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Consortium analysis of gene and gene-folate interactions in purine and pyrimidine metabolism pathways with ovarian carcinoma risk

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Abstract

Scope—We re-evaluated previously reported associations between variants in pathways of one-carbon (folate) transfer genes and ovarian carcinoma (OC) risk, and in related pathways of purine and pyrimidine metabolism, and assessed interactions with folate intake.

Methods and Results—Odds ratios (OR) for 446 genetic variants were estimated among 13,410 OC cases and 22,635 controls and among 2,281 cases and 3,444 controls with folate information. Following multiple testing correction, the most significant main effect associations were for *DPYD* variants rs11587873 (OR=0.92, P=6x10^{-5}) and rs828054 (OR=1.06, P=1x10^{-4}). Thirteen variants in the pyrimidine metabolism genes, *DPYD, DPYS, PPAT* and *TYMS*, also interacted significantly with folate in a multi-variant analysis (corrected P=9.9x10^{-6}) but collectively explained only 0.2% of OC risk. Although no other associations were significant after multiple testing correction, variants in *SHMT1* in one-carbon transfer, previously reported with OC, suggested lower risk at higher folate ($P_{interaction}$=0.03-0.006).

Conclusions—Variation in pyrimidine metabolism genes, particularly *DPYD*, which was previously reported to be associated with OC, may influence risk; however, stratification by folate intake is unlikely to modify disease risk appreciably in these women. *SHMT1* SNP-byfolate interactions are plausible but require further validation. Polymorphisms in selected genes in purine metabolism were not associated with OC.

Keywords

case-control; *DPYD*; folate; polymorphism; *SHMT1*

Introduction

Global statistics estimated that ovarian carcinoma (OC) afflicted 225,000 women and resulted in 140,000 deaths in 2008 [1]. There are no specific screening methods or unique symptoms to detect OC in early stages [2]. Risk stratification strategies may have appreciable impact in reducing the incidence and suffering from OC by identifying those women at greatest risk of developing the disease who would benefit from preventive measures [3]. Germline mutations in high-risk genes (e.g., *BRCA1* and *BRCA2*) remain the

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Conflict of Interest Statement
The authors declare that there are no financial or commercial conflicts of interest.

best-defined genetic risk factors [4], but explain just 10-15% of all OC [5-7]. About 4% of the polygenic risk is explained by common, but poorly understood, low-risk polymorphisms [8-11] and most non-genetic risk factors (oral contraceptive use [12-14], parity [15, 16], breast-feeding [15, 17], tubal ligation [18], endometriosis [19] and smoking [20]) are not conducive to public health recommendations for risk modification.

Stratification of genetic risk by dietary factors may prevent some cancers. For example, folates participate in one-carbon (1-C) transfer reactions that are essential for the biosynthesis of purines (adenine and guanine) and pyrimidines (cytosine, thymine and uracil), which are incorporated into DNA and RNA, as well as for the biosynthesis of methyl groups for DNA methylation [21] (**Figure 1**). Perturbation of the coenzymatic role of folate, or of key enzymes in 1-C transfer or purine or pyrimidine metabolism, can have broad consequences that lead to tumor initiation [22] and progression [23] and thus could alter risk among a substantial proportion of individuals. Numerous genetic disorders of purine and pyrimidine metabolism have been characterized in humans [24]. Although these are rare and inherited in Mendelian fashion, genes encoding enzymes in these pathways have been associated with various cancers [25-30]. We previously examined associations between 180 tagging common single nucleotide polymorphisms (tagSNPs) in 21 genes involved in 1-C transfer and risk of OC in 1,770 participants [31] and also reported risk modification by multivitamin intake, a proxy for folate intake [31, 32]. Ten SNPs in eight genes (AHCYL1, DNMT3A, DPYD, MTHFD1, MTHFS, SHMT1, SLC19A1 and *TYMS*) were associated with OC at P≤0.05 in either ordinal or co-dominant genetic risk models [31] and eight SNPs in five genes (*DNMT3A, DNMT1, MTHFR, MTHFD1* and *ATIC*) were associated with OC at P≤0.05 in interaction analyses with multivitamin use [31, 32]. In those studies, the strongest evidence for association was for a haplotype and a single SNP (rs9909104) in *SHMT1* for which we calculated a false positive report probability of 9% to 16%, respectively [31], and for two SNPs in *ATIC* interacting with multivitamin use at a false discovery rate <0.25 and P<0.05 [32]. SNPs in *DNMT3A, DPYD, MTHFD1* and *MTHFS* did not replicate in a subsequent genotyping effort among 16,000 participants [33].

