. [PubMed: 20103664]
39. Bellance N, Benard G, Furt F, Begueret H, Smolkova K, Passerieux E, et al. Bioenergetics of lung tumors: alteration of mitochondrial biogenesis and respiratory capacity. Int J Biochem Cell Biol. 2009; 41:2566–2577. [PubMed: 19712747]
40. Formentini L, Martinez-Reyes I, Cuezva JM. The mitochondrial bioenergetic capacity of carcinomas. IUBMB Life. 2010; 62:554–560. [PubMed: 20552634]
41. Lopez-Lluch G, Irusta PM, Navas P, de Cabo R. Mitochondrial biogenesis and healthy aging. Exp Gerontol. 2008; 43:813–819. [PubMed: 18662766]
42. Venkatesh S, Kumar M, Sharma A, Kriplani A, Ammini AC, Talwar P, et al. Oxidative stress and ATPase6 mutation is associated with primary ovarian insufficiency. Arch Gynecol Obstet. 2010
43. Roberti M, Polosa PL, Bruni F, Manzari C, Deceglie S, Gadaleta MN, et al. The MTERF family proteins: mitochondrial transcription regulators and beyond. Biochim Biophys Acta. 2009; 1787:303–311. [PubMed: 19366610]
44. Biswas M, Chan JY. Role of Nrf1 in antioxidant response element-mediated gene expression and beyond. Toxicol Appl Pharmacol. 2010; 244:16–20. [PubMed: 19665035]
45. Hu R, Saw CL, Yu R, Kong AN. Regulation of Nrf2 Signaling for Cancer Chemoprevention: Antioxidant Coupled with Anti-inflammatory. Antioxid Redox Signal. 2010
46. Schreiber SN, Emter R, Hock MB, Knutti D, Cardenas J, Podvinec M, et al. The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. Proc Natl Acad Sci U S A. 2004; 101:6472–6477. [PubMed: 15087503]
47. Luo H, Rankin GO, Liu L, Daddysman MK, Jiang BH, Chen YC. Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells. Nutr Cancer. 2009; 61:554–563. [PubMed: 19838928]
48. Sun P, Sehouli J, Denkert C, Mustea A, Konsgen D, Koch I, et al. Expression of estrogen receptor-related receptors, a subfamily of orphan nuclear receptors, as new tumor biomarkers in ovarian cancer cells. J Mol Med. 2005; 83:457–467. [PubMed: 15770498]
49. Davidson B, Hadar R, Stavnes HT, Trope CG, Reich R. Expression of the peroxisome proliferator-activated receptors-alpha, -beta, and -gamma in ovarian carcinoma effusions is associated with poor chemoresponse and shorter survival. Hum Pathol. 2009; 40:705–713. [PubMed: 19157507]
50. Lavalle CR, George KM, Sharlow ER, Lazo JS, Wipf P, Wang QJ. Protein kinase D as a potential new target for cancer therapy. Biochim Biophys Acta. 2010
51. Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY, et al. Genetic polymorphisms in the estrogen receptor beta (ESR2) gene and the risk of epithelial ovarian carcinoma. Cancer Causes Control. 2009; 20:47–55. [PubMed: 18704709]
52. Gaillard S, Dwyer MA, McDonnell DP. Definition of the molecular basis for estrogen receptor-related eceptor-alpha-cofactor interactions. Mol Endocrinol. 2007; 21:62–76. [PubMed: 17053040]
53. Agarwal A, Gupta S, Sekhon L, Shah R. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. Antioxid Redox Signal. 2008; 10:1375–1403. [PubMed: 18402550]
54. Stafford P, Chen-Quin EB. The pattern of natural selection in somatic cancer mutations of human mtDNA. J Hum Genet. 2010

55. Fasching PA, Gayther S, Pearce L, Schildkraut JM, Goode E, Thiel F, et al. Role of genetic polymorphisms and ovarian cancer susceptibility. Mol Oncol. 2009; 3:171–181. [PubMed: 19383379]

56. Schildkraut JM, Iversen ES, Wilson MA, Clyde MA, Moorman PG, Palmieri RT, et al. Association between DNA damage response and repair genes and risk of invasive serous ovarian cancer. PLoS One. 2010; 5:e10061. [PubMed: 20386703]

