



Regular multivitamin supplement use, single nucleotide polymorphisms in *ATIC*, *SHMT2*, and *SLC46A1*, and risk of ovarian carcinoma

Linda E. Kelemen^{1,2,3*}, Qinggang Wang¹, Irina Dinu⁴, Robert A. Vierkant⁵, Ya-Yu Tsai⁶, Julie M. Cunningham⁷, Catherine M. Phelan⁶, Brooke L. Fridley⁵, Ernest K. Amankwah¹, Edwin S. Iversen⁸, Andrew Berchuck⁸, Joellen M. Schildkraut⁸, Ellen L. Goode⁵ and Thomas A. Sellers⁶

¹ Department of Population Health Research, Alberta Health Services-Cancer Care, Calgary, AB, Canada

² Department of Medical Genetics, University of Calgary, Calgary, AB, Canada

³ Department of Oncology, University of Calgary, Calgary, AB, Canada

⁴ Department of Public Health Sciences, University of Alberta, Edmonton, AB, Canada

⁵ Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA

⁶ Division of Cancer Prevention and Control, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

⁷ Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

⁸ Department of Community and Family Medicine, Duke University Medical Center, Durham, NC, USA

Edited by:

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*Correspondence:

Linda E. Kelemen, Alberta Health Services-Cancer Care, Population Health Research, 2210 2nd Street SW, Calgary, AB, Canada T2S 3C3.
e-mail: lkelemen@post.harvard.edu

ATIC, *SHMT2*, and *SLC46A1* have essential roles in one-carbon (1-C) transfer. The authors examined whether associations between ovarian carcinoma and 15 variants in these genes are modified by regular multivitamin use, a source of 1-C donors, among Caucasian participants from two US case-control studies. Using a phased study design, variant-by-multivitamin interactions were tested, and associations between variants and ovarian carcinoma were reported stratified by multivitamin supplement use. Per-allele risk associations were modified by multivitamin use at six variants among 655 cases and 920 controls (Phase 1). In a larger sample of 968 cases and 1,265 controls (Phases 1 and 2), interactions were significant ($P \leq 0.03$) for two variants, particularly among regular multivitamin users: *ATIC* rs7586969 [odds ratio (OR) = 0.7, 95% confidence interval (CI) = 0.6–0.9] and *ATIC* rs16853834 (OR = 1.5, 95% CI = 1.1–2.0). The two *ATIC* single nucleotide polymorphisms (SNPs) did not share the same haplotype; however, the haplotypes they comprised mirrored their SNP risk associations among regular multivitamin supplement users. A multi-variant analysis was also performed by comparing the observed likelihood ratio test statistic from adjusted models with and without the two *ATIC* variant-by-multivitamin interaction terms with a null distribution of test statistics generated by permuting case status 10,000 times. The corresponding observed P value of 0.001 was more extreme than the permutation-derived P value of 0.009, suggesting rejection of the null hypothesis of no association. In summary, there is little statistical evidence that the 15 variants are independently associated with risk of ovarian carcinoma. However, the statistical interaction of *ATIC* variants with regular multivitamin intake, when evaluated at both the SNP and gene level, may support these findings as relevant to ovarian health and disease processes.

Keywords: autophagy, effect modification, haplotype, one-carbon transfer, permutation, purines

INTRODUCTION

The one-carbon (1-C) transfer pathway refers to biologic reactions that depend on 1-C transfers mediated by folate coenzymes: these transfers play essential roles in many major cellular processes including nucleic acid biosynthesis and methyl group generation (Ulrich et al., 2008). Consequently, perturbations in this pathway caused by folate or 1-C donor nutrient deficiency can compromise DNA synthesis and methylation and can lead to tumorigenesis (Choi and Mason, 2000). Polymorphisms in genes involved in the 1-C transfer pathway may mimic folate or 1-C donor deficiency and influence the risk of cancers. However, risk may be modified by dietary factors such as folate and other 1-C donor

nutrients (Ma et al., 1997, 2009; Maruti et al., 2009; Levine et al., 2010).

We previously examined the association between single nucleotide polymorphisms (SNPs) in several genes in the 1-C transfer pathway and ovarian carcinoma risk, but found little evidence in secondary analyses for interactions with regular multivitamin supplement use as a source of folate and other B-vitamins (Kelemen et al., 2008). In that study, the smallest false positive report probabilities (FPRP) for interaction analyses were between 0.54 and 0.66 for associations between three SNPs in *DNMT3A* and risk of ovarian carcinoma among multivitamin supplement users (Kelemen et al., 2008).

In the present study, we elucidated the potential contribution of variants in three genes for which we hypothesized, *a priori*, would have interactive effects with multivitamin supplement use as a source of folate and other 1-C donor nutrients. Aminoimidazole carboxamide ribonucleotide transformylase/inosine monophosphate cyclohydrolase (*ATIC*) is a single bifunctional protein that catalyzes the penultimate and final steps of the *de novo* purine biosynthesis via a 1-C transfer from reduced folate (Wolan et al., 2004). The *ATIC* gene is highly conserved across several species from *Escherichia coli* to humans (Cheong et al., 2004). Serine hydroxymethyltransferase 2 (*SHMT2*) is the mitochondrial isoenzyme of cytoplasmic *SHMT1*. Both enzymes catalyze the reversible transfer of a single carbon from serine to tetrahydrofolate to form glycine and 5,10 methylene-tetrahydrofolate, which is required for *de novo* pyrimidine synthesis (Tibbetts and Appling, 2010). Polymorphisms in *SHMT1* have been reported to alter the risk of ovarian carcinoma (Kelemen et al., 2008), squamous cell carcinoma of the head and neck (Zhang et al., 2005), and lung carcinoma (Wang et al., 2007). Solute carrier protein 46A1 (*SLC46A1*) plays a critical role in the absorption of folate from diet by functioning as a proton-coupled folate transporter and as a mediator of folate receptor endocytosis (Qiu et al., 2006; Zhao et al., 2009). Nucleotide substitutions in *SLC46A1* that result in point mutations or a splice variant in the corresponding protein have recently been described in patients exhibiting hereditary folate malabsorption (Zhao et al., 2007).

We present here our findings for the associations between 15 common SNPs in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma risk, stratified by regular multivitamin supplement use as an estimate of B-vitamin intake, using data from two case-control studies including 968 cases and 1,265 controls.

MATERIALS AND METHODS

STUDY POPULATION

The study population consisted of a combination of two individual studies of epithelial ovarian cancer from Mayo Clinic and Duke University. Details of the study protocols have been published previously (Sellers et al., 2005). Briefly, participants included Caucasians and African-Americans enrolled from June 1999 onward. Mayo Clinic cases were 20 years of age or older and resident in a six-state region (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, and South Dakota) that defined the primary catchment area. Controls were women seeking general medical care at Mayo Clinic. Duke University cases were between 20 and 74 years of age and identified from a 48-county region in North Carolina. Controls were identified from the same region using population-based list-assisted random digit dialing. In both studies, cases were newly diagnosed, histologically confirmed, of either borderline or invasive behavior, and enrolled within 1 year of diagnosis. Controls had at least one intact ovary, no history of ovarian cancer and were frequency-matched to cases on age (5-year age categories), race, and state of residence. The response among cases and controls, respectively, was high at Mayo Clinic (83 and 74%) and Duke University (75 and 64%). All participants provided written informed consent and procedures followed were in accordance with the Institutional Review Board of Mayo Clinic and the Institutional Review Board of Duke University Medical Center.