Our objectives in the current investigation were to re-evaluate previously reported genetic associations in a larger sample size, evaluate additional variants in the related pathways of purine and pyrimidine metabolism and to assess effect modification by dietary folate intake. We performed these analyses in over 36,000 women contributing DNA in the Ovarian Cancer Association Consortium (OCAC).

Materials and Methods

Gene and SNP selection

Genes were selected according to two categories. The first category consisted of six genes (*ATIC, DNMT3B, DPYD, MTR, SHMT1* and *TYMS*) with previously observed SNP associations with OC [31, 32] and were included for replication. These SNPs were selected with high gene coverage using minor allele frequency (MAF) ≥0.01 and pair-wise linkage disequilibrium (LD) threshold of r^2<0.8 (*ATIC, DNMT3B, DPYD* and *MTR*) or <0.9 (*SHMT1* and *TYMS*). The second category consisted of nine genes related to purine metabolism (*ADSL, ADSS, DCK, GART, GMPS, IMPDH1, IMPDH2, PAICS* and *PFAS*) and

11 genes involved in pyrimidine metabolism (AK3, CAD, CMPK1, CTPS, DHODH, DPYS, NME6, PPAT, PRPS2, RRM2B and *UMPS*) (**Supplementary Figure 1**). These SNPs were selected with MAF≥0.05 and LD threshold of r^2<0.8. All SNPs within 5kb up- and downstream of the largest cDNA isoform (Human Genome build 36) of each gene was selected using information from 60 unrelated individuals of European ancestry sequenced in the pilot phase of the 1000 Genomes Project [34] and binned using the Haploview program [35]. We prioritized tagSNPs for genotyping that were coding SNPs, had the highest MAF in each bin and, if available, met criteria for predicted likelihood of successful genotyping based on Illumina quality score metrics. In January 2010, 803 tagSNPs were submitted for genotyping: 31% of these SNPs were unique to the 1,000 Genomes Project and not found in dbSNP.

Study Subjects

Subjects (n=47,630) from 43 individual studies participating in OCAC were grouped into 34 geographically similar study strata [11]. Of 44,308 subjects whose DNA passed genotyping quality control criteria (see below), we further excluded subjects with borderline tumors, subjects of non-European ancestry and those with prior history of cancer other than non-melanoma skin cancer, leaving 36,045 eligible subjects (13,410 cases and 22,635 controls) for analysis. Informed consent was obtained in each of the individual studies and local human research investigations committees approved each study.

Genotyping and Quality Control (QC)

Details of the genotyping have been described elsewhere [11]. In brief, we used an Illumina Infinium custom iSelect BeadChip developed for the international Collaborative Oncology Gene-environment Study (iCOGS). Centralized genotyping calls and QC were performed at the University of Cambridge. Quality control for samples has been detailed previously [11]. We excluded SNPs that failed genotyping, had call rates <95% and MAF >0.05 or call rates <99% and MAF <0.05, departed from Hardy-Weinberg equilibrium (P value $<10^{-7}$), had discordant genotypes >2% between duplicates and monomorphic SNPs. Of 803 tagSNPs submitted for genotyping, 203 SNPs failed genotyping, 127 were monomorphic and 27 had MAF <0.01 leaving 446 SNPs that passed QC. Genotyping failures and monomorphic SNPs reflected the large number of polymorphisms that were subsequently found to be falsely positive in the pilot phase sequencing data of the 1000 Genomes Project.

Covariate and Dietary Data

Key clinical, demographic and questionnaire data were harmonized across study centers and merged into a common dataset. Dietary intakes of folate and total energy were estimated with validated food frequency questionnaires (FFQs) in six studies (AUS [36], DOV [37], HAW and STA [38], NEC [39] and NJO [40]) pertaining to the year preceding recruitment or for the time period approximately four years before the reference date (DOV). Data on the use of multivitamins and single vitamin and mineral supplements were also available and total folate intake was estimated by summing intakes from both food sources and from supplements. Nutrient and genotype data were available for 2,281 cases and 3,444 controls of European ancestry.

