Mathematical Modeling of Folate Metabolism: Predicted Effects of Genetic Polymorphisms on Mechanisms and Biomarkers Relevant to Carcinogenesis

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Abstract

Low-folate status and genetic polymorphisms in folate metabolism have been linked to several cancers. Possible biological mechanisms for this association include effects on purine and thymidine synthesis, DNA methylation, or homocysteine concentrations. The influence of genetic variation in folate metabolism on these putative mechanisms or biomarkers of cancer risk has been largely unexplored. We used a mathematical model that simulates folate metabolism biochemistry to predict (*a*) the effects of polymorphisms with defined effects on enzyme function (*MTHFR* and *TS*) and (*b*) the effects of potential, as-of-yet-unidentified polymorphisms in a comprehensive set of folate-metabolizing enzymes on biomarkers and mechanisms related to cancer risk. The model suggests that there is substantial robustness in the pathway. Our predictions were consistent with measured effects of known polymorphisms in *MTHFR* and *TS* on biomarkers. Polymorphisms that alter enzyme function of *FTD*, *FTS*, and *MTCH* are expected to affect purine synthesis, *FTS* more so under a low-folate status. In addition, *MTCH* polymorphisms are predicted to influence thymidine synthesis. Polymorphisms in methyltransferases should affect both methylation rates and thymidylate synthesis. Combinations of polymorphisms in *MTHFR*, *TS*, and *SHMT* are expected to affect nucleotide synthesis in a nonlinear fashion. These investigations provide information on effects of genetic polymorphisms on biomarkers, including those that cannot be measured well, and highlight robustness and sensitivity in this complex biological system with regard to genetic variability. Although the proportional changes in biomarkers of risk with individual polymorphisms are frequently small, they may be quite relevant if present over an individual's lifetime. (Cancer Epidemiol Biomarkers Prev 2008;17(7):1822–31)

Introduction

Folate-mediated one-carbon metabolism (FOCM) is unequivocally linked to multiple health outcomes, including birth defects, several types of cancer, and possibly cardiovascular disease and cognitive function. For cancer risk, associations for both folate status and genetic polymorphisms in FOCM are strongest for gastrointestinal and hematopoietic malignancies but have also been observed for pancreatic and other cancers (1-6). The exact mechanisms linking FOCM to cancer risk are unknown; possibilities include effects on global and promoter-specific DNA methylation, effects on thymidylate and purine synthesis, as well as possible oxidative effects of homocysteine (7). For example, FOCM can affect DNA methylation because the balance between S-adenosylhomocysteine and S-adenosyl methionine is dependent on the conversion of homocysteine to methionine via the methionine synthase reaction (8). Multiple studies have shown that folate availability affects global DNA methylation, which largely reflects CpG sites at repetitive regions (9-16). However, the effect of folate status on promoter methylation, a mechanism of gene silencing, is currently less well defined (17-21). The *de novo* synthesis of thymidine [via thymidylate synthase (TS)] and purines [through aminoimidazolecarboxamide ribonucleotide transferase (AICART)] is also a folate-dependent reaction.

Genetic polymorphisms in FOCM can affect enzyme function and folate homeostasis (5, 6). They have been identified as risk or preventive factors for cancer, both as independent predictors of risk and as modifiers of dietary associations (gene-diet interactions; ref. 5). However, the functional effect of many polymorphisms on biomarkers in the pathway and on putative mechanisms related to cancer risk is currently unknown. In fact, for some key mechanisms (e.g., purine synthesis),

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we lack reliable, reproducible biomarkers for use in human studies. Information on the influence of genetic variants on folate-related biomarkers of carcinogenesis can solidify the associations that have been observed between polymorphisms and cancer risk and provide a critical piece in our understanding of the role of FOCM in cancer.

It is important to recognize that more than 20 proteins play important roles in FOCM and the pathway is characterized by multiple interconnected cycles with multiple regulatory mechanisms (8). For targeting epidemiologic investigations, as well as experimental studies, information on which polymorphisms are more likely to disrupt folate homeostasis or result in changes in a cancer-related biological mechanism would be of great utility.