ASSESSMENT OF REGULAR MULTIVITAMIN SUPPLEMENT USE AND OTHER RISK FACTORS

Multivitamin supplement use and information on established risk factors such as reproductive history, family history of cancer, medical history, and lifestyle habits were obtained from all subjects through in-person interviews using study-specific questionnaires. Variables were harmonized across studies for this analysis. The Mayo Clinic study began collecting information on multivitamin intake 3 years after the start of the study, while the Duke University study elicited this information from the start of the study. At Duke University, participants indicated their regularity of multivitamin use (none, <1 pill per week, 1–2 per week, 3–4 per week, 5–6 per week, or daily) during the previous 5 years. We defined users as those who consumed at least three pills per week for at least 48 months during the past 5 years (e.g., allowing 80% adherence). At Mayo Clinic, participants responded yes or no to whether they consumed multivitamins at least four times per week during the previous year for controls and 1 year before diagnosis for cases; they also estimated their total number of years' duration of use. To create a "multivitamin user" definition that compared to the Duke University subjects, we defined Mayo Clinic users as women consuming at least four pills per week for at least 48 months' duration of lifetime use.

GENETIC ANALYSIS

DNA was obtained from peripheral blood samples using salt extraction protocols (Sellers et al., 2005; Cunningham et al., 2008). In addition, whole genome amplification (WGA) using the REPLI-G WGA protocol (Qiagen Inc., Valencia, CA, USA) was used to enrich DNA samples from Duke University due to limited quantities. Excellent (>99%) concordance between genomic and WGA DNA genotype calls for these samples has been reported previously (Cunningham et al., 2008).

TagSNPs were chosen using SNP information from unrelated Caucasian samples within HapMap Consortium's release 22 (The International HapMap Consortium, 2003) as previously described (Kelemen et al., 2008), and also for their predicted likelihood of successful genotyping using the Illumina Golden Gate Assay™. We selected nine tagSNPs in *ATIC* (rs3772078, rs2372536, rs1880586, rs1983462, rs16853826, rs7586969, rs16853834, rs1404772, and rs7604984) from among 45 individual SNPs, two tagSNPs in *SHMT2* (rs7301155 and rs2229716) from among two individual SNPs and four tagSNPs in *SLC46A1* (rs9894260, rs739439, rs2239908, and rs17719944) from among 10 individual SNPs using criteria of minor allele frequency (MAF) ≥ 0.05 and pairwise linkage disequilibrium (LD) of $r^2 < 0.8$. The SNPs were located within, and 5 kb upstream and downstream of, each gene region.

The 15 tagSNPs were genotyped using a phased study design. In the first phase (Phase 1), we genotyped the SNPs as part of a larger investigation of 1,152 SNPs in a variety of pathways using the Illumina GoldenGate™ assay and Illumina BeadStudio software (Oliphant et al., 2002; Fan et al., 2005; Steemers and Gunderson, 2005). Genotyping was attempted on 897 genomic DNA samples from Mayo Clinic and 1,279 WGA samples from Duke University (total = 2,176 including 129 duplicate samples) for subjects recruited through March 2006 as well as 65 laboratory controls. Of these samples, we excluded 44 (one Mayo Clinic

sample and 43 Duke University samples) with call rates < 90% and Illumina quality control (GenCall) scores < 0.25, and 22 samples where the cancer was not of epithelial origin or the samples were mislabeled, resulting in 1,981 unique samples that were successfully genotyped. The sample call rate was 99.74% and the concordance for duplicate samples was 99.99%. All 15 tagSNPs in *AT1C*, *SHMT2*, and *SLC46A1* were genotyped successfully, except *SHMT2* rs7301155 that failed in Duke University samples. In Phase 2, we genotyped or imputed the 15 tagSNPs as part of a genome-wide association study (GWAS) of ovarian carcinoma using the Illumina 610 K chip on 186 genomic DNA samples from Mayo Clinic and 477 WGA samples from Duke University for subjects recruited after March 2006. Genotyping call rates for these SNPs were $\geq 99\%$. Imputation was performed using the MACH software (Li et al., 2009) and the phase II HapMap (release 22) referent genotypes for Caucasian samples for eight SNPs [*AT1C* (rs2372536, rs1880586, rs1983462, rs16853826, and rs7586969), *SHMT2* (rs7301155 and rs2229716), and *SLC46A1* (rs9894260)]. Because *SHMT2* rs7301155 failed in Duke University samples from Phase 1, we included the imputed genotypes for this SNP from both Phase 1 and Phase 2 Duke University samples represented on the GWAS. Squared correlations between imputed and true genotypes were between 0.93 and 0.99, except $r^2 = 0.43$ for *SHMT2* rs2229716, which was likely due to its rare frequency (Table A1 in Appendix).

STATISTICAL ANALYSIS

Analyses were restricted to subjects who were self-reported Caucasian controls or cases with invasive epithelial ovarian carcinoma, resulting in a final sample size of 1,575 participants in Phase 1 from Mayo Clinic (312 cases and 439 controls) and Duke University (343 cases and 481 controls) and 658 participants in Phase 2 from Mayo Clinic (89 cases and 96 controls) and Duke University (224 cases and 249 controls). Genotypes of participants were used to estimate allele frequencies and chi squared tests were used to examine deviations of genotype frequencies from those expected under Hardy–Weinberg equilibrium (HWE) in control subjects. Pairwise LD was examined across all SNPs with the r^2 statistic using Haploview (Barrett et al., 2005). Data from Mayo Clinic and Duke University were combined following confirmation of no statistical heterogeneity in SNP associations between study sites.

Our primary hypothesis was that SNP associations would be modified by regular multivitamin supplement use as a source of folate or other nutrients involved 1-C transfer. We evaluated this first in Phase 1 samples. Interactions were evaluated with the Wald test in models that included a one degree-of-freedom product term for regular multivitamin supplement use (yes/no) and the ordinal coding for genotype, in addition to the covariates age (<40, 40–49, 50–59, 60–69, and 70+ years), region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina), SNP, regular multivitamin supplement use, body mass index, age at menarche, oral contraceptive use, postmenopausal status, postmenopausal hormone use, parity/age at first birth, smoking, and education (see Table 2 for categories). To determine the degree of falsely positive findings due to the number of interaction tests that were performed, we calculated

q -values to estimate false discovery rates (FDR; Hochberg and Benjamini, 1990). Associations between SNPs and ovarian carcinoma were stratified by regular multivitamin supplement use and estimated as odds ratios (OR) with 95% confidence intervals (CI) using multivariable-adjusted unconditional logistic regression under ordinal genetic models. For SNPs where the tests for interaction had higher FDR q -values (e.g., >0.23 in our data), suggesting a higher likelihood of falsely positive tests, we estimated associations between the genetic main effects and ovarian carcinoma under both co-dominant and ordinal genetic models. Models evaluating the main effects of SNPs were adjusted for age and region of residence. Adjustment for additional covariates did not change the ORs (data not shown).

We performed the same analyses using Phase 2 samples as an independent replication, and then combined the Phase 1 and Phase 2 samples to increase statistical power. To account for the potential correlations among SNPs within genes, we performed both a multi-variant analysis and haplotype analysis. The multi-variant analysis was performed by calculating a likelihood ratio test (LRT) statistic comparing a regression model consisting of the main effects of those SNPs exhibiting significant interactions with multivitamin supplement use, their corresponding interaction terms, and potential confounding variables to a model without the SNP-by-multivitamin interaction terms. SNP main effects and their interaction terms were regressed under the ordinal (log-additive) genetic model. Permutation-based tests were then used to compute P values from a null distribution of the LRT statistic generated by permuting case status 10,000 times. The corresponding P value threshold was calculated as the probability of observing a permuted LRT statistic as extreme or more extreme than the observed LRT statistic. We also estimated haplotype frequencies for each gene, stratified and unstratified by regular multivitamin supplement use, by computing the posterior probabilities of all possible haplotypes for an individual, conditioned on the observed genotypes, using an expectation–maximization algorithm (Schaid et al., 2002). We used these probabilities to define haplotype design variables that estimated the number of each of the haplotypes carried by an individual, and retained only those with estimated frequencies of > 1%. We first tested whether differences among the haplotypes within each gene were associated statistically with ovarian carcinoma overall ($P < 0.016$, Bonferroni-corrected for three genes). Next, associations between individual haplotypes and risk were estimated under ordinal genetic models (e.g., carriage of 0, 1, or 2 copies of a particular haplotype; Schaid, 2011). Individual associations were not considered statistically significant in the absence of global significance.