Statistical Analysis

Genotypes were used to estimate allele frequencies and pair-wise LD between SNPs was estimated with r^2 values using Haploview [35]. Data from the 34 study strata were combined into a single dataset following confirmation of no statistical heterogeneity in SNP associations across study sites. We estimated odds ratios (OR) and 95% confidence intervals (CI) for each SNP using unconditional logistic regression treating the number of variant alleles carried as an ordinal (log-additive) variable. Secondary analyses also considered co-dominant (non-additive) risk models. Interactions between each SNP and total folate intake were evaluated with the Wald test in models that also included a one degree-of-freedom product term for the ordinal coding for genotype and total folate intake group (below/above the energy-adjusted median intake: ≤484 μg/d vs >484 μg/d ≈ approximately the dietary reference intake of 400 μg/d for folate, which is also the folic acid content of a typical multivitamin supplement). Risk models were adjusted for age (continuous), study stratum and the first five eigenvalues from principal components analysis to account for sub-strata of European ancestry across the 34 international studies (see ref [11]). Additional adjustment for non-genetic risk factors did not change estimates and these variables were excluded from the models (data not shown).

Multi-variant analysis—Because some of the genes selected for replication belonged to either the purine (*ATIC*) or pyrimidine (*DPYD* and *TYMS*) metabolism pathways, while *DNMT3B*, *MTR* and *SHMT1* belonged to 1-C transfer, we evaluated associations according to these three pathways. However, we considered evidence for replication if SNPs in these six genes reached statistical significance according to the criteria described below.

To assess the likelihood of false-positive findings, we performed a multi-variant analysis that accounted for the potential correlations between SNPs within genes in a pathway. Since our primary interest was to evaluate SNP-by-folate interactions, we prioritized these associations for evaluation of multiple testing as follows. A likelihood ratio test (LRT) statistic was calculated by comparing a regression model with and without significant SNP-by-folate interaction terms. 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 generation of a null distribution was performed five times, each time with a different seed. For evaluation of individual genes that showed SNP associations at $P<0.05$, we applied a conservative Bonferroni correction of the Type I error using the number of SNPs tested in that gene's pathway (44 SNPs in 1-C transfer, 100 SNPs in purine metabolism and 302 SNPs in pyrimidine metabolism). The corresponding thresholds were $P=0.001$ for 1-C transfer, $P=5\times10^{-4}$ for purine metabolism and $P=1.6\times10^{-4}$ for pyrimidine metabolism.

We also estimated haplotype frequencies of >1% for selected genes with and without stratification by total folate intake using an expectation-maximization algorithm [41] as described in detail elsewhere [32]. The generation of haplotypes using 129 *DPYD* tagSNPs resulted in an infinite recursion so we selected 29 tagSNPs, one for each haplotype block constructed according to the Gabriel criteria [42] in Haploview [35] and two located outside of a haplotype block. These tagSNPs were selected based on significant P values in main

effect or interaction analyses, highest MAF or highest D' values with other tagSNPs in the haplotype block. Individual haplotype associations were interpreted carefully in the absence of global haplotype significance.

To assess the population importance of the SNP-by-folate interactions, we used the Genome-wide Complex Trait Analysis (GCTA) program to estimate the percent variance in risk of OC explained by the SNP-by-folate interaction terms [43]. In principle, the GCTA program can be used to evaluate a subset of SNPs or SNP-by-environment interactions and has been used by the developers in this context (J Yang, personal communication, December 2013). Briefly, we first estimated the pairwise genetic relationship matrix (GRM) of the subjects using the SNPs of interest and then fitted the GRM in a regression model that also included age, study stratum, five eigenvalues, total folate intake group and SNP-by-folate interaction terms. Restricted maximum likelihood was applied to deconstruct the phenotypic variance into the percentages explained by the SNPs, the SNP-by-folate interaction terms and residual environmental component.

Statistical tests were two-sided and, unless stated otherwise, were implemented with SAS version 9 (SAS Institute, NC), R [44] and Plink v1.07 [45] software.

Results

The distribution of cases and controls stratified by study is shown in **Supplementary Table 1**. Descriptive information on the 446 SNPs is provided in **Supplementary Table 2**.

Twenty-three SNPs were associated with risk of OC at P<0.05, including two SNPs in *SHMT1* (1-C transfer) and 12 SNPs in *DPYD* (pyrimidine metabolism) (**Table 1**). We reported associations with SNPs in both of these genes previously, although with different variants [31]. In the current study, the two SNPs with the smallest P value were in *DPYD* in pyrimidine metabolism: rs11587873 (OR, 0.92; 95% CI, 0.89-0.96; $P=6x10^{-5}$) and rs828054 (OR, 1.06; 95% CI, 1.03-1.10; $P=1x10^{-4}$). These two SNPs remained statistically significant at the corrected $P=1.6x10^{-4}$. The other 10 *DPYD* SNPs were correlated with either rs11587873 or rs828054. There was no statistical heterogeneity in ORs across study strata. Associations were similar when restricted to high-grade serous OC histology (**Table 1**). Associations for the remaining SNPs are shown in **Supplementary Table 3**.