To this end, we developed a mathematical simulation model of FOCM to investigate the effect of genetic polymorphisms on various mechanisms relevant to carcinogenesis (22). This model uses information on enzyme kinetics and regulatory mechanisms to derive predictions for the effects of genetic polymorphisms thought to affect enzyme function or gene transcription. We have investigated the predicted effect of multiple known polymorphisms, as well as hypothetical polymorphisms in FOCM, on thymidine synthesis, purine synthesis, methylation rate, and homocysteine concentrations. We further explored gene-gene interactions between multiple genetic variants. Our model predictions are consistent with the published literature for known polymorphism-biomarker relationships and provide new insights into the effects on biomarkers/mechanisms that are not easily measured. In addition, the modeling offers predictions on the effect of genetic variability in genes that have not yet been thoroughly screened for polymorphisms on key mechanisms relevant to carcinogenesis.

Materials and Methods

Overview of the Model. We used a mathematical model of FOCM that has been previously described (Fig. 1; ref. 22). Briefly, the model was built based on known biochemistry and standard reaction kinetics using differential equations to describe each enzymatic reaction in the context of variable substrate availability. Data on known regulatory mechanisms (e.g., substrate inhibition or long-range inhibition; ref. 23) have also been incorporated. All the long-range interactions between the folate and methionine cycles that are known to regulate the properties of one-carbon metabolism have been included (22, 23). The model is based on published data from different species and tissues on folate-enzyme kinetics and regulatory mechanisms. This model was used to predict (*a*) the effect of known polymorphisms with established functional significance (e.g., *MTHFR* and *TS*) and (*b*) the potential effect of functional polymorphisms in other enzymes in FOCM (e.g., *DHFR* and *MAT-II*) on biomarkers/mechanisms relevant to carcinogenesis (e.g., homocysteine, methylation rate, thymidine synthesis, and purine synthesis). We used a model for hepatic FOCM for the predictions on homocysteine concentrations and a model for epithelial FOCM for the predictions on other cancer-related mechanisms/