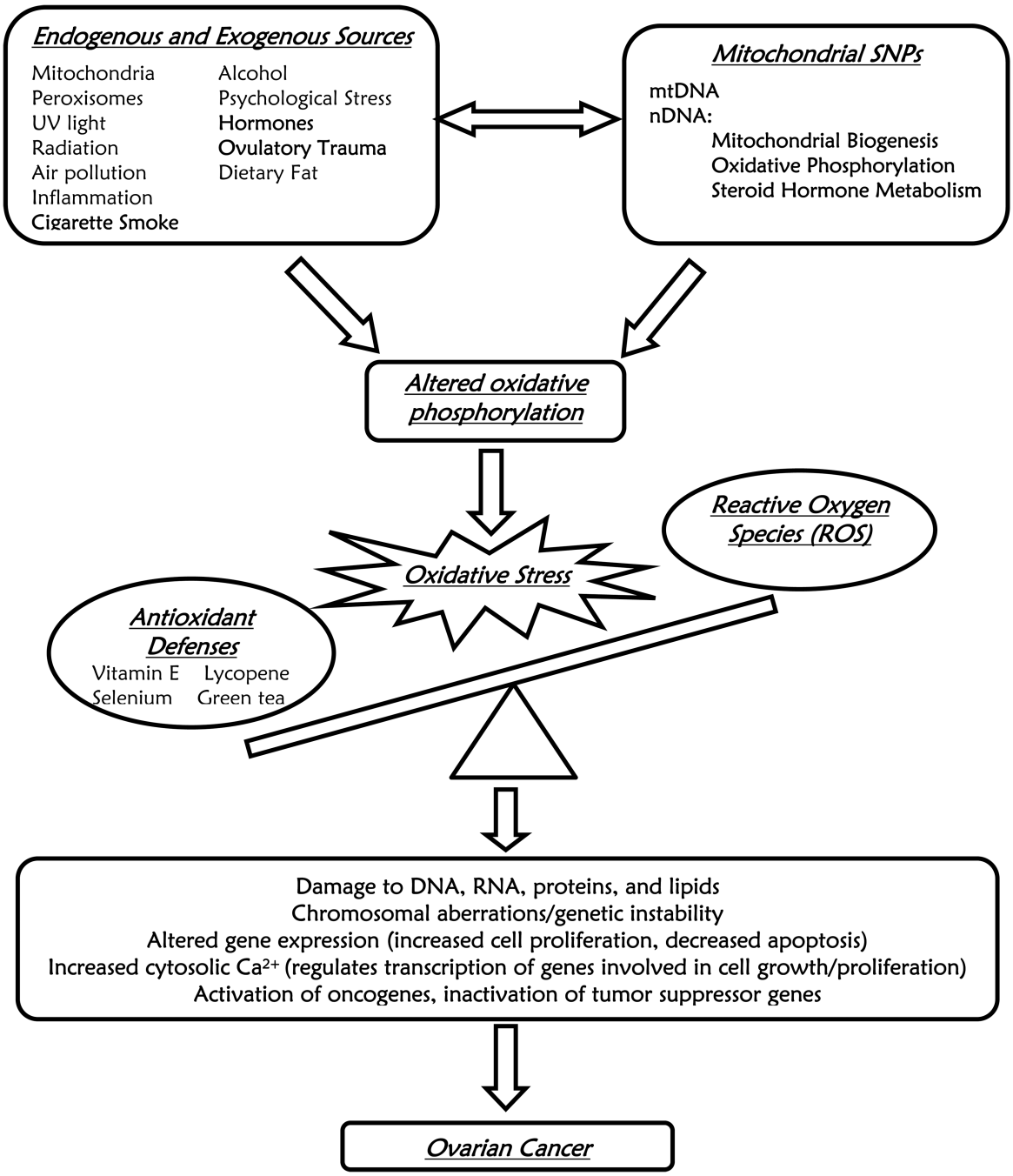

Figure 1.

Schema illustrating how mitochondrial SNPs and other factors may contribute to oxidative stress and ovarian carcinogenesis. Polymorphisms in mtDNA and/or several categories of nDNA may interact with one another or with other non-genetic endogenous and exogenous exposures to contribute to slight changes in encoded proteins over time, leading to altered OXP activity and oxidative stress. An accumulation of ROS may promote DNA damage and genetic instability, increased concentrations of calcium, and altered expression of genes involved in cellular proliferation and apoptosis, including oncogenes and tumor suppressor genes. This process may ultimately promote ovarian cancer development.

Table 1

Characteristics of participating epithelial ovarian cancer case-control studies

Study	Study Period (month/ year)	Age range at diagnosis/ Interview (years)	Case ascertainment	Control ascertainment	% White, Non-Hispanic	Participation Rates[a] (Cases vs. Controls)
MAY	1/00-11/07	20+	Mayo Clinic's gynecologic surgery & oncology departments; residents of six-state surrounding region (MN, IA, WI, IL, ND, SD).	Women from the same geographic region seeking general medical examination through Mayo's departments of primary care and internal medicine.	95	84% vs 65%
NCO	1/99-11/07	20–74	Rapid case ascertainment (i.e. frequent contact with local hospitals and physicians); residents of contiguous 48-county region.	Random digit dialing targeted towards women residing in the same region	83	70% vs 63%
TBO	12/00-9/03 and 12/05-2/10	18–80	Rapid ascertainment (i.e. frequent contact with 7 regional gynecologic oncologists); residents of a 2-county, heavily populated region.	Women attending a local screening clinic for routine mammography	91	87% vs. NA
TOR	1/95-12/99 and 1/03-12/04	20–79	Ontario Cancer Registry.	Women receiving routine screening (i.e. blood pressure checks, mammograms, cholesterol screening) at the Women's College Hospital	93	64% vs. 95%