When SNPs were examined by interactions with total folate intake (**Table 2**), 22 SNPs showed interactions at P<0.05, including two SNPs that were associated with OC risk overall (*SHMT1* rs4925179 and *DPYD* rs7522938). Three of four *SHMT1* SNPs in 1-C transfer (rs56001517, rs7216214 and rs2273026) were associated with a 23% to 30% decreased risk of OC at higher total folate intake (smallest P=0.006) and these three SNPs were correlated with each other (r^2 = 0.61 to 0.92) but did not pass the multiple testing significance threshold of P=0.001. Fifteen of the 22 SNPs (60%) were in pyrimidine metabolism genes and a large proportion of these were in four genes (*DPYS*, *DPYD*, *PPAT* and *TYMS*) that encode enzymes in the sub-pathway of fluoropyrimidine metabolism, which is an important pharmacogenomics pathway targeted by anti-folate chemotherapy. We, therefore, evaluated the 13 SNP-by-folate interactions in these four genes collectively in a

multi-variant analysis. Despite generating five null distributions, none achieved a LRT statistic that included the observed LRT statistic: permuted maximum χ^2 = 37.95 to 45.74 with 13 degrees-of-freedom (df; smallest P=1.6x10^{-5}) compared to observed χ^2 = 46.92 with 13 df (P=9.9x10^{-6}). This suggested the observed value was more extreme than would be expected. Associations for the remaining SNP-by-folate interactions are shown in **Supplementary Table 4**. We estimated the percentage of variance in risk of OC explained by the 13 SNP-by-folate interaction terms in pyrimidine metabolism to be 0.1994 % (95% CI, 0.1991 to 0.1997) compared to 1x10^{-4} % (95% CI, 9.9x10^{-5} to 1x10^{-4}) explained by the 13 SNPs alone.

There were no significant associations of haplotypes at the global (gene) level with risk of OC for *DPYD*, *DPYS* or *SHMT1* (**Supplementary Table 5**). Interestingly, *SHMT1* haplotype #6 comprised minor alleles of the three correlated *SHMT1* SNPs (rs56001517, rs7216214 and rs2273026) mentioned above and showed a decreased risk with OC at higher total folate intake (haplotype OR=0.68, 95% CI=0.53-0.87, P=0.002) that mirrored those of the individual SNP findings in **Table 2**. The selection of 29 haplotype block tagSNPs produced a single haplotype that was not significant, while several individual haplotypes of low frequency in *DPYS* were observed at P<0.05. Folate intake was not independently associated with risk of OC in multivariable-adjusted models (OR for >484 μg/d vs ≤484 μg/d = 1.04, 95% CI=0.91-1.18), nor when using different total folate intake cutpoints (OR for >400 μg/d vs ≤400 μg/d = 1.00, 95% CI=0.88-1.13 and OR for >683 μg/d [>75% percentile] vs ≤683 μg/d = 0.98, 95% CI=0.84-1.14).

Discussion

The results of the current study suggested a potential role for inherited variation in *DPYD* in pyrimidine metabolism with risk of OC. Folate intake may modify genetic risk of OC in the pyrimidine metabolism pathway, but the population effect is likely to be small. A possible role for *SHMT1* SNP-by-folate interactions in the one-carbon transfer pathway may exist, but requires further validation. Selected genes in purine metabolism were not associated with risk of OC.

In the current study, 12 SNPs with additive effects in *DPYD* were found to be associated with OC and the strongest association (rs11587873) suggested a modest 8% decreased risk. We previously reported that the main effect of another *DPYD* SNP, rs1801265 (Arg29Cys), was associated with increased risk of OC among homozygous rare allele carriers [31], although that association was not replicated elsewhere [33] or in the current study. *DPYD* was represented by five SNPs in our earlier study of 1,770 participants [31] and these participants were also included in the current investigation of 129 SNPs. A haplotype analysis did not support an association of a single *DPYD* haplotype with risk of OC; however, this may be due, in part, from selecting 29 haplotype block tagSNPs to overcome the infinite recursion when using all 129 tagSNPs. We, therefore, cannot rule out a role for *DPYD* in risk of OC.