Statistical tests were two-sided, and were implemented with SAS (SAS Institute, NC), R (R Development Core Team, 2011), and Haplo.stats (Schaid, 2011) software.

RESULTS

There was no evidence of deviation from HWE in controls from both studies for any of the SNPs. The MAFs among controls ranged from 0.04 to 0.48 and were similar across study sites in Phase 1 (Table A1 in Appendix), Phase 2 (data not shown), and Phases 1 and 2 combined, with imputed SNPs in Phases 1 and 2 combined showing similar MAFs as typed SNPs in Phase 1 (Table A1

in Appendix). The distribution of some covariates varied among cases and controls at both Mayo Clinic and Duke University in Phase 1 (Table A2 in Appendix), Phase 2 (data not shown), and Phases 1 and 2 combined (Table 1); however, histological subtypes of tumors were distributed comparably for both studies.

In Phase 1, the associations between six SNPs and ovarian carcinoma risk were modified by regular multivitamin supplement use at the smallest FDR q -value of 0.23: *ATIC* rs16853826, rs7586969 and rs16853834, *SHMT2* rs7301155, and *SLC46A1* rs9894260 and rs739439 (Table 2). In stratified analyses, among women who took multivitamins regularly, the strongest associations were observed between ovarian carcinoma and *ATIC* rs16853834 (OR = 1.5, 95% CI = 1.1–2.1, $P = 0.01$) and *SHMT2* rs7301155 (OR = 1.7, 95% CI = 1.1–2.6, $P = 0.01$). Among non-regular supplement users, a trend was observed between ovarian carcinoma and *ATIC* rs16853826 (OR = 0.7, 95% CI = 0.5–1.0, $P = 0.06$). Comparable stratified analyses are shown in Table A3 in Appendix for Phase 2 samples, with none of the aforementioned SNPs showing statistically significant interactions in this smaller sample, although the direction of association among multivitamin supplement users was consistent for *ATIC* rs16853834 (OR = 1.6, 95% CI = 0.7–3.7). Combining Phase 1 and Phase 2 samples (968 cases and 1,265 controls; Table 3), statistically significant interactions were observed between regular multivitamin supplement use and *ATIC* rs7586969 (OR = 0.7, 95% CI = 0.6–0.9) and *ATIC* rs16853834 (OR = 1.5, 95% CI = 1.1–2.0).

A multi-variant analysis was performed by calculating a LRT statistic comparing adjusted models that included *ATIC* rs7586969 and *ATIC* rs16853834, multivitamin use and the SNP-by-multivitamin interaction terms to a model without *ATIC* SNP-by-multivitamin interaction terms, and comparing this to a null distribution of the LRT statistic generated by permuting case status 10,000 times. The observed P value for *ATIC* ($\chi^2 = 12.98$, 2 df, $P = 0.001$) was more extreme than the P value derived from permutation testing ($P = 0.009$), suggesting the evidence is inconsistent with the null hypothesis of no association between *ATIC* SNP-by-multivitamin interactions and risk of ovarian carcinoma.

In haplotype analyses, only the *ATIC* gene was statistically significantly associated with ovarian carcinoma among regular multivitamin supplement users (Table 4: global haplotype test $P = 0.01$; data not shown for *SHMT2* and *SLC46A1*). The two *ATIC* SNPs, rs7586969, and rs16853834, whose minor alleles showed opposing risks, did not share the same haplotype; however, the haplotypes they comprised mirrored their SNP risk associations among regular multivitamin supplement users. Haplotype #1 accounted for 24% of all estimated haplotypes, comprised the minor allele of *ATIC* rs7586969 and was associated with a 24% decreased risk. Haplotype #9 accounted for 13% of all estimated haplotypes, comprised the minor allele of *ATIC* rs16853834 and was associated with a 39% increased risk. A third haplotype, haplotype #10, with 8% frequency, comprised common alleles for *ATIC* rs7586969 and rs16853834 and was associated with a 42% increased risk. Among non-users, haplotype #5 was associated with a 27% decreased risk, but the global haplotype test was not statistically significant. None of the associations were statistically significant in the unstratified data. Squared correlations among the SNPs are shown in Figure A1 in Appendix.

For remaining SNPs with FDR q -values > 0.23 in Phase 1, we estimated main effect associations with ovarian carcinoma (Table A4 in Appendix). An increased risk was observed among 12 carriers homozygous for the rare variant *SLC46A1* rs9894260 compared to carriers homozygous for the wildtype allele (OR = 5.4, 95% CI = 1.4–20.5). Similar results were seen using Phase 2 samples (data not shown), as well as Phase 1 and Phase 2 samples combined (Table A5 in Appendix). The main effect association between regular multivitamin supplement use and ovarian carcinoma in Phase 1 and Phase 2 samples combined was not statistically significant at $P < 0.05$ (multivariable OR = 0.86, 95% CI = 0.70–1.06, $P = 0.15$; data not shown).

DISCUSSION

We evaluated the association between 15 SNPs in *ATIC*, *SHMT2*, and *SLC46A1* in the 1-C transfer pathway and ovarian carcinoma risk, and the modification of risk by regular multivitamin supplement use among 968 cases and 1,265 controls. There was little statistical evidence that SNPs or haplotypes in these genes were independently associated with ovarian carcinoma risk. However, the associations of *ATIC* rs7586969 and *ATIC* rs16853834 with ovarian carcinoma, when stratified by regular multivitamin supplement use and evaluated further in multi-variant and haplotype analyses, suggest the potentially important role that lifestyle factors may have in modifying genetic susceptibility.

We calculated that six interactions between SNPs and regular multivitamin use had an FDR q -value of 0.23 indicating that, out of 100 SNPs with this FDR q -value, 23 will be falsely positive, or approximately one in four. Further analyses in a larger sample showed consistent associations for two of these SNPs at $P < 0.05$. *ATIC* rs7586969 and *ATIC* rs16853834 have MAF in our data of 0.39 and 0.16, respectively, but are weakly correlated with each other (pairwise LD $r^2 = 0.12$). The power to detect these associations was relatively high: with an average exposure in our control group of 43% for regular multivitamin supplement use, we achieved close to 80% power needed with 1,054 cases using a ratio of 1:1.3 case-control pairs (actual: 968 cases and 1,265 controls) to detect an OR of 0.7 (*ATIC* rs7586969) at $\alpha = 0.05$, and we would require 1,161 case-control pairs to detect an OR of 1.5 (*ATIC* rs16853834) under the log-additive genetic model (Quanto version 1.2, CA, USA, <http://hydra.usc.edu/gxe>). The power to detect the haplotype associations, where the greatest frequency was 24% (haplotype #1), was lower. The presence of two SNPs in the same gene with relatively independent (and opposing) effects was resolved with haplotype analysis: the two SNPs do not share the same haplotype. Both the haplotype analysis and the multi-variant, permutation-based test suggest the data are inconsistent with the null hypothesis of no association between *ATIC* SNP-by-multivitamin interactions and risk of ovarian carcinoma. The aforementioned *ATIC* SNPs are intronic and, unlike exons, little is known of their role in the regulation of the final functional protein. However, because we genotyped tagSNPs, which merely identify a region in the gene at which an association is found, additional genotyping of the region (finemapping) and association testing is required to determine the precise causal variant(s). *In vitro* cell culture studies, where the nutrient medium can be manipulated, could

Table 1 | Characteristics of 2,233 Caucasian subjects (Phases 1 and 2), Mayo Clinic, and Duke University.