Abbreviations: MAY=Mayo Clinic Ovarian Cancer Study (Rochester, MN); NCO=North Carolina Ovarian Cancer Study (Durham, NC); TOR=Familial Ovarian Tumor Study (Ontario, Canada); TBO= Tampa Bay Ovarian Cancer Study (Tampa, FL); NA=Not available

[a]Indicates % of those invited to participate (after excluding those who had died, were too sick, unable to provide informed consent, etc

Table 2

List of investigated mtDNA and nDNA genes and number of genotyped SNPs analyzed by pathway

Pathway	Gene abbreviations	# SNPs on 610K array	# SNPs passing QC	# SNPs with MAF ≥ 0.05 [a]
mtDNA (N=22 genes + 8 regions)				
Polypeptides (n=12)	*MT-ATP6, MT-COI, MT-COII, MT-COIII, MT-CYTB, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND4L, MT-ND5, MT-ND6*	91	85	10
tRNAs (n=8)	*MT-TR, MT-TN, MT-TD, MT-TC, MT-TQ, MT-TG, MT-TL2, MT-TT*	10	10	2
rRNAs (n=2)	*MT-RNR1, MT-RNR2*	14	13	5
Displacement (D)-Loop region	*MT-DLOOP, MT-CSB-1, MT-HV2, MT-HV3, MT-TFX*	19	16	5
Miscellaneous	*MT-ATT, MT-NCL, MT-NC7*	4	4	2
Subtotal		**138**	**128** [b]	**24**
nDNA (N=142 genes)				
Mitochondrial Biogenesis (BIO) (n=35)	*ATF2, C10ORF2, CAMK2A, CAMK2B, CAMK2D, CAMK2G, CAMK4, CREB1, ESRRA, HCFC1, MAPK1, MTERF, MT01, MYEF2, NFE2L2, NR1H3, NRF1, PPARA, PPARG, PPARGC1A, PPARGC1B, PPRC1, PRKAA1, PRKAA2, PRKAB1, PRKCA, SP1, SSBP1, TFAM, TFB1M, TFB2M, THRA, THRB, TRNT1, YY1*	1194	1135	1051
Steroid Hormone Metabolism (HOR) (n=14)	*AR, ESR1, ESR2, HSD17B1, HSD17B3, HSD17B4, HSD17B8, NR3C1, PGR, POMC, RARA, RARB, RARG, SRD5A2*	635	584	532
Oxidative Phosphorylation (OXP) (n=93)	*ATP5A1, ATP5C1, ATP5D, ATP5F1, ATP5G1, ATP5G2, ATP5G3, ATP5H, ATP5J, ATP5L, ATP5O, ATPAF1, COX10, COX11, COX15, COX17, COX4I1, COX4I2, COX5A, COX5B, COX6A1, COX6B1, COX6B2, COX6C, COX7A1, COX7A2, COX7A2L, COX7B, COX7B2, COX7C, COX8A, COX8C, GAPDH, GFM1, LRPPRC, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA12L, NDUFA3, NDUFA4, NDUFA5, NDUFA6, NDUFA7, NDUFA8, NDUFA9, NDUFAB1, NDUFAF1, NDUFB1, NDUFB10, NDUFB11, NDUFB2, NDUFB3, NDUFB4, NDUFB5, NDUFB6, NDUFB7, NDUFB8, NDUFB9, NDUFC1, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS5, NDUFS6, NDUFS8, NDUFV1, NDUFV2, NDUFV3, NOX1, POLG, PPA2, SCO1, SDHA, SDHB, SDHC, SDHD, SLC25A14, SLC25A4, TSFM, TUFM, UCP1, UCP3, UCRC, UQCRB, UQCRC1, UQCRC2, UQCRFS1, UQCRH*	1235	1120	1043
Subtotal		**3064**	**2839** [c]	**2626**
TOTAL		**3202**	**2967**	**2650**

Abbreviations: QC=quality control; MAF=minor allele frequency among controls

[a] Number of successfully genotyped SNPs having MAF ≥ 0.05; data from these common SNPs were utilized in gene-level and pathway-level analyses.

[b] 128 mtDNA SNPs from all evaluated polypeptides, tRNAs, and rRNAs passed QC (only *MT-TFX* SNPs in the *D-Loop* region did not pass QC)

[c] 2839 nDNA SNPs from a total of 138 nDNA genes (35 BIO, 90 OXP, 13 HOR) passed QC; of these 2839 SNPs, 133 (4.7%) deviated from Hardy Weinberg Equilibrium (HWE) among controls (P_{HWE}<0.05); 142 were expected by chance.