During pyrimidine metabolism, uracil and thymine concentrations are determined, in part, by the availability of 1-C units from folates and are catabolized to β-alanine and to valine/

leucine/isoleucine, respectively (**Supplementary Figure 1**). *DPYD* encodes dihydropyrimidine dehydrogenase, the initial and rate-limiting step, whereas *DPYS* encodes dihydropyrimidinase, which catalyzes the secondary step. DPYD and DPYS enzyme deficiency or inhibition can cause decreased production of β-alanine or accumulation of the pyrimidines, uracil/dihydrouracil and thymine/dihydrothymine, and has shown considerable phenotypic variation ranging from severe neurological and developmental disorders associated with inborn errors to milder symptoms of lethargy, dizziness [46-48] and gastrointestinal abnormalities (gastroesophageal reflux, malabsorption) [49]. The pyrimidine metabolism pathway is identical for the degradation of fluoropyrimidines including 5-fluorouracil (5-FU), one of the most commonly prescribed chemotherapeutic agents in cancers [50, 51]. DPYD or DPYS enzyme deficiency results in toxicity among cancer patients from the inability to metabolize 5-FU [52, 53]. Screening programs for inborn errors of pyrimidine degradation have also identified individuals without symptoms, indicating an incomplete knowledge of the full spectrum of genetic, gene-environment, biochemical and clinical manifestations of DPYD and DPYS impairment [46, 49]. It will, therefore, be important to also investigate these associations with survival outcomes.

We had also previously reported increased risk with the main effect of *SHMT1* variant rs9909104 [31], but could not replicate the main effect association here. The expanded analysis of *SHMT1* SNPs and haplotypes in the present study suggested that risk may be modified by higher folate intake, although these associations did not meet the criteria of significance following multiple testing correction. *SHMT1* encodes the serine hydroxymethyltransferase 1 (soluble) enzyme that catalyzes the reversible conversion of glycine and tetrahydrofolate to serine and 5,10 methylenetetrahydrofolate in the cytoplasm for the synthesis of methionine, pyrimidines (e.g., thymidylate) and purines [54]. Serine synthesis, nucleotide synthesis and the pentose phosphate pathway, which generates ribose-5-P (see **Supplementary Figure 1**), are implicated as important mechanisms of metabolic reprogramming in cancer cells [55]. Polymorphisms in *SHMT1* have also been associated with carcinomas of the lung [56] and head and neck [57] and have been shown to interact with dietary folate to alter risk of non-Hodgkin lymphoma [58].

The strengths of this investigation include the gene-environment risk analysis in a targeted pathway using a large assembly of women with OC and the rigorous centralized genotyping and quality control standards. We improved upon our previous work [31, 32] by refining the associations using total folate intake instead of multivitamin supplement use. The median cutpoint for total folate intake approximated the dietary reference intake of 400 μg/d; therefore, the SNP-by-folate interactions can be interpreted as comparing women who meet or exceed recommendations to those who do not. There are also limitations to the current study. Folate intake was assessed at time of diagnosis using a FFQ that asked about average intake over the last year that may not represent habitual intake and may be affected by recall bias. Another potential limitation of the SNP-by-folate interactions is the analysis of all tumor types without stratification by tumor histology. This was decided *a priori* to maximize statistical power, although the evaluation of SNP main effects suggested no significant differences in risk estimates across the histological types for most SNPs. Another limitation is the inability to distinguish potentially causal SNPs at this time. We genotyped

tagSNPs and some genes, such as *DPYD,* were large and were represented by several correlated tagSNPs that suggested either decreased or increased risk. The evaluation of a *DPYD* haplotype did not satisfactorily overcome this challenge and will need further clarification.

Conclusions

SNPs in *DPYD* may have modest effects on risk of OC and will require further evaluation in order to disentangle putative causal variants. Our findings suggest that exceeding the recommendations for folate intake does not negatively modify susceptibility in selected genes in pyrimidine metabolism to influence risk of OC. A possible role for *SHMT1* SNP-by-folate interactions in the one-carbon transfer pathway may exist, but will require further validation. Polymorphisms in selected genes in purine metabolism do not appear to be associated with OC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