	Mayo Clinic		Duke University	
	Cases (<i>n</i> = 401)	Controls (<i>n</i> = 535)	Cases (<i>n</i> = 567)	Controls (<i>n</i> = 730)
Age, year [mean (SD)]	61.0 (12.5)	60.7 (13.2)	57.3 (10.3)	55.3 (12.0)
BODY MASS INDEX, kg/m²				
<23 [<i>n</i> (%)]	80 (20.5)	127 (24.8)	152 (27.6)	203 (28.4)
23–26 [<i>n</i> (%)]	100 (25.6)	150 (29.3)	131 (23.8)	173 (24.2)
26–29 [<i>n</i> (%)]	75 (19.2)	99 (19.3)	113 (20.5)	141 (19.7)
≥29 [<i>n</i> (%)]	136 (34.8)	136 (26.6)	155 (28.1)	199 (27.8)
AGE AT MENARCHE, year				
<12 [<i>n</i> (%)]	63 (19.0)	77 (15.3)	139 (24.7)	132 (18.1)
12 [<i>n</i> (%)]	84 (25.3)	111 (22.0)	160 (28.4)	210 (28.8)
13 [<i>n</i> (%)]	93 (28.0)	149 (29.5)	139 (24.7)	206 (28.3)
≥14 [<i>n</i> (%)]	92 (27.7)	168 (33.3)	126 (22.3)	181 (24.8)
ORAL CONTRACEPTIVE USE, months				
Never [<i>n</i> (%)]	182 (47.5)	200 (39.8)	194 (40.3)	207 (31.1)
1–48 [<i>n</i> (%)]	101 (26.4)	107 (21.3)	142 (29.5)	181 (27.2)
≥48 [<i>n</i> (%)]	100 (26.1)	196 (39.0)	145 (30.2)	278 (41.7)
POSTMENOPAUSAL STATUS				
Yes [<i>n</i> (%)]	290 (74.7)	393 (76.0)	251 (46.9)	502 (69.8)
POSTMENOPAUSAL HORMONE USE, months				
Never [<i>n</i> (%)]	241 (61.6)	287 (58.5)	211 (39.2)	407 (58.4)
1–60 [<i>n</i> (%)]	71 (18.2)	91 (18.5)	166 (30.9)	147 (21.1)
≥60 [<i>n</i> (%)]	79 (20.2)	113 (23.0)	161 (29.9)	143 (20.5)
PARITY, <i>n</i>/age at first birth, year				
Nulliparous [<i>n</i> (%)]	69 (17.4)	77 (15.0)	113 (19.9)	94 (12.9)
1–2/≤20 [<i>n</i> (%)]	29 (7.3)	26 (5.1)	80 (14.1)	92 (12.6)
1–2/≥20 [<i>n</i> (%)]	115 (29.0)	155 (30.1)	208 (36.7)	314 (43.0)
≥3/≤20 [<i>n</i> (%)]	77 (19.4)	74 (14.4)	80 (14.1)	92 (12.6)
≥3/≥20 [<i>n</i> (%)]	106 (26.8)	183 (35.5)	86 (15.2)	138 (18.9)
FAMILY HISTORY OF OVARIAN CANCER^a				
Yes [<i>n</i> (%)]	50 (12.6)	42 (8.1)	56 (9.9)	46 (6.3)
SMOKING, pack-years				
None [<i>n</i> (%)]	242 (63.7)	333 (68.5)	303 (55.7)	376 (53.5)
≤20 [<i>n</i> (%)]	75 (19.7)	100 (20.6)	137 (25.2)	187 (26.6)
>20 [<i>n</i> (%)]	63 (16.6)	53 (10.9)	104 (19.1)	140 (19.9)
EDUCATION				
No diploma [<i>n</i> (%)]	22 (5.8)	21 (4.1)	49 (8.6)	66 (9.0)
High School diploma [<i>n</i> (%)]	136 (35.9)	133 (25.7)	179 (31.6)	192 (26.3)
Post-high school education [<i>n</i> (%)]	221 (58.3)	364 (70.3)	339 (59.8)	472 (64.7)
MULTIVITAMIN USE^b				
Yes [<i>n</i> (%)]	119 (44.9)	259 (52.4)	210 (38.1)	260 (36.4)
TUMOR HISTOLOGY, CASES				
Serous [<i>n</i> (%)]	255 (63.9)		329 (58.1)	
Mucinous [<i>n</i> (%)]	11 (2.8)		26 (4.6)	
Endometrioid [<i>n</i> (%)]	77 (19.3)		85 (15.0)	
Clear cell [<i>n</i> (%)]	25 (6.3)		60 (10.6)	
Other [<i>n</i> (%)]	31 (7.8)		66 (11.7)	

Counts do not total to 2,233 subjects due to missing data for some variables.

^aIn first- or second-degree relative.

^bAt least four pills per week during the previous year for at least 48 months' duration of lifetime use (Mayo Clinic subjects) or at least three pills per week for at least 48 months during the past 60 months (Duke University subjects).

Table 2 | Odds ratios (OR) and 95% CIs for the association between variants in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma stratified by multivitamin supplement use among 1,575 Caucasian subjects (Phase 1), Mayo Clinic, and Duke University.

Gene/SNP rsID	Multivitamin non-users				Multivitamin users ^a				<i>P</i> _{int} ^c	FDR ^d
	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele Cases/controls	Ordinal (per minor allele) OR (95%CI) ^b	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele Cases/controls	Ordinal (per minor allele) OR (95%CI) ^b		
<i>ATIC</i>										
rs3772078	212/319	95/160	6/14	0.9 (0.7–1.2)	122/241	58/110	7/16	1.0 (0.7–1.4)	0.54	0.63
rs2372536	137/225	156/226	28/55	1.0 (0.8–1.2)	98/167	64/162	25/44	0.9 (0.7–1.2)	0.52	0.63
rs1880586	76/131	176/268	71/108	1.1 (0.9–1.3)	52/107	84/188	51/83	1.1 (0.9–1.4)	0.79	0.79
rs1983462	147/235	146/221	31/51	1.0 (0.8–1.3)	97/166	73/180	18/32	0.8 (0.6–1.1)	0.27	0.45
rs16853826	258/378	65/122	1/7	0.7 (0.5–1.0)	139/291	46/80	3/7	1.2 (0.8–1.7)	0.09	0.23
rs7586969	115/199	161/235	47/72	1.1 (0.9–1.4)	81/135	83/185	24/58	0.8 (0.6–1.0)	0.07	0.23
rs16853834	227/356	85/140	12/11	1.0 (0.8–1.4)	119/281	60/87	9/10	1.5 (1.1–2.1)	0.07	0.23
rs1404772	279/450	43/55	2/2	1.3 (0.9–1.9)	167/325	20/51	1/2	0.8 (0.5–1.3)	0.17	0.31
rs7604984	106/189	172/241	46/77	1.1 (0.9–1.3)	81/128	77/187	30/62	0.8 (0.6–1.1)	0.12	0.26
<i>SHMT2</i>										
rs7301155 ^e	46/76	48/101	16/25	1.0 (0.7–1.4)	23/104	42/93	8/20	1.7 (1.1–2.6)	0.08	0.23
rs2229716	300/462	23/44	1/1	0.8 (0.5–1.4)	174/348	14/29	0/0	0.9 (0.5–1.9)	0.70	0.75
<i>SLC46A1</i>										
rs9894260	265/419	57/86	1/2	1.0 (0.7–1.4)	151/319	32/58	4/1	1.5 (1.0–2.3)	0.08	0.23
rs739439	216/350	93/148	14/9	1.2 (0.9–1.5)	126/237	56/124	5/17	0.8 (0.6–1.1)	0.09	0.23
rs2239908	98/159	163/253	62/94	1.0 (0.8–1.3)	57/102	96/203	34/72	0.9 (0.7–1.2)	0.54	0.63
rs17719944	270/426	50/75	4/6	1.0 (0.7–1.5)	152/298	34/77	1/3	0.9 (0.6–1.3)	0.46	0.63

^aAt least 4 pills per week during the previous year for at least 48 months' duration of lifetime use (Mayo Clinic subjects) or at least three pills per week for at least 48 months during the past 60 months (Duke University subjects).

^bAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ years), region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina), body mass index, age at menarche, oral contraceptive use, postmenopausal status, postmenopausal hormone use, parity/age at first birth, smoking, and education (see **Table 1** for categories). SNP effect was determined using a log-additive logistic regression model.

^c*P* for interaction.

^dFalse discovery rate *q*-value.

^eMayo Clinic subjects only.

Table 3 | Odds ratios (OR) and 95% CIs for the association between variants in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma stratified by multivitamin supplement use among 2,233 subjects (Phases 1 and 2), Mayo Clinic and Duke University.

Gene/SNP rsID	Multivitamin non-users				Multivitamin users ^a				<i>P</i> _{int} ^c
	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele Cases/controls	Ordinal (per minor allele) OR (95%CI) ^b	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele Cases/controls	Ordinal (per minor allele) OR (95%CI) ^b	
<i>ATIC</i>									
rs3772078	323/438	141/217	12/20	0.9 (0.7–1.1)	214/337	102/149	12/22	1.0 (0.7–1.3)	0.51
rs2372536 ^d	224/323	213/289	47/76	1.0 (0.8–1.2)	159/232	122/222	47/60	1.0 (0.8–1.3)	0.87
rs1880586 ^d	125/191	253/342	108/156	1.0 (0.9–1.2)	83/146	149/249	96/124	1.2 (0.9–1.4)	0.47
rs1983462 ^d	208/315	222/298	57/76	1.1 (0.9–1.3)	174/230	125/237	30/52	0.8 (0.6–1.0)	0.05
rs16853826 ^d	384/516	98/164	5/9	0.8 (0.6–1.0)	245/400	80/111	4/8	1.1 (0.8–1.6)	0.11
rs7586969 ^d	166/261	239/323	81/104	1.1 (0.9–1.3)	142/185	149/252	38/82	0.7 (0.6–0.9)	0.01
rs16853834	349/483	121/186	16/18	1.0 (0.8–1.3)	214/384	102/121	13/14	1.5 (1.1–2.0)	0.03
rs1404772	425/609	59/77	3/3	1.2 (0.8–1.7)	290/447	38/70	1/2	0.7 (0.5–1.2)	0.20
rs7604984	179/275	239/306	69/108	1.0 (0.9–1.3)	130/184	142/248	57/85	0.9 (0.7–1.2)	0.35
<i>SHMT2</i>									
rs7301155 ^d	204/283	187/296	42/66	0.9 (0.7–1.1)	133/239	147/211	28/42	1.3 (1.0–1.6)	0.08
rs2229716 ^d	458/638	28/49	1/2	0.8 (0.5–1.3)	309/488	20/30	0/0	1.0 (0.5–2.0)	0.51
<i>SLC46A1</i>									
rs9894260 ^d	405/575	78/111	3/3	1.0 (0.7–1.3)	272/433	50/83	6/3	1.1 (0.7–1.6)	0.62
rs739439	323/475	143/201	20/13	1.1 (0.9–1.4)	206/336	109/160	13/23	1.0 (0.7–1.3)	0.46
rs2239908	139/213	249/348	98/127	1.1 (0.9–1.3)	82/144	178/268	68/106	1.0 (0.8–1.2)	0.64
rs17719944	404/576	78/104	5/9	1.0 (0.7–1.3)	261/421	65/94	1/4	1.1 (0.7–1.6)	0.66

^aAt least four pills per week during the previous year for at least 48 months' duration of lifetime use (Mayo Clinic subjects) or at least three pills per week for at least 48 months during the past 60 months (Duke University subjects).

^bAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ years), region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina), body mass index, age at menarche, oral contraceptive use, postmenopausal status, postmenopausal hormone use, parity/age at first birth, smoking, and education (see **Table 1** for categories). SNP effect was determined using a log-additive logistic regression model.

^c*P* for interaction.

^dSNP imputed from GWAS for all Duke University subjects (Phase 1 and Phase 2).

Table 4 | Association^a between haplotypes derived from ATIC variants and ovarian carcinoma with and without stratification by multivitamin supplement use among 2,233 subjects (Phases 1 and 2), Mayo Clinic, and Duke University.

Haplotype ^b	Multivitamin non-users			Multivitamin users			All subjects		
	Global haplotype test <i>P</i> value	Estimated haplotype frequency	OR (95% CI)	Global haplotype test <i>P</i> value	Estimated haplotype frequency	OR (95% CI)	Global haplotype test <i>P</i> value	Estimated haplotype frequency	OR (95% CI)
	0.56			0.01			0.70		
001101000		0.26	1.04 (0.86–1.26)		0.24	0.76 (0.59–0.97)		0.25	0.93 (0.81–1.07)
010000001		0.26	0.98 (0.81–1.19)		0.25	0.82 (0.65–1.04)		0.26	0.94 (0.82–1.08)
100001011		0.05	1.23 (0.84–1.79)		0.05	0.74 (0.46–1.19)		0.05	1.05 (0.80–1.38)
000101000		0.07	1.16 (0.89–1.51)		0.07	0.96 (0.68–1.35)		0.06	1.01 (0.82–1.23)
100010000		0.10	0.73 (0.55–0.98)		0.10	1.10 (0.78–1.55)		0.10	0.95 (0.78–1.16)
101001011		0.01	0.69 (0.29–1.66)		0.02	1.28 (0.53–3.05)		0.01	0.77 (0.42–1.40)
101010000		0.02	1.13 (0.63–2.03)		0.03	1.50 (0.79–2.87)		0.02	1.17 (0.78–1.75)
000000100		0.03	0.87 (0.56–1.38)		0.03	1.52 (0.90–2.56)		0.03	1.02 (0.73–1.43)
001000100		0.13	1.00 (0.78–1.29)		0.13	1.39 (1.04–1.87)		0.13	1.16 (0.97–1.38)
011000001		0.06	1.07 (0.79–1.45)		0.08	1.42 (1.04–1.95)		0.06	1.12 (0.91–1.39)

^aAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ yrs), residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina), body mass index, age at menarche, oral contraceptive use, postmenopausal status, postmenopausal hormone use, parity/age at first birth, smoking, and education (see **Table 1** for categories). Haplotype associations were estimated using log-additive logistic regression models.

^bEstimated using both cases and controls; SNPs that formed ATIC haplotypes were, in positional order, rs3772078, rs2372536, rs1880586, rs1983462, rs16853826, **rs7586969**, **rs16853834**, rs1404772, rs7604984, where 0 = common allele and 1 = rare allele. Bold indicates the two significant SNPs with opposing effects from **Table 3**.

then begin to unravel the functional impact of SNP-by-nutrient interactions.

ATIC catalyzes the final two steps in *de novo* purine biosynthesis, which is the main source of purine nucleotides (Kondo et al., 2000; Wolan et al., 2004). The final atom of the purine ring is supplied by folate, producing 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). Not only can AICAR induce apoptosis under certain conditions (Hallstrom et al., 2008), but as the substrate for ATIC, it can be catabolized to the purine “parent” nucleotide, inosine monophosphate (IMP), that ultimately generates two of the four bases, adenine and guanine. The bases are incorporated into DNA and RNA and also function as important sources of energy that drive most biologic reactions (e.g., ATP, GTP, UTP, CTP; Rodwell, 2000). Conceivably, ATIC may link nutrient metabolism vis-à-vis the 1-C transfer pathway to enable autophagy, which is the cellular catabolic degradation response to starvation or stress whereby cellular proteins, organelles, and cytoplasm are engulfed, digested, and recycled to sustain cellular metabolism (Mathew et al., 2007). For example, amino acid starvation is a potent inducer of autophagy (Mizushima, 2007), and defects in autophagy have been linked to tumorigenesis (Mathew et al., 2007).