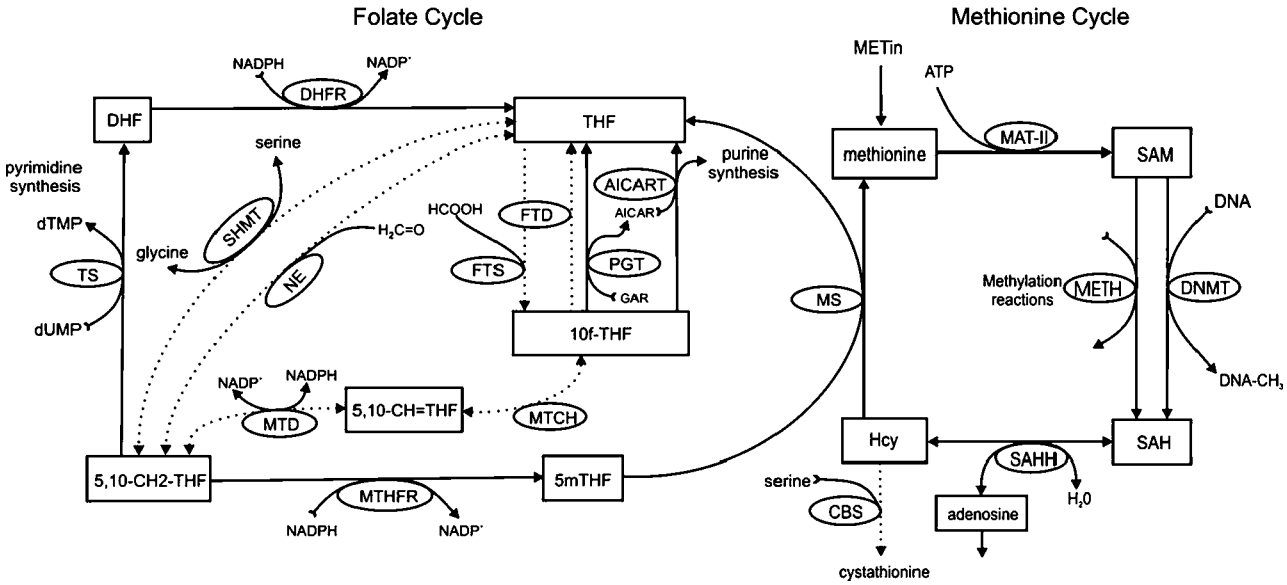

Figure 1. Epithelial folate and methionine metabolism. Substrates are enclosed in rectangular boxes, and enzymes are shown in ellipses. *AICART*, aminoimidazolecarboxamide ribonucleotide transferase; *CBS,* cystathionine β-synthase; *DHFR,* dihydrofolate reductase; *DNMT,* DNA-methyltransferase; *FTD,* 10-formyltetrahydrofolate dehydrogenase; *FTS,* 10-formyltetrahydrofolate synthase; *MAT,* methionine adenosyl transferase; *MS,* methionine synthase; *MTD,* 5,10-methylenetetrahydrofolate dehydrogenase; *MTCH,* 5,10-methenyltetrahydrofolate cyclohydrolase; *MTHFR,* 5,10-methylenetetrahydrofolate reductase; *NE,* nonenzymatic interconversion of THF and 5,10-CH_2-THF; *PGT,* phosphoribosyl glycinamidetransformalase; *SAHH,* S-adenosylhomocysteine hydrolase; *SHMT,* serinehydroxymethyltransferase; *TS*, thymidylate synthase.

Table 1. Comparison between biomarker concentrations observed in the literature and those predicted by the mathematical model of FOCM for genes having polymorphisms with known functional influence on enzymatic activity

Gene	Polymorphism	Genotype	Observed functional effect* (% wild-type activity)	Homocysteine (% wild-type)			Methylation rate (% wild-type)			Purine synthesis (% wild-type)			Thymidylate synthesis (% wild-type)		
				Observed† (range)	Predicted‡		Observed§ (range)	Predicted		Observed (range)	Predicted		Observed‖ (range)	Predicted	
					Folate status			Folate status			Folate status			Folate status	
					High	Low		High	Low		High	Low		High	Low
MTHFR	C677T (29, 42-53)	CC	100	100	100	100	100	100	100	100	100	100	100	100	100
		CT	60	100-110	106	106	(99)-102	92	91	NI	102	105	(108)	105	109
		TT	30¶	113-169	116	123	62-(95)	80	63	141	105	119	(101)-128	112	137
	A1298C (42, 43, 54)	AA	100	100	100	100	100	100	100	NI	100	100	100	100	100
		AC	90	74-(98)	101	101	(99)	98	98		101	101	(99)-(116)	101	102
		CC	68	(103)-115	105	104	(84)-(101)	94	93		102	104	(94)	104	107
TS	TSER (45, 55)	3rpt/3rpt	100	100	100	100	NI	100	100	NI	100	100	NI	100	100
		3rpt/2rpt	58	(107)	99	99		101	101		103	102		62	61
		2rpt/2rpt	42	(98)-(105)	99	99		102	102		104	103		47	45
	1494*del*6 (56)	+6bp/+6bp	100	100	100	100	NI	100	100	NI	100	100	NI	100	100
		+6bp/−6bp	48	(97)	99	99		102	101		104	103		53	51
		−6bp/−6bp	24	78**	98	99		103	102		106	104		28	27

NOTE: Modeled under ''normal-folate'' (=high) status (=20 μmol/L) and ''low-folate'' status (=10 μmol/L).

Abbreviation: NI, no information on this polymorphism/biomarker combination in the literature.

*Functional influence of the polymorphism on enzymatic activity as described in the literature.

† Observed values were calculated as a percentage of wild-type from the published literature in healthy individuals with normal-folate status. Data for low-folate status is reported in the text. Nonstatistically significant ($P > 0.05$) results are reported in parentheses.

‡ Genotypes were modeled as percent increases or decreases in enzymatic activity relative to wild-type.

§ For studies reporting changes in [^{3}H]methyl group acceptance, an inverse measure of global methylation, the inverse of the % change was taken.

‖ Uracil misincorporation was used as an indirect, inverse measure of thymidine synthesis. % change is inverse of the uracil misincorporation data.

¶ MTHFR 677TT was modeled as having 7% of wild-type activity under low-folate status as in ref. 57.