1-C	one-carbon
ADSL	adenylosuccinate lyase
ADSS	adenylosuccinate synthase
AK3	adenylate kinase 3
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase
CMPK1	cytidine monophosphate (UMP-CMP) kinase 1, cytosolic
CTPS	CTP synthase 1
DCK	deoxycytidine kinase
DHODH	dihydroorotate dehydrogenase (quinone)
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta
DPYD	dihydropyrimidine dehydrogenase
DPYS	dihydropyrimidinase
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase
GCTA	Genome-wide Complex Trait Analysis
GMPS	guanine monphosphate synthase
iCOGS	international Collaborative Oncology Gene-environment Study
IMPDH1	IMP (inosine 5'-monophosphate) dehydrogenase 1
IMPDH2	IMP (inosine 5'-monophosphate) dehydrogenase 2
LD	linkage disequilibrium
LRT	likelihood ratio test
MAF	minor allele frequency
NME6	NME/NM23 nucleoside diphosphate kinase 6
OC	Ovarian carcinoma
OCAC	Ovarian Cancer Association Consortium
OR	odds ratio

PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase
PFAS	phosphoribosylformylglycinamidine synthase
PPAT	phosphoribosyl pyrophosphate amidotransferase
PRPS2	phosphoribosyl pyrophosphate synthetase 2
QC	quality control
RRM2B	ribonucleotide reductase M2 B (TP53 inducible)
SHMT1	serine hydroxymethyltransferase 1 (soluble)
SNP	single nuctleotide polymorphism
TYMS	thymidylate synthase
UMPS	uridine monophosphate synthetase

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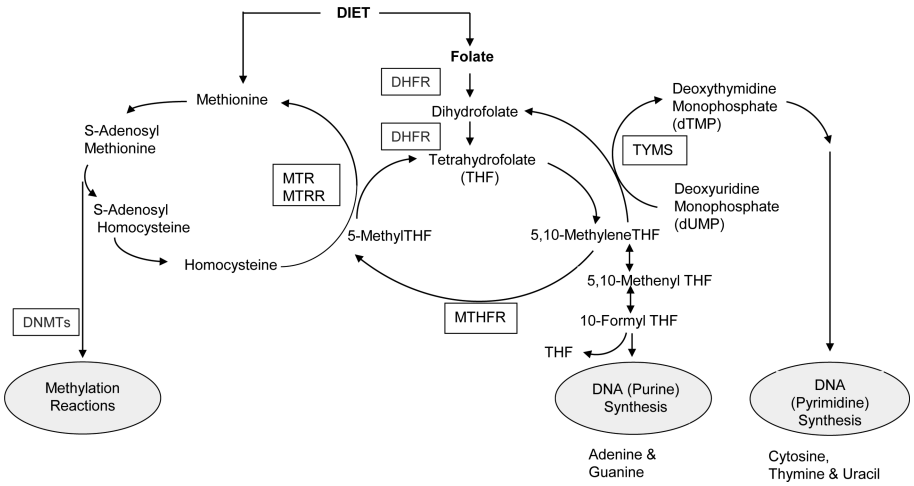

Figure 1.

Overview of the role of folate and key enzymes involved in one-carbon transfer for DNA synthesis and methylation reactions. DHFR, dihydrofolate reductase; DNMTs, DNA methyltransferases; MTHFR, methylenetetrahydrofolate reductase; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; MTRR, 5-methyltetrahydrofolatehomocysteine methyltransferase reductase; TYMS, thymidylate synthase.

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Table 1

Per-allele (log-additive) associations[a)] between variants in genes in 1-C transfer and purine and pyrimidine metabolism pathways and risk of ovarian carcinoma: 13,410 cases (5,813 high-grade serous-only) and 22,635 controls of European ancestry in OCAC.