Most individuals do not meet their Dietary Reference Intakes (DRI) for certain nutrients from foods alone. For example, among non-supplement users, folate intake (on average, <300 µg/day; Zhang et al., 1999; Feigelson et al., 2003; Kelemen et al., 2004; Ericson et al., 2007) is less than the DRI (400 µg/day). A typical multivitamin contains a minimum of 400 µg of folic acid, thereby increasing some individuals’ intakes to >600 µg/day (Zhang et al., 1999; Feigelson et al., 2003). Pharmacologic doses of vitamin

intake are hypothesized to be both chemo-preventive and chemo-progressive, and the mechanism(s) for these alterations may be related to the presence of existing pre-cancerous lesions, where vitamins like folate are required for growth by proliferating cells including tumors (Kim, 2003), and/or genetic susceptibility. Perhaps the best known example where nutrients influence gene-product function is that of high folate levels physically stabilizing, and thereby rescuing, MTHFR enzyme function in the presence of the MTHFR C677T SNP (Frosst et al., 1995; Anonymous, 1999; Guenther et al., 1999). Individuals with this SNP who also have higher folate intakes have lower risks of CVD (Klerk et al., 2002) and cancers of the colon (Giovannucci, 2002; Sharp and Little, 2004) than those with the SNP who have lower folate intakes.

Candidate SNP studies and GWAS examining main effect associations between SNPs and cancer risk have revealed relatively few causal variants. Given that some environmental exposures are known to play a role in the development of cancer (World Cancer Research Fund/American Institute for Cancer Research, 2007), it is entirely possible that the genetic effects assumed present but not yet found may be hidden within specific subsets of individuals defined by their environmental exposures, as shown in the current study. The SNPs in this investigation would not have been identified had we only focused on main effects, supporting the need for more studies such as this one to fully discern the interplay between genes and environment on risk of cancer. Large international consortia with well-annotated exposure and phenotype information, such as the international Ovarian Cancer Association Consortium, are well-positioned to provide the large sample sizes needed to detect effect modification, especially for SNPs with low MAF.

The strengths of this analysis include the use of stringent quality control for genotyping that resulted in few excluded or failed samples, with remaining call rates over 99%, and the large sample to investigate our findings for the SNP-by-multivitamin interactions, especially for *ATIC* SNPs. Population structure is unlikely, since analyses were restricted to Caucasians and there was no statistical heterogeneity in SNP associations between study sites. There are also potential limitations. For most other SNPs, the power to detect gene–environment interactions was low. Although we applied the FDR method to estimate the number of falsely positive interactions, the application of FDR has been traditionally to high dimensionality data and may not be applicable to smaller datasets. Nevertheless, the consistent associations in the combined Phases 1 and 2 sample of two of the six SNPs with lower FDR values from Phase 1, in addition to the multi-variant and haplotype analyses of *ATIC* SNP-by-multivitamin interactions, suggests the associations may not be due to chance. Another potential limitation is that we did not have information on dietary intake to verify which nutrient(s) was related to the modifying effects of multivitamins. Although self-reported multivitamin supplement use adequately represents the higher nutrient status among consumers compared

to non-consumers (Zhang et al., 1999, 2003; Kelemen et al., 2004), the potential for misclassification of this exposure may still exist in our study. The reference period for multivitamin use differed by site resulting in a slightly different definition in the Mayo Clinic and the Duke University studies. Our results for the interaction *P* values were somewhat attenuated compared to analyses that disregarded the information on duration of use (data not shown). Ostensibly, misclassification of exposure, although present, did not appear to influence our findings substantially.

In summary, this report provides little statistical evidence that SNPs in *ATIC*, *SHMT2*, and *SLC46A1* are independently associated with ovarian carcinoma risk. The statistical interaction of *ATIC* SNPs with regular multivitamin intake and *ATIC*'s role in cellular metabolism and autophagy may support these findings as relevant to ovarian health and disease processes.

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APPENDIX

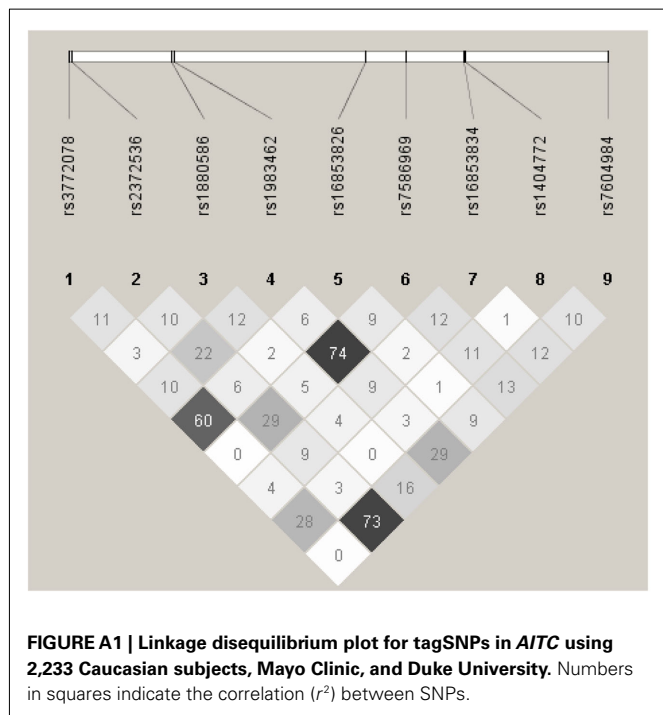


Table A1 | Descriptive information and test statistics for SNPs in *ATIC*, *SHMT2*, and *SLC46A1* among Caucasian controls, Mayo Clinic, and Duke University.

Gene	Chr	Chr Position (bp) ^a	SNP rsID	Location	Phase 1						Phases 1 and 2					
					P HWE ^b			MAF ^c			P HWE ^b			MAF ^c		
					Mayo	Duke	All	Mayo	Duke	All	Mayo	Duke	All	Mayo	Duke	All
<i>ATIC</i>	2	215898090	rs3772078	Intron	0.88	0.88	0.74	0.19	0.19	0.19	1.00	0.80	0.85	0.20	0.18	0.19
	2	215898265	rs2372536 ^d	Exon ^e	0.23	0.54	0.71	0.32	0.34	0.33	0.32	0.40	0.18	0.32	0.33	0.32
	2	215903416	rs1880586 ^d	Intron	0.77	0.36	0.35	0.48	0.48	0.48	0.94	0.46	0.51	0.49	0.48	0.48
	2	215903526	rs1983462 ^d	Intron	0.58	0.30	0.23	0.32	0.32	0.32	0.77	0.61	0.90	0.32	0.33	0.33
	2	215913412	rs16853826 ^d	Intron	1.00	0.69	0.77	0.13	0.13	0.13	1.00	0.30	0.38	0.13	0.13	0.13
	2	215915470	rs7586969 ^d	Intron	0.42	0.38	0.94	0.39	0.37	0.38	0.47	0.53	1.00	0.39	0.39	0.39
<i>SHMT2</i>	2	215918481	rs16853834	Intron	0.36	0.49	1.00	0.15	0.15	0.15	0.61	0.89	0.60	0.15	0.16	0.16
	2	215918544	rs1404772	Intron	1.00	0.65	0.78	0.07	0.06	0.06	1.00	0.73	1.00	0.07	0.06	0.06
	2	215925884	rs7604984	3' Flanking	0.84	0.70	0.94	0.40	0.40	0.40	0.53	0.24	0.19	0.39	0.39	0.39
	12	55909088	rs7301155 ^d	5' Flanking	0.67	-	0.67	0.34	-	0.34	0.70	0.39	0.39	0.34	0.30	0.32
	12	55912849	rs2229716 ^d	Exon ^f	1.00	1.00	1.00	0.04	0.04	0.04	1.00	0.22	0.67	0.04	0.04	0.04
	17	23743752	rs9894260 ^d	Intron	0.34	0.56	0.19	0.08	0.09	0.08	1.00	0.36	0.36	0.08	0.09	0.08
<i>SLC46A1</i>	17	23747949	rs739439	3' UTR	0.72	0.16	0.44	0.17	0.20	0.18	0.66	0.38	0.71	0.18	0.19	0.18
	17	23749392	rs2239908	3' UTR	0.24	0.65	0.23	0.44	0.46	0.45	0.67	0.50	0.40	0.45	0.45	0.45
	17	23753580	rs17719944	Intron	0.55	1.00	0.70	0.09	0.10	0.10	0.80	0.51	0.51	0.09	0.09	0.09

^a Base pairs on genome build 36; UTR, untranslated region.