**Among individuals in the highest quartile of RBC folate only.

biomarkers. This approach was used because the preponderance of homocysteine metabolism occurs in liver, whereas epithelial FOCM is a topic of major interest in many cancers (e.g., colorectal). The predictions derived from these two approaches were similar. For enzymes with less well-defined polymorphisms, we modeled the effects of a 150%, 60%, and 30% enzyme activity relative to wild-type (=100%). We also modeled the effect of polymorphisms both under a normal/high (20 μmol/L) and low (10 μmol/L) folate status. These folate levels were chosen because they lie within reasonable ranges of hepatic folate concentrations (24-26). However, data on epithelial folate concentration are quite limited. The current model probably overestimates the nonenzymatic conversion of THF to methylene THF. Yet, this overestimation does not alter the modeling results; when the nonenzymatic reaction is eliminated entirely from the model, the results change minimally (generally <1%). In addition, the model is based on the S phase of the cell cycle, reflecting elevated production of nucleotides. More rapidly proliferating tissues, including colon epithelium, or hematopoietic cells will be more appropriately represented by this model.

We compared model predictions for known polymorphisms with established functional effects to data from the published literature. The literature was reviewed for publications giving quantitative results on the relationship between polymorphisms in genes involved in FOCM and biomarkers relevant to folate status. A literature search was done in Medline for articles published between January 1966 and March 2007. Each FOCM gene known to have polymorphisms (*CBS*, *cSHMT*, *MS*, *MTHFR*, *MTRR*, *RFC*, *TCII*, and *TS*) was crossed with the terms ''polymorphism or mutant or variant'' and ''homocysteine or methylation or purine or pyrimidine or thymidine,'' and the relevant articles were reviewed for quantitative data. Additional references were obtained from these articles and from several reviews. In the case of methylation, only articles that reported on global methylation, not promoter-specific hypermethylation, were included. Finally, all literature observations presented here pertain to healthy individuals; only results for the control group (for case-control studies) or baseline time point (for dietary intervention or depletion/repletion studies) were considered. A total of 38 publications provided relevant data for the comparison of model predictions to observed data. If more than three studies were available, only the results from the largest studies are listed in the tables as published ranges.

Results

Table 1 illustrates the observed and predicted effect of polymorphisms in *MTHFR* and *TS*. Our modeling predictions are consistent with the reported literature on associations with global DNA methylation and replicate the greater influence of the *MTHFR* variant under a low-folate status (15, 27-32). The effects of the *MTHFR* 677TT genotype on homocysteine concentrations were predicted to be somewhat lower than what the published literature suggests (27-30). We hypothesize that this is due to the fact that our model predicts epithelial, intracellular concentrations of homocysteine, whereas the literature refers to plasma concentrations. In life, and in our model, homocysteine is rapidly exported from epithelial cells, so the intracellular steady-state concentration is expected to be lower than the plasma concentration, and plasma homocysteine (total homocysteine) is predominantly in a bound form. The *MTHFR* 677TT genotype also was predicted to increase purine and thymidylate synthesis. As expected, the variant had a greater effect under a low-folate status (e.g., thymidylate synthesis was increased by 27% under normal-folate status compared with 63% under low-folate status). This is in part attributable to greater rate of inactivation of the enzyme under low-folate status.

We modeled two functional polymorphisms in *TS*, including one in the promoter-enhancer region and one in the 3′ untranslated region, both of which are decreasing TS protein levels (33, 34). As expected, *TS* variants significantly altered thymidylate synthesis yet had a relatively modest influence on purine synthesis (~10% change). The effects of *TS* polymorphisms on the methylation rate and homocysteine concentrations were negligible, which corresponds to the inconsistent reports in the literature.

For most FOCM genes, the functional effect of potential polymorphisms on enzymatic activity or transcription is not well defined. Thus, we modeled the effects of these polymorphisms under three different scenarios in the epithelium and hepatocyte: a 50% increase in activity (150% of wild-type activity), a 40% decrease in activity (60% of wild-type), and a 70% decrease in activity (30% of wild-type) of the enzyme of interest (Tables 2 and 3).

Some key findings in the epithelial model are that polymorphisms that alter enzyme function of FTD, FTS, and MTCH are expected to affect purine synthesis (predicted changes ± 12-31% at 30% modeled enzyme activity and ± 6-13% at 60% modeled enzyme activity). *MTCH* polymorphisms also should have a substantial influence on thymidylate synthesis with ~25% increase in thymidylate synthesis at 60% modeled enzyme activity, increasing to >59% increase in thymidylate synthesis when enzyme function is modeled at 30%. Polymorphisms that reduce methionine synthase/methionine synthase reductase function are predicted to alter purine and thymidylate synthesis (30% enzyme activity: purine synthesis: −30% under normal folate and −37% under a low-folate status; thymidylate synthesis: −49% under normal folate and −50% under a low-folate status) and, to some degree, the methylation rate and homocysteine concentrations. Polymorphisms in methyltransferases should affect not only methylation rates but also thymidylate synthesis. Under a low-folate status, the effect on thymidylate synthesis is exacerbated, whereas methylation rates remain more stable. However, there are a multitude of methyltransferases, and we currently modeled these as a single reaction.