		All cases					High-Grade Serous cases				
Gene	**SNP**	**OR**	**95% CI lower**	**95% CI upper**	**P-value**	**P-value (SNP*site interaction)**	**OR**	**95% CI lower**	**95% CI upper**	**P-value**	**P-het[b)]**
1-C transfer											
DNMT3B	rs4911256	1.04	1.00	1.07	0.025	0.88	1.04	0.99	1.09	0.082	0.26
SHMT1	rs669340	0.96	0.93	0.99	0.021	0.58	0.94	0.90	0.98	0.006	0.09
SHMT1	rs4925179	0.97	0.93	1.00	0.040	0.69	0.95	0.91	1.00	0.037	0.21
Purine metabolism											
ATIC	pos215884573	1.05	1.00	1.10	0.036	0.34	1.08	1.01	1.14	0.016	0.08
IMPD2	rs4974081	0.96	0.93	1.00	0.043	0.68	0.96	0.92	1.01	0.169	0.66
Pyrimidine metabolism											
AK3[c)]	rs691941	0.95	0.91	0.99	0.020	0.13	0.94	0.89	1.00	0.061	0.11
DPYD	rs828054	1.06	1.03	1.10	0.0001	0.80	1.07	1.03	1.12	0.002	0.02
DPYD	rs11587873	0.92	0.89	0.96	0.00006	0.08	0.93	0.88	0.98	0.008	0.01
DPYD	rs12120388	0.94	0.91	0.97	0.0004	0.83	0.94	0.90	0.98	0.004	0.02
DPYD	rs676686	1.06	1.02	1.09	0.001	0.85	1.07	1.02	1.12	0.003	0.01
DPYD	rs914959	1.05	1.01	1.08	0.007	0.65	1.05	1.00	1.10	0.028	0.08
DPYD	rs7537668	1.04	1.01	1.08	0.011	0.61	1.05	1.01	1.10	0.026	0.03
DPYD	rs4128474	0.95	0.90	1.00	0.031	0.99	0.96	0.90	1.02	0.178	0.34
DPYD	rs494271	1.04	1.00	1.07	0.034	0.80	1.04	1.00	1.10	0.052	0.02
DPYD	rs6678858	0.95	0.91	1.00	0.034	1.00	0.97	0.91	1.03	0.321	0.70
DPYD	rs7555294	0.96	0.92	1.00	0.041	0.90	0.97	0.91	1.02	0.229	0.73
DPYD	rs4434871	0.95	0.91	1.00	0.041	0.97	0.98	0.92	1.04	0.491	0.65
DPYD	rs7522938	1.03	1.00	1.07	0.048	0.72	1.02	0.98	1.07	0.330	0.26
DHODH[d)]	rs3213423	1.05	1.01	1.09	0.018	0.10	1.01	0.96	1.06	0.757	0.77
DHODH	rs11864453	0.97	0.93	1.00	0.037	0.19	0.97	0.93	1.01	0.170	0.54
NME6	rs7651161	0.97	0.94	1.00	0.048	0.73	0.96	0.92	1.01	0.089	0.49

		All cases					High-Grade Serous cases				
Gene	**SNP**	**OR**	**95% CI lower**	**95% CI upper**	**P-value**	**P-value (SNP*site interaction)**	**OR**	**95% CI lower**	**95% CI upper**	**P-value**	**P-het** *b)*
RRM2B	pos103299244	2.67	1.15	6.19	0.022	0.92	2.42	0.78	7.48	0.126	0.27
TYMS	pos656897	1.27	1.09	1.48	0.002	0.27	1.31	1.07	1.62	0.010	0.008

a) Adjusted for age (continuous), study stratum and the first five eigenvalues from principal components analysis; $P < 0.05$.

b) P-value for tumor heterogeneity comparing odds ratios between all controls and each of high-grade serous OC, low-grade serous OC, mucinous OC, endometrioid OC and clear cell OC.

c) SNP is located 3′ upstream of gene.

d) SNP is located in flanking 5′ region of gene; all other SNPs are located in introns; ‘pos’ SNPs are novel and identified by chromosomal position.

Table 2

Associations[a)] between variants in genes in 1-C transfer and purine and pyrimidine metabolism pathways and risk of ovarian carcinoma stratified by total folate intake: 2,281 cases and 3,444 controls of European ancestry participants in OCAC.