^b P values from the chi-square test assessing deviation of genotype frequencies among controls from those expected under Hardy-Weinberg Equilibrium.

^c Minor allele frequency.

^d Imputed SNPs in Phase 2 samples.

^e Non-synonymous polymorphism.

^f Synonymous polymorphism.

Table A2 | Characteristics of 1,575 Caucasian subjects (Phase 1), Mayo Clinic, and Duke University.

	Mayo Clinic		Duke University	
	Cases (<i>n</i> = 312)	Controls (<i>n</i> = 439)	Cases (<i>n</i> = 343)	Controls (<i>n</i> = 481)
Age, years [mean (SD)]	60.8 (12.8)	60.0 (12.9)	56.8 (10.4)	54.6 (12.0)
BODY MASS INDEX, kg/m²				
<23 [<i>n</i> (%)]	57 (18.5)	103 (23.7)	96 (29.0)	132 (28.1)
23–26 [<i>n</i> (%)]	77 (24.9)	125 (28.8)	85 (25.7)	117 (25.0)
26–29 [<i>n</i> (%)]	61 (19.7)	88 (20.3)	75 (22.7)	110 (23.5)
≥29 [<i>n</i> (%)]	114 (36.9)	118 (27.2)	75 (22.7)	110 (23.5)
AGE AT MENARCHE, year				
<12 [<i>n</i> (%)]	46 (18.5)	67 (15.7)	88 (25.7)	85 (17.7)
12 [<i>n</i> (%)]	60 (24.1)	97 (22.8)	99 (29.0)	142 (29.5)
13 [<i>n</i> (%)]	66 (26.5)	126 (29.6)	82 (24.0)	140 (29.1)
≥14 [<i>n</i> (%)]	77 (30.9)	136 (31.9)	73 (21.4)	114 (23.7)
ORAL CONTRACEPTIVE USE, months				
Never [<i>n</i> (%)]	150 (49.7)	166 (38.9)	116 (34.5)	148 (31.0)
1–48 [<i>n</i> (%)]	75 (24.8)	90 (21.1)	105 (31.3)	133 (27.8)
≥48 [<i>n</i> (%)]	77 (25.5)	171 (40.1)	115 (34.2)	197 (41.2)
POSTMENOPAUSAL STATUS				
Yes [<i>n</i> (%)]	222 (73.0)	328 (75.1)	251 (80.2)	312 (66.1)
POSTMENOPAUSAL HORMONE USE, months				
Never [<i>n</i> (%)]	193 (62.7)	244 (58.5)	108 (33.4)	270 (59.3)
1–60 [<i>n</i> (%)]	54 (17.5)	79 (18.9)	114 (35.3)	96 (21.1)
≥60 [<i>n</i> (%)]	61 (19.8)	94 (22.5)	101 (31.3)	89 (19.6)
PARITY, <i>n</i>/age at first birth, year				
Nulliparous [<i>n</i> (%)]	56 (18.0)	64 (14.7)	71 (20.7)	64 (13.3)
1–2/≤20 [<i>n</i> (%)]	20 (6.4)	24 (5.5)	46 (13.4)	57 (11.9)
1–2/≥20 [<i>n</i> (%)]	91 (29.2)	130 (29.9)	125 (36.4)	211 (43.9)
≥3/≤20 [<i>n</i> (%)]	59 (18.9)	63 (14.5)	49 (14.3)	61 (12.7)
≥3/≥20 [<i>n</i> (%)]	86 (27.6)	154 (35.4)	52 (15.2)	88 (18.3)
FAMILY HISTORY OF OVARIAN CANCER^a				
Yes [<i>n</i> (%)]	41 (13.1)	33 (7.5)	33 (9.6)	26 (5.4)
SMOKING, pack-years				
None [<i>n</i> (%)]	194 (65.3)	279 (68.2)	190 (57.8)	250 (54.1)
≤20 [<i>n</i> (%)]	56 (18.9)	82 (20.1)	80 (24.3)	121 (26.2)
>20 [<i>n</i> (%)]	47 (15.8)	48 (11.7)	59 (17.9)	91 (19.7)
EDUCATION				
No diploma [<i>n</i> (%)]	18 (6.1)	19 (4.3)	26 (7.6)	42 (8.7)
High School diploma [<i>n</i> (%)]	107 (36.2)	116 (26.5)	105 (30.6)	131 (27.2)
Post-high school education [<i>n</i> (%)]	171 (57.8)	303 (69.2)	212 (61.8)	308 (64.0)
MULTIVITAMIN USE^b				
Yes [<i>n</i> (%)]	73 (39.9)	217 (51.7)	115 (35.0)	161 (34.6)
TUMOR HISTOLOGY, CASES				
Serous [<i>n</i> (%)]	191 (61.2)		206 (60.2)	
Mucinous [<i>n</i> (%)]	8 (2.6)		19 (5.7)	
Endometrioid [<i>n</i> (%)]	63 (20.2)		53 (15.5)	
Clear cell [<i>n</i> (%)]	20 (6.4)		28 (8.2)	
Other [<i>n</i> (%)]	30 (9.6)		36 (10.5)	

Counts do not total to 1,575 subjects due to missing data for some variables.

^aIn first- or second-degree relative.

^bAt least four pills per week during the previous year for at least 48 months' duration of lifetime use (Mayo Clinic subjects) or at least three pills per week for at least 48 months during the past 60 months (Duke University subjects).

Table A3 | Odds ratios (OR) and 95% CIs for the association between variants in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma stratified by multivitamin supplement use among 658 subjects included in the GWAS (Phase 2), Mayo Clinic, and Duke University.

Gene/SNP rsID	Regular multivitamin users ^a				Regular multivitamin non-users				<i>P</i> _{int} ^c
	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele OR (95%CI) ^b	Ordinal (per minor allele) Cases/controls	Homozygous major allele (referent) Cases/controls	Heterozygous Cases/controls	Homozygous minor allele OR (95%CI) ^b	Ordinal (per minor allele)	
<i>ATIC</i>									
rs3772078	92/96	44/39	5/6	0.9 (0.4–2.1)	111/119	46/57	6/6	0.9 (0.4–1.7)	0.92
rs2372536	61/65	58/60	22/16	1.1 (0.6–2.1)	87/98	57/63	19/21	1.2 (0.7–2.1)	0.85
rs1880586	31/39	65/61	45/41	0.9 (0.5–1.7)	49/60	77/74	37/48	1.1 (0.6–1.9)	0.90
rs1983462	77/64	52/57	12/20	0.7 (0.4–1.4)	61/80	76/77	26/25	1.0 (0.6–1.8)	0.85
rs16853826	106/109	34/31	1/1	0.9 (0.3–2.4)	126/138	33/42	4/2	0.8 (0.3–1.8)	0.77
rs7586969	61/50	66/67	14/24	0.7 (0.4–1.4)	51/62	78/88	34/32	1.0 (0.5–1.7)	0.82
rs16853834	95/103	42/34	4/4	1.6 (0.7–3.7)	122/127	36/46	4/7	1.0 (0.4–2.1)	0.73
rs1404772	123/122	18/19	0/0	0.6 (0.1–2.8)	146/159	16/22	1/1	0.8 (0.2–2.6)	0.99
rs7604984	49/56	65/61	27/23	1.1 (0.6–2.2)	73/86	67/65	23/31	1.2 (0.7–2.1)	0.60
<i>SHMT2</i>									
rs7301155	65/69	63/60	13/12	0.6 (0.3–1.3)	85/80	64/82	14/20	0.6 (0.3–1.1)	0.43
rs2229716	135/140	6/1	0/0	5.8 (0.3–111.9)	185/176	5/5	0/1	1.6 (0.2–13.2)	0.68
<i>SLC46A1</i>									
rs9894260	121/114	18/25	2/2	0.7 (0.2–1.9)	140/156	21/25	2/1	0.6 (0.2–1.8)	0.56
rs739439	80/99	53/36	8/6	1.0 (0.4–2.2)	107/125	50/53	6/4	1.0 (0.5–2.1)	0.39
rs2239908	25/42	82/65	34/34	0.9 (0.5–1.8)	41/54	86/95	36/33	1.6 (0.9–2.8)	0.03
rs17719944	109/123	31/17	0/1	4.0 (1.1–14.8)	1,340/150	28/29	1/3	0.5 (0.2–1.4)	0.02

^aAt least four pills per week during the previous year for at least 48 months' duration of lifetime use (Mayo Clinic subjects) or at least three pills per week for at least 48 months during the past 60 months (Duke University subjects).

^bAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ years), region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina), body mass index, age at menarche, oral contraceptive use, postmenopausal status, postmenopausal hormone use, parity/age at first birth, smoking, and education. SNP effect was determined using a log-additive logistic regression model.

^c*P* for interaction.

Table A4 | Odds ratios (OR) and 95% CIs for the main effect association between variants in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma among 1,575 Caucasian subjects (Phase 1), Mayo Clinic, and Duke University.

Gene/SNP rsID	Homozygous major allele (referent)		Heterozygous			Homozygous minor allele			P value (2 df)	Ordinal (per minor allele)	
	Cases	Controls	Cases	Controls	OR (95%CI) ^a	Cases	Controls	OR (95%CI) ^a		OR (95%CI) ^a	P-trend
<i>ATIC</i>											
rs3772078	415	583	201	279	1.0 (0.8–1.3)	20	30	0.9 (0.5–1.7)	0.98	1.0 (0.8–1.2)	0.93
rs2372536	304	409	279	399	1.0 (0.8–1.2)	67	103	0.9 (0.6–1.3)	0.76	0.9 (0.8–1.1)	0.46
rs1880586	167	245	336	473	1.0 (0.8–1.3)	149	201	1.1 (0.8–1.4)	0.88	1.0 (0.9–1.2)	0.61
rs1983462	319	417	275	417	0.9 (0.7–1.1)	60	86	0.9 (0.6–1.3)	0.42	0.9 (0.8–1.1)	0.30
rs16853826	497	696	150	210	1.0 (0.8–1.3)	8	14	0.8 (0.3–2.0)	0.93	1.0 (0.8–1.2)	0.84
rs7586969	259	348	301	436	0.9 (0.7–1.1)	93	134	0.9 (0.7–1.3)	0.76	1.0 (0.8–1.1)	0.55
rs16853834	442	657	187	241	1.1 (0.9–1.4)	26	22	1.7 (0.9–3.1)	0.13	1.2 (1.0–1.4)	0.06
rs1404772	568	807	84	108	1.1 (0.8–1.5)	3	4	1.1 (0.2–5.0)	0.86	1.1 (0.8–1.4)	0.59
rs7604984	242	332	317	442	1.0 (0.8–1.2)	96	144	0.9 (0.7–1.2)	0.84	1.0 (0.8–1.1)	0.64
<i>SHMT2</i>											
rs7301155	121	187	153	202	1.2 (0.9–1.6)	37	49	1.2 (0.7–1.9)	0.56	1.1 (0.9–1.4)	0.33
rs2229716	601	838	51	79	0.9 (0.6–1.3)	2	1	2.4 (0.2–27.0)	0.63	0.9 (0.7–1.3)	0.70
<i>SLC46A1</i>											
rs9894260	531	768	111	148	1.1 (0.8–1.4)	9	3	5.4 (1.4–20.5)	0.04	1.2 (0.9–1.5)	0.14
rs739439	435	610	191	283	0.9 (0.8–1.2)	26	27	1.3 (0.8–2.3)	0.48	1.0 (0.8–1.2)	0.87
rs2239908	197	270	324	473	0.9 (0.7–1.2)	131	175	1.0 (0.8–1.4)	0.76	1.0 (0.9–1.2)	0.99
rs17719944	534	753	113	157	1.0 (0.8–1.3)	7	9	1.1 (0.4–3.1)	0.96	1.0 (0.8–1.3)	0.81

^aAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ years) and region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina).

Table A5 | Odds ratios (OR) and 95% CIs for the main effect association between variants in *ATIC*, *SHMT2*, and *SLC46A1* and ovarian carcinoma among 2,233 Caucasian subjects (Phases 1 and 2), Mayo Clinic, and Duke University.

Gene/SNP rsID	Homozygous major allele (referent)		Heterozygous			Homozygous minor allele			P value (2 df)	Ordinal (per minor allele)	
	Cases	Controls	Cases	Controls	OR (95%CI) ^a	Cases	Controls	OR (95%CI) ^a		OR (95%CI) ^a	P-trend
<i>ATIC</i>											
rs3772078	623	810	294	384	1.0 (0.8–1.2)	32	43	1.0 (0.6–1.6)	0.98	1.0 (0.8–1.2)	0.85
rs2372536	455	583	399	530	1.0 (0.8–1.2)	109	143	1.0 (0.7–1.3)	0.95	1.0 (0.9–1.1)	0.84
rs1880586	248	348	482	619	1.1 (0.9–1.3)	235	297	1.1 (0.9–1.4)	0.69	1.0 (0.9–1.2)	0.48
rs1983462	463	573	406	559	0.9 (0.8–1.1)	98	133	0.9 (0.7–1.2)	0.54	0.9 (0.8–1.1)	0.32
rs16853826	734	957	220	291	1.0 (0.8–1.2)	14	17	1.1 (0.5–2.3)	0.92	1.0 (0.8–1.2)	0.97
rs7586969	377	469	448	602	0.9 (0.8–1.1)	141	192	0.9 (0.7–1.2)	0.66	0.9 (0.8–1.1)	0.39
rs16853834	665	902	268	328	1.1 (0.9–1.3)	34	33	1.4 (0.8–2.2)	0.32	1.1 (1.0–1.3)	0.15
rs1404772	846	1,107	118	152	1.0 (0.8–1.3)	4	5	1.1 (0.3–4.1)	0.99	1.0 (0.8–1.3)	0.98
rs7604984	367	483	455	577	1.0 (0.9–1.3)	146	202	1.0 (0.7–1.2)	0.75	1.0 (0.9–1.1)	0.87
<i>SHMT2</i>											
rs7301155	398	545	404	530	1.0 (0.9–1.3)	86	114	1.0 (0.8–1.4)	0.87	1.0 (0.9–1.2)	0.64
rs2229716	902	1,175	63	86	0.9 (0.7–1.3)	2	2	1.1 (0.1–7.7)	0.92	0.9 (0.7–3.3)	0.73
<i>SLC46A1</i>											
rs9894260	799	1,056	152	202	1.0 (0.8–1.2)	13	6	3.1 (1.1–8.3)	0.08	1.1 (0.9–1.3)	0.44
rs739439	627	845	298	281	1.0 (0.9–1.3)	40	39	1.4 (0.9–2.2)	0.36	1.1 (0.9–1.3)	0.25
rs2239908	267	370	495	642	1.1 (0.9–1.3)	203	251	1.1 (0.9–1.4)	0.73	1.1 (0.9–1.2)	0.43
rs17719944	784	1,040	174	211	1.1 (0.9–1.4)	8	13	0.9 (0.4–2.1)	0.66	1.1 (0.9–1.3)	0.53

^aAdjusted for age (<40, 40–49, 50–59, 60–69, and 70+ years) and region of residence (Minnesota, Iowa, Wisconsin, Illinois, North Dakota, South Dakota, and North Carolina).