Table 3

Selected characteristics of study participants, stratified by study site and case-control status

Variable	MAY (N=879)		NCO (N=1149)		TBO (N=396)		TOR (N=1291)		TOTAL (N=3715)	
	Cases (n=359)	**Controls (n=520)**	**Cases (n=494)**	**Controls (n=655)**	**Cases (n=227)**	**Controls (n=169)**	**Cases (n=735)**	**Controls (n=556)**	**Cases (n=1815)**	**Controls (n=1900)**
Age (y), [b,d, e] mean (SD)	61.2 (12.4)	60.5 (13.3)	57.0 (10.6)	55.1 (11.9)	61.4 (11.5)	59.6 (10.7)	61.0 (11.5)	54.4 (9.9)	60.0 (11.6)	56.8 (12.0)
Education achieved [a,c,d,e], N (%)										
No diploma	19 (5)	18 (3)	42 (9)	59 (9)	12 (5)	3 (2)	217 (30)	74 (13)	290 (16)	154 (8)
High school diploma	123 (34)	125 (24)	160 (32)	177 (27)	85 (37)	13 (8)	189 (26)	134 (24)	556 (31)	449 (24)
Post high school	196 (55)	343 (66)	292 (59)	419 (64)	104 (46)	84 (50)	271 (37)	345 (62)	860 (47)	1191 (63)
Missing	21 (6)	34 (7)	0 (0)	0 (0)	26 (11)	69 (41)	58 (8)	3 (1)	109 (6)	106 (6)
Lifetime ovulatory cycles ^{2}, [a,b,d,e], N (%)										
≤ 332	55 (15)	130 (25)	104 (21)	217 (33)	18 (8)	26 (15)	73 (10)	165 (30)	250 (14)	538 (28)
333–460	114 (32)	164 (32)	207 (42)	281 (43)	17 (7)	24 (14)	121 (16)	236 (42)	459 (25)	705 (37)
461+	88 (25)	87 (17)	157 (32)	141 (22)	18 (8)	11 (7)	365 (5)	139 (25)	628 (35)	378 (20)
Missing	102 (28)	139 (27)	26 (5)	16 (2)	174 (77)	108 (64)	176 (24)	16 (3)	478 (26)	279 (15)
Parity ^{1}, [b,c,d,e], N (%)										
Nulliparous	60 (17)	74 (14)	99 (20)	88 (14)	33 (14)	21 (12)	21 (3)	94 (17)	213 (12)	277 (15)
1–2 pregnancies	121 (34)	171 (33)	240 (49)	369 (56)	94 (41)	45 (27)	324 (44)	283 (51)	779 (43)	868 (46)
3+ pregnancies	169 (47)	239 (46)	151(31)	198 (30)	100 (44)	43 (25)	217 (30)	179 (32)	637 (35)	659 (35)
Missing	9 (3)	36 (7)	4(1)	0 (0)	0 (0)	60 (36)	173 (24)	0 (0)	186 (10)	96 (5)
OC use [a,b,c,e], N (%)										
Never	159 (44)	186 (36)	170 (34)	181 (28)	95 (42)	29 (17)	302 (41)	267 (48)	726 (40)	663 (35)
Ever	190 (53)	295 (57)	324 (66)	474 (72)	110 (48)	75 (44)	376 (51)	286 (51)	999 (55)	1130 (60)
Missing	10 (3)	39 (8)	0 (0)	0 (0)	23 (10)	65 (38)	57 (8)	3 (1)	90 (5)	107 (6)
History of Breastfeeding [b,c, e], N (%)										
Never	44 (12)	54 (10)	356 (72)	426 (65)	111(49)	33 (20)	227 (31)	210 (38)	738 (41)	723 (38)
Ever	NA	NA	136 (28)	229 (35)	60 (26)	50 (30)	310 (42)	346 (62)	506 (28)	625 (33)
Missing	315 (88)	466 (90)	2 (0.4)	0 (0)	56 (25)	86 (51)	198 (27)	0 (0)	571 (31)	552 (29)
Menopausal status at diagnosis/interview [b,c,d,e], N (%)										
Pre/peri	88 (25)	117 (23)	135 (27)	241 (37)	15 (7)	35 (21)	486 (66)	201 (36)	723 (40)	595 (31)

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2012 June 01.