However, overall, the model suggests that there is substantial robustness in the pathway toward genetic variation at a single site. In particular, polymorphisms in *AICART* and *MAT-II* are not expected to alter biomarkers of cancer risk. This can be explained by the fact that in these cases, changes in enzyme activity are matched by reciprocal changes in the steady-state concentrations of their substrates so that the flux carried by these enzymes remains unchanged.

Table 2. Predicted effect of potential functional polymorphisms in other enzymes in epithelial FOCM on biomarkers/mechanisms relevant to carcinogenesis

Gene	Modeled functional effect (% of wild-type)	Modeled biomarkers (% of wild-type)							
		Homocysteine		Methylation rate		Purine synthesis		Thymidylate synthesis	
		Normal folate	Low folate	Normal folate	Low folate	Normal folate	Low folate	Normal folate	Low folate
AICART	150	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100
	60	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100
CBS	150	86	89	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100
	60	116	112	100	100	100	100	101	100
	30	132	123	99	100	100	100	101	101
FTD	150	100	100	101	101	89	88	104	103
	100	100	100	100	100	100	100	100	100
	60	101	101	99	99	112	112	95	96
	30	101	101	98	98	122	124	90	92
FTS	150	100	101	99	99	108	112	97	97
	100	100	100	100	100	100	100	100	100
	60	100	100	100	101	94	90	102	103
	30	100	99	101	101	88	82	104	105
MAT-II	150	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100
	60	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100
MTCH	150	103	103	96	96	108	109	80	83
	100	100	100	100	100	100	100	100	100
	60	97	97	105	105	88	87	130	125
	30	94	93	111	110	69	70	170	159
MTD	150	100	100	100	100	100	100	99	99
	100	100	100	100	100	100	100	100	100
	60	100	100	101	100	99	99	103	102
	30	99	99	102	102	97	97	110	107
MTHFD	150	104	103	95	95	115	120	77	79
	100	100	100	100	100	100	100	100	100
	60	96	96	106	106	79	76	135	131
	30	94	92	112	112	51	47	182	172
MTR/MTRR	150	99	99	102	102	104	106	108	110
	100	100	100	100	100	100	100	100	100
	60	102	103	97	96	92	88	84	81
	30	110	110	87	86	70	63	51	50
PGT	150	99	99	101	101	129	125	105	104
	100	100	100	100	100	100	100	100	100
	60	101	101	99	99	68	70	95	95
	30	102	102	98	98	37	40	89	89
SAHH	150	108	110	105	103	98	98	97	97
	100	100	100	100	100	100	100	100	100
	60	92	87	91	95	102	103	105	105
	30	87	78	72	82	107	110	117	118
CSHMT	150	97	97	105	105	109	109	125	122
	100	100	100	100	100	100	100	100	100
	60	105	104	94	94	87	89	72	75
	30	112	110	84	85	71	76	45	49
Methyltransferases	150	102	103	119	115	94	90	88	84
	100	100	100	100	100	100	100	100	100
	60	94	91	78	83	106	110	113	118
	30	86	77	49	57	112	122	130	142

Abbreviations: AICART, aminoimidazolecarboxamide ribonucleotide transferase; CBS, cystathionine β-synthase; FTD, formyltetrahydrofolate dehydrogenase; FTS, formyltetrahydrofolate synthase; MAT-II, methionine adenosyl transferase-II; MTCH, 5,10-methylenetetrahydrofolate cyclohydrolase; MTD, 5,10-methylenetetrahydrofolate dehydrogenase; MTHFD, trifunctional enzyme (independent effects for MTD, MTCH, and FTS above); MTR/MTRR, methionine synthase/methionine synthase reductase; PGT, phosphoribosyl glycinamide transformylase; SAHH, S-adenosylhomocysteine hydrolase; CSHMT, cytosolic SHMT.