		Total folate Intake ≤ 484 μg/d										Total folate intake > 484 μg/d										
		Controls			Cases							Controls			Cases							
Gene	**SNP**	**AA[b)]**	**AB**	**BB**	**AA**	**AB**	**BB**	**OR**	**95% CI lower**	**95% CI upper**	**P value**	**AA**	**AB**	**BB**	**AA**	**AB**	**BB**	**OR**	**95% CI lower**	**95% CI upper**	**P value**	**P int[c)]**
1-C transfer																						
SHMT1[d)]	rs56001517	1506	232	8	991	162	5	1.05	0.85	1.29	0.656	1475	215	6	1011	108	2	0.70	0.55	0.89	0.004	0.012
SHMT1	rs7216214	1394	335	18	907	237	15	1.08	0.91	1.28	0.396	1341	340	13	925	182	12	0.76	0.63	0.92	0.004	0.006
SHMT1	rs4925179	711	786	250	501	526	131	0.88	0.79	0.99	0.03	703	775	217	469	509	144	1.03	0.92	1.16	0.606	0.048
SHMT1	rs2273026	1409	323	15	931	213	15	1.03	0.86	1.22	0.767	1363	322	11	938	172	11	0.77	0.64	0.94	0.008	0.027
Purine metabolism																						
IMPD1[d)]	rs6948333	991	653	101	624	453	77	1.09	0.96	1.23	0.171	950	637	105	638	435	46	0.91	0.80	1.03	0.139	0.039
PFAS	rs11649742	1049	606	92	721	378	60	0.93	0.82	1.06	0.257	1020	592	84	624	443	55	1.14	1.00	1.30	0.045	0.029
PFAS	pos8110739	1659	85	0	1122	37	0	0.64	0.43	0.96	0.03	1630	64	2	1068	51	1	1.18	0.81	1.71	0.384	0.034
Pyrimidine metabolism																						
CTPS	rs6675122	1030	611	106	615	475	69	1.18	1.04	1.33	0.009	960	619	118	624	442	56	0.98	0.86	1.11	0.706	0.034
CTPS	rs41268101	1121	521	92	677	402	61	1.16	1.02	1.32	0.022	1026	568	88	670	387	45	0.95	0.83	1.09	0.441	0.034
DPYD	rs667565	671	810	266	378	573	207	1.18	1.06	1.31	0.003	614	814	268	439	508	175	0.92	0.82	1.03	0.127	0.001
DPYD	rs4520446	501	879	363	318	566	274	1.09	0.98	1.21	0.135	464	824	397	331	555	234	0.90	0.81	1.00	0.057	0.016
DPYD	rs7522938	752	745	216	440	540	164	1.15	1.03	1.29	0.011	712	760	208	498	469	145	0.96	0.86	1.08	0.482	0.019
DPYD	rs2811182	562	887	297	413	538	208	0.96	0.86	1.07	0.43	617	784	296	360	553	209	1.12	1.00	1.25	0.041	0.042
DPYS	rs1962267	550	872	325	417	555	187	0.87	0.78	0.97	0.014	586	802	309	378	535	209	1.02	0.92	1.14	0.689	0.033
DPYS	rs2853160	489	889	369	303	580	276	1.08	0.97	1.20	0.158	460	839	398	317	569	236	0.93	0.83	1.03	0.169	0.036
DPYS	rs17834440	1295	427	25	908	239	12	0.82	0.69	0.97	0.018	1318	359	20	853	250	19	1.11	0.94	1.31	0.223	0.008
DPYS	rs2853178	859	750	138	601	475	83	0.93	0.83	1.05	0.242	889	680	128	571	448	103	1.10	0.97	1.24	0.142	0.049
DPYS	rs13263121	751	782	212	530	492	136	0.93	0.84	1.04	0.227	764	751	181	480	496	145	1.13	1.01	1.27	0.034	0.02
DPYS	rs1319371	1053	613	81	672	422	65	1.10	0.97	1.25	0.157	1011	592	94	683	387	52	0.90	0.79	1.02	0.106	0.03

		Total folate Intake ≤ 484 µg/d										Total folate intake > 484 µg/d										
		Controls			Cases							Controls			Cases							
Gene	**SNP**	**AA**[b]	**AB**	**BB**	**AA**	**AB**	**BB**	**OR**	**95% CI lower**	**95% CI upper**	**P value**	**AA**	**AB**	**BB**	**AA**	**AB**	**BB**	**OR**	**95% CI lower**	**95% CI upper**	**P value**	**P int**[c]
DPYS	rs35450967	1034	626	87	654	435	70	1.11	0.98	1.26	0.1	982	616	99	671	397	54	0.88	0.77	1.01	0.061	0.012
PPAT	rs13135046	516	857	374	362	579	216	0.91	0.82	1.01	0.079	519	837	338	314	569	239	1.08	0.96	1.20	0.198	0.023
TYMS	rs2298582	1336	393	18	914	234	11	0.86	0.73	1.02	0.09	1356	327	14	864	235	22	1.21	1.02	1.44	0.027	0.006

[a] Adjusted for age (continuous), study stratum and the first five eigenvalues from principal components analysis. SNP effect was determined using a log-additive logistic regression model.

[b] Allele counts: AA=homozygous wildtype allele carriers; AB=heterozygous allele carriers; BB=homozygous variant allele carriers.

[c] P-value for interaction.

[d] SNP is located 5′ downstream of gene; all other SNPs are located in introns; ‘pos’ SNPs are novel and identified by chromosomal position.