	MAY (N=879)		NCO (N=1149)		TBO (N=396)		TOR (N=1291)		TOTAL (N=3715)	
Variable	**Cases (n=359)**	**Controls (n=520)**	**Cases (n=494)**	**Controls (n=655)**	**Cases (n=227)**	**Controls (n=169)**	**Cases (n=735)**	**Controls (n=556)**	**Cases (n=1815)**	**Controls (n=1900)**
Post	256 (71)	367 (71)	355 (72)	412 (63)	186 (82)	114 (67)	191 (26)	350 (63)	988 (54)	1243 (65)
Missing	15 (4)	36 (7)	4 (1)	2 (0.3)	26 (11)	20 (12)	58 (8)	5 (1)	104 (6)	62 (3)
Body Mass Index *3*, [a] (kg/m^2), mean (SD)	27.8 (6.1)	26.8 (5.5)	27.3 (6.4)	27.2 (6.5)	26.5 (6.5)	26.4 (5.3)	26.6 (5.2)	26.5 (4.5)	27.1 (5.9)	26.8 (5.6)
Duration HRT use (mo)[b,d,e], N (%)										
60+	50 (14)	106 (20)	140 (28)	133 (20)	65 (29)	32 (19)	104 (14)	12 (2)	359 (20)	283 (15)
1–59	32 (9)	59 (11)	126 (26)	109 (17)	23 (10)	23 (14)	146 (20)	166 (30)	326 (18)	357 (19)
Never	133 (37)	241 (46)	204 (41)	388 (59)	101 (44)	28 (17)	484 (66)	378 (68)	1004 (55)	1076 (57)
Missing	144 (40)	114 (22)	24 (5)	25 (4)	38 (17)	86 (51)	1 (0.1)	0 (0)	126 (7)	184 (10)
Family history of ovarian or breast cancer in ≥1 FDR, N (%)										
No	302 (84)	431 (83)	439 (89)	607 (93)	217 (96)	165 (98)	NA	NA	958 (89)	1203 (90)
Yes	57 (16)	89 (17)	51 (10)	48 (7)	10 (4)	4 (2)			118 (11)	141 (11)
Missing	0 (0)	0 (0)	4 (0.8)	0 (0)	0 (0)	0 (0)			4 (0.4)	0 (0)
Pack-years smoked·[a,d,e], N (%)										
≥ 20	60 (17)	49 (9)	97 (20)	128 (20)	26 (11)		195 (27)	36 (6)	378 (21)	213 (11)
<20	63 (18)	96 (18)	110 (22)	166 (25)	20 (9)	NA	168 (23)	165 (30)	361 (20)	427 (23)
None	216 (60)	310 (60)	265 (54)	338 (52)	56 (25)		315 (43)	322 (58)	852 (47)	970 (51)
Missing	20 (6)	65 (13)	22 (5)	23 (4)	125 (55)		57 (8)	33 (6)	224 (12)	290 (15)
Histology, N (%)										
Serous	228 (63.5)		294 (59.5)		146 (65.8)		402 (54.7)		1070 (59.1)	
Mucinous	11 (3.1)		20 (4.1)		13 (5.9)		57 (7.8)		101 (5.6)	
Endometrioid	71 (19.8)	-	81 (16.4)	-	28 (12.6)	-	154 (21.0)	-	334 (18.5)	-
Clear Cell	24 (6.7)		58 (11.7)		12 (5.4)		47 (6.4)		141 (7.8)	
Mixed Cell	21 (5.9)		0 (0)		23 (10.4)		9 (1.2)		53 (2.9)	
Other	4 (1.1)		11 (2.2)		0 (0)		66 (9.0)		116 (6.1)	
Stage, N (%)										
I	67 (18.8)		77 (15.6)		38 (16.7)				182 (19.0)	
II	26 (7.3)		84 (17.1)		25 (11.0)				135 (12.6)	
III	203 (57.2)	-	328 (66.7)	-	154 (67.8)	-	NA	-	685 (63.8)	-
IV	59 (16.6)		0 (0.0)		10 (4.4)				69 (6.4)	

Variable	MAY (N=879)		NCO (N=1149)		TBO (N=396)		TOR (N=1291)		TOTAL (N=3715)	
	Cases (n=359)	**Controls (n=520)**	**Cases (n=494)**	**Controls (n=655)**	**Cases (n=227)**	**Controls (n=169)**	**Cases (n=735)**	**Controls (n=556)**	**Cases (n=1815)**	**Controls (n=1900)**
Unknown	0 (0)		3(0.3)		0 (0)				3 (0.3)	
Grade, N (%)										
I	13 (3.6)		65 (13.5)		11 (5.5)				89 (8.5)	
II	45 (12.5)		138 (28.7)		32 (3.1)				215 (20.6)	-
III	174 (48.5)	-	277 (57.6)	-	157 (77.7)	-	NA	-	608 (58.4)	
IV	121 (33.7)		1 (0.21)		2 (1.0)				124 (11.9)	
Unknown	6 (1.7)		0 (0)		0 (0)				6 (0.6)	

Percentages were rounded and may not add up to 100.

Abbreviations: NA= Data not available; OC=Oral Contraceptive; HRT=Hormone Replacement Therapy; FDR=First degree relative

Case-control differences with $p<0.05$ at MAY, NCO, TBO, TOR, or overall are denoted by superscripts a, b, c, d, and e, respectively.

[1]Parity for MAY, NCO, and TOR was based on the number of full-term births resulting from pregnancies lasting at least 6 months. For TBO, parity was based on the number of pregnancies, regardless of length or outcome.

[2]Distribution is based on tertiles among controls.

[3]Body mass index was calculated using usual adult or current weight for subjects from TOR and TBO, and was calculated using weight 1 year prior to the age at diagnosis/interview for MAY and NCO subjects.

Table 4

Top ranked SNP-epithelial ovarian cancer risk associations

Gene (locus)	SNP (Major/Minor Allele)	Location [a]	MAF	P_{HWE}	Model [b]	All subtypes (1815 cases, 1900 controls)					
						OR_{simple} (95% CI)[c]	P_{simple} [d]	$EMP1_{simple}$ [e]	OR_{full} (95% CI) [f]	P_{full} [g]	$EMP1_{full}$ [h]
MT-CO1 **(MT)**	MitoT6777C (A/G)	-	0.06	-	AL	0.68 (0.51–0.92)	0.013	0.006	0.57 (0.38–0.83)	0.003	0.007
NRF1 **(7q32)**	rs10245560 (G/A)	flanking 5'UTR	0.45	0.18	R	0.80 (0.68 –0.95)	0.010	0.016	0.77 (0.62 –0.94)	0.010	0.012
	rs2402976 (A/G) [i]	intron	0.46	0.46	A	1.12 (1.02 –1.23)	0.016	0.009	1.19 (1.06 –1.33)	0.003	0.004
	rs1882095 (G/A)	flanking 3'UTR	0.34	0.61	A	1.11 (1.00 –1.21)	0.045	0.029	1.13 (1.00 –1.27)	0.044	0.043
MTERF **(7q21–q22)**	rs2540571 (G/A)	flanking 3'UTR	0.50	0.46	A	0.89 (0.81 –0.97)	0.012	0.015	0.91 (0.81 –1.02)	0.107	0.069
	rs2540592 (G/A) [j]	flanking 3'UTR	0.38	0.03	D	1.19 (1.03 –1.36)	0.015	0.012	1.13 (0.96 –1.34)	0.139	0.055
	rs12673563 (G/A)	flanking 3'UTR	0.10	0.02	R	2.20 (1.03 –4.79)	0.043	0.038	2.30 (0.97 –5.42)	0.059	0.031
	rs10488506 (G/A) [k]	flanking 3'UTR	0.07	0.60	A	1.26 (1.06 –1.50)	0.008	0.021	3.12 (1.33 –7.40)	0.009	0.009
	rs6950538 (A/G) [l]	flanking 3'UTR	0.39	0.73	A	0.90 (0.81 –0.99)	0.031	0.036	0.86 (0.77 –0.97)	0.015	0.041
	rs974508 (G/A)	flanking 3'UTR	0.09	0.16	R	2.90 (1.32 –6.36)	0.008	0.014	3.84 (1.58 –9.36)	0.003	0.001
	rs2269811 (A/G) [m]	intron	0.10	0.02	R	2.89 (1.31 –6.34)	0.008	0.015	3.30 (1.39 –7.82)	0.007	0.014
PPARGC1A **(4p15.1)**	rs13151220 (C/A) [n]	flanking 3'UTR	0.08	1.00	A	1.21 (1.02 –1.42)	0.028	0.025	1.11 (0.91 –1.35)	0.317	0.073
	rs1509241 (A/G)	flanking 3'UTR	0.45	0.71	R	0.84 (0.71 –1.00)	0.048	0.010	0.92 (0.75 –1.13)	0.427	0.110
	rs12498626 (G/A)	flanking 3'UTR	0.12	0.75	D	1.17 (1.01 –1.37)	0.042	0.042	1.26 (1.05 –1.52)	0.014	0.014
	rs6857002 (A/C)	flanking 3'UTR	0.24	0.35	D	1.18 (1.03–1.35)	0.015	0.020	1.26 (1.07 –1.48)	0.005	0.012
	rs4697041 (G/A)	flanking 3'UTR	0.37	0.46	A	1.12 (1.02 –1.24)	0.021	0.026	1.11 (0.98 –1.25)	0.092	0.023
	rs2970882 (A/G)	flanking 3'UTR	0.35	0.76	A	0.90 (0.82 –0.99)	0.044	0.030	0.87 (0.77 –0.98)	0.023	0.026
	rs7682765 (A/G)	intron	0.06	0.34	R	3.84 (1.37 –10.8)	0.011	0.005	5.14 (1.62 –16.3)	0.005	0.009
	rs4550905 (A/G) [o]	intron	0.32	0.96	A	0.87 (0.78 –0.96)	0.005	0.009	0.87 (0.77 –0.98)	0.025	0.017
	rs4361373 (A/G)	intron	0.18	0.47	A	0.84 (0.74 –0.95)	0.006	0.013	0.85 (0.73 –0.98)	0.029	0.024
	rs10030083 (C/A)	flanking 5'UTR	0.10	1.00	D	1.19 (1.01 –1.41)	0.038	0.034	1.30 (1.06 –1.60)	0.011	0.105
	rs13108219 (C/A)	flanking 5'UTR	0.11	0.49	A	1.22 (1.06 –1.40)	0.007	0.004	1.21 (1.02 –1.44)	0.028	0.038
	rs4697429 (A/G)	flanking 5'UTR	0.20	0.15	A	1.13 (1.01 –1.27)	0.031	0.008	1.17 (0.99 –1.38)	0.066	0.070
	rs9291455 (G/A) [p]	flanking 5'UTR	0.15	0.59	A	1.15 (1.01 –1.30)	0.039	0.039	1.16 (0.99 –1.36)	0.061	0.073

Gene (locus)	SNP (Major/Minor Allele)	Location [a]	MAF	P HWE	Model [b]	All subtypes (1815 cases, 1900 controls)					
						OR_{simple} (95% CI)[c]	P_{simple} [d]	EMP1 $_{simple}$ [e]	OR_{full} (95% CI) [f]	P_{full} [g]	EMP1 full[h]
	rs16874778 (C/A)	flanking 5'UTR	0.29	0.06	D	1.17 (1.03 –1.34)	0.018	0.019	1.28 (1.09 –1.51)	0.003	0.015
	rs640050 (G/A)	flanking 5'UTR	0.38	0.36	A	1.11 (1.01 –1.22)	0.031	0.020	1.25 (1.06 –1.48)	0.009	0.048
	rs614457 (C/A)	flanking 5'UTR	0.34	0.09	D	1.17 (1.02 –1.33)	0.026	0.010	1.22 (1.03 –1.43)	0.018	0.021
	rs7680610 (G/A)	flanking 5'UTR	0.35	$3E^{-3}$	R	0.78 (0.63 –0.95)	0.015	0.025	0.79 (0.61 –1.01)	0.059	0.005
***ESRRA* (11q13)**	rs11600990 (G/A)	intron	0.16	0.40	D	0.83 (0.72 –0.97)	0.016	0.042	0.91 (0.76 –1.09)	0.299	0.418
***CAMK2D* (4q26)**	rs3733619 (A/G)	flanking 3'UTR	0.10	0.90	A	1.22 (1.05 –1.42)	0.009	0.014	1.18 (0.98 –1.41)	0.079	0.044
	rs7684418 (G/A)	flanking 3'UTR	0.09	0.05	R	2.57 (1.02 –6.46)	0.045	0.034	5.11 (1.34 –19.5)	0.017	1.000
	rs1525000 (A/G)	intron	0.40	0.08	R	0.80 (0.66 –0.96)	0.017	0.008	0.76 (0.61 –0.96)	0.019	0.124
	rs7697831 (G/A)	intron	0.20	0.14	D	1.17 (1.02 –1.34)	0.028	0.009	1.14 (0.99 –1.31)	0.057	0.023
	rs17630328 (A/C) [q]	intron	0.07	0.39	A	1.24 (1.04 –1.47)	0.016	0.020	1.30 (1.06 –1.60)	0.014	0.021
	rs10033037 (A/G)	intron	0.15	0.24	A	1.17 (1.03 –1.32)	0.018	0.020	1.13 (0.97 –1.32)	0.114	0.086
	rs13107662 (A/G)	intron	0.35	0.45	A	0.88(0.80 –0.98)	0.018	0.014	0.90 (0.77 –1.06)	0.204	0.036
	rs1029471 (A/G)	intron	0.18	0.88	A	1.13 (1.01–1.28)	0.039	0.039	1.14 (0.97 –1.35)	0.123	0.223
	rs764830 (A/G)	intron	0.37	0.07	R	1.23 (1.02 –1.49)	0.034	0.040	1.19 (0.95 –1.50)	0.133	0.025
	rs6533711 (A/G)	intron	0.38	1.00	R	1.22 (1.02 –1.46)	0.032	0.028	1.21 (0.97 –1.50)	0.093	0.011
	rs2040742 (A/G) [r]	intron	0.08	1.00	A	0.82 (0.69 –0.98)	0.030	0.045	0.77 (0.62 –0.96)	0.019	0.017
***ESR2* (14q23.2)**	rs1256062 (A/G)	intron	0.10	0.21	A	1.19 (1.03 –1.38)	0.021	0.025	1.16 (0.97 –1.39)	0.106	0.319
	rs1256061 (C/A) [s]	intron	0.49	0.71	R	0.82 (0.69 –0.95)	0.010	0.012	0.81 (0.67 –0.98)	0.033	0.017
	rs6573553 (A/C)	intron	0.48	0.68	A	1.11 (1.01 –1.22)	0.032	0.021	1.12 (1.00 –1.26)	0.053	0.019
***TUFM* (16p11.2)**	rs9972768 (A/C)	flanking 5'UTR	0.38	0.63	A	1.11 (1.00 –1.22)	0.043	0.031	1.14 (1.01 –1.28)	0.029	0.055
***COX7A1* (19q13.1)**	rs753420 (A/C)	5'UTR	0.26	0.63	R	1.37 (1.07 –1.75)	0.013	0.007	1.48 (1.10 –1.99)	0.010	0.001
***COX8C* (14q32.12)**	rs6575325 (G/A) [t]	flanking 3'UTR	0.29	0.41	A	1.16 (1.04 –1.28)	0.005	0.011	1.14 (1.01 –1.29)	0.038	0.022
***NDUFB10* (16p13.3)**	rs758335 (G/A) [u]	intron	0.19	0.05	D	0.80 (0.69 –0.92)	0.002	0.004	0.82 (0.70 –0.98)	0.027	0.021
***NDUFA11* (19p13.3)**	rs1674159 (G/A)	flanking 3'UTR	0.13	0.10	D	1.21 (1.04 –1.41)	0.017	0.044	1.22 (1.01 –1.46)	0.036	0.062
	rs10421538 (G/A)	intron	0.21	0.83	A	1.17 (1.05 –1.32)	0.006	0.019	1.23 (1.08 –1.41)	0.003	0.010
***NDUFA12* (12q22)**	rs10777629 (A/G)	flanking 3'UTR	0.22	0.23	D	0.83 (0.73 –0.96)	0.009	0.023	0.85 (0.72 –1.00)	0.050	0.050
	rs10777647 (A/G)	flanking 3'UTR	0.40	0.47	A	1.12 (1.02 –1.24)	0.020	0.021	1.15 (1.03 –1.30)	0.018	0.045

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2012 June 01.

Gene (locus)	SNP (Major/Minor Allele)	Location [a]	MAF	P_{HWE}	Model [b]	All subtypes (1815 cases, 1900 controls)					
						OR_{simple} (95% CI)[c]	P_{simple} [d]	$EMP1_{simple}$ [e]	OR_{full} (95% CI) [f]	P_{full} [g]	$EMP1_{full}$ [h]
	rs6538566 (C/A)	flanking 3'UTR	0.23	0.56	A	0.87 (0.77 –0.97)	0.015	0.018	0.63 (0.43 –0.92)	0.016	0.034
	rs7965624 (G/A) [v]	flanking 3'UTR	0.38	0.01	R	0.75 (0.62 –0.90)	0.003	0.005	0.72 (0.57 –0.91)	0.005	0.003
	rs2052741 (A/G)	flanking 3'UTR	0.48	$1E^{-3}$	R	0.74 (0.63 –0.87)	$2E^{-4}$	0.001	0.68 (0.56 –0.83)	$1E^{-4}$	$2E^{-4}$
	rs2081595 (C/A)	flanking 3'UTR	0.49	0.18	A	1.13 (1.03 –1.24)	0.010	0.017	1.13 (1.01 –1.27)	0.029	0.011
	rs2033092 (G/A)	flanking 3'UTR	0.39	0.25	D	0.83 (0.72 –0.95)	0.008	0.011	0.82 (0.73 –0.92)	0.001	0.001
	rs7133343 (C/A)	flanking 3'UTR	0.33	0.88	A	0.91 (0.81 –1.00)	0.042	0.024	0.86 (0.76 –0.97)	0.014	0.010
	rs11107851 (A/G)	intron	0.21	0.45	R	1.44 (1.06 –1.96)	0.019	0.045	1.45 (1.01 –2.07)	0.042	0.027

Sorted by nDNA pathway-level significance and gene-level significance (Supplementary Table 1) and then by SNP position (base pair). Only SNPs in genes with gene-level P < 0.05 are listed, and only SNPs with *P*<0.05 and *EMP1*<0.05 are listed. All *P*-values are two-sided.

Abbreviations: MT=Mitochondrial DNA; MAF=minor allele frequency among controls; P_{HWE} =P-value for deviation from Hardy Weinberg Equilibrium among controls (P_{HWE} <0.05); OR (CI) = Odds ratio (confidence interval);

[a] SNP location was derived from the Illumina Infinium 610K Array annotation file that obtains this information from GenBank, a genetic sequence database, along with dbSNP;

[b] Best-fitting genetic model (A = Log-additive; D = Dominant; R = Recessive; AL=Allelic);

[c] OR and 95% CI adjusted for the simple model (age at diagnosis/interview (continuous)and study site (indicator variable for NCO, TOR, and TBO);

[d] Asymptotic P-value for testing the genetic effects related to overall ovarian cancer risk before permutation testing using the simple model. *P-value* under an additive model represents *P-trend*;

[e] Empirical point-wise *P*-value for testing the genetic effects related to overall ovarian cancer risk using the simple model;

[f] OR and 95% CI adjusted for the full model (age at diagnosis/interview (continuous), study site (indicator variable for NCO, TOR, and TBO), LOC category (low, medium, high), duration of hormone replacement therapy (HRT) use (continuous), and pack-years of cigarettes smoked (continuous));

[g] Asymptotic P-value for testing the genetic effects related to ovarian cancer risk before permutation testing using the full model;

[h] Empirical point-wise *P*-value for testing the genetic effects related to ovarian cancer risk using the full model;

[i] rs2402976 is in linkage disequilibrium (LD) (r^2=1.0) with imputed SNP rs10954252;

[j] rs2540592 is in LD (r^2=1.0) with rs2540552;

[k] rs10488506 is in LD ($0.76 \le r^2 \le 0.87$) rs12668203 and rs12667992;

[l] rs6950538 is in LD ($0.97 \le r^2 \le 0.99$) with rs6949881, rs13229505, rs2049314, and rs9656002;

[m] rs2269811 is in LD (r^2=0.97) with rs7781767;

[n] rs13151220 is in LD (r^2=1.0) with rs13143346;

[o] rs4550905 is in LD ($0.95 \leq r^2 \leq 1.0$) with imputed SNPs rs13106578, rs13131226, and rs6838600;

[p] rs9291455 is in LD ($0.86 \leq r^2 \leq 1.0$) with rs16874486 and rs16874509;

[q] rs17630328 is in LD (r^2=1.0) with imputed SNP rs7657655;

[r] rs2040742 is in LD (r^2=1.0) with rs6842343;

[s] rs1256061 is in LD (r^2=0.86) with rs12435857;

[t] rs6575325 is in LD(r^2=1.0) with rs1503959;

[u] rs758335 is in LD (r^2=0.96) with imputed SNP rs397435;

[v] rs7965624 is in LD (r^2=1.0) with rs7306940.