Table 3. Predicted effect of potential functional polymorphisms in enzymes in hepatic FOCM on biomarkers/mechanisms relevant to carcinogenesis

Gene	Modeled functional effect (% of wild-type)	Modeled biomarkers (% of wild-type)							
		Homocysteine		Methylation rate		Purine synthesis		Thymidylate synthesis	
		Normal folate	Low folate	Normal folate	Low folate	Normal folate	Low folate	Normal folate	Low Folate
BHMT	150	97	92	101	101	101	102	103	104
	100	100	100	100	100	100	100	100	100
	60	103	109	99	98	99	98	98	96
	30	106	120	98	97	98	96	95	93
GNMT	150	102	104	95	93	99	97	97	95
	100	100	100	100	100	100	100	100	100
	60	98	96	106	108	101	103	104	105
	30	95	90	112	117	103	106	107	111
MAT-I	150	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100
	60	100	100	100	100	100	100	100	100
	30	100	100	100	100	100	100	100	100
Methyltransferases	150	103	106	131	131	97	95	92	91
	100	100	100	100	100	100	100	100	100
	60	96	94	66	66	102	104	106	107
	30	94	90	34	35	104	106	110	111

Abbreviations: BHMT, betaine homocysteine methyltransferase; GNMT, glycine N-methyltransferase; MAT-I, methionine adenosyl transferase-I.

The mathematical simulation model provides an easy tool for the simultaneous investigation of multiple polymorphisms concurrently (Figs. 2 and 3). We have investigated here two sets of gene-gene interactions that have been proposed to be of particular interest to cancer researchers because of their relevance to nucleotide and, specifically, purine synthesis (35, 36).

First, we simulated gene-gene interactions between *MTHFR* and *TS* by modeling a continuous change in MTHFR and TS activity (Fig. 2, scaling changes depending on biomarker to allow for a representation of the surface). As expected, there was little contribution of variation in TS on homocysteine concentrations. However, thymidylate synthesis, and to a greater extent purine synthesis, was affected by changes in both enzymes in a nonlinear fashion, suggesting gene-gene interactions between these two enzymes on biomarkers of cancer risk.

Second, we modeled the combined effects of polymorphisms in *MTHFR* and serine hydroxymethyltransferase (*SHMT*; Fig. 3). Again, note that scaling is adapted to show the surface, whereas the circle indicates a wild-type genotype (100% enzyme activity for both). Changes in both MTHFR and SHMT levels contributed to variations in homocysteine levels. The effects of simultaneous changes in MTHFR and SHMT activity are nonlinear, suggesting gene-gene interactions between these two enzymes. Furthermore, *SHMT* variations contributed to both purine and thymidine synthesis, with higher SHMT activity increasing synthesis.

Discussion

These results from our mathematical simulation model of FOCM illustrate its utility in predicting where genetic variability could have the greatest effect on homeostasis and biologically relevant outputs. Considering that high-throughput genotyping largely alleviates the need for targeted investigations in epidemiologic studies, these results will be mostly useful for (*a*) aiding the interpretation of epidemiologic findings and (*b*) targeting experimental studies, such as dietary intervention studies that select individuals by genotype or targeted knock-in mouse studies.

Our model suggests that FOCM is generally quite robust toward changes in enzyme function. This is not unexpected because FOCM is an integral part of the machinery for essential cellular processes, such as the synthesis of nucleotides. In fact, FOCM is an ''ancient'' pathway and occurs, with variations, in animals, plants, fungi, and bacteria. Strong evolutionary pressure against genetic instability in this system must have existed, arguing that most polymorphisms will not influence the FOCM outputs unless the system is under stress because of disturbances at other sites (e.g., gene-gene interaction) or a low-folate status (gene-diet interaction). Indeed, our recent work predicts that several regulatory mechanisms in this pathway have evolved to protect the ''methylation capacity'' against fluctuations in methionine input (23). In most cases, we observed a greater effect of the variants on biological outputs under a low-folate status. This argues that for genetic investigations of a complex pathway with many regulatory properties, sole reliance on the ''main effects'' of polymorphisms may miss many important relationships and yield false-negative results.

On the other hand, the model predictions indicate where there is sensitivity in the system and thus suggest where genetic polymorphisms may play a larger role. Many genes in FOCM have not been systematically sequenced for polymorphism discovery and our results suggest that polymorphisms in *MS*, *MTRR*, *FTD*, *FTS*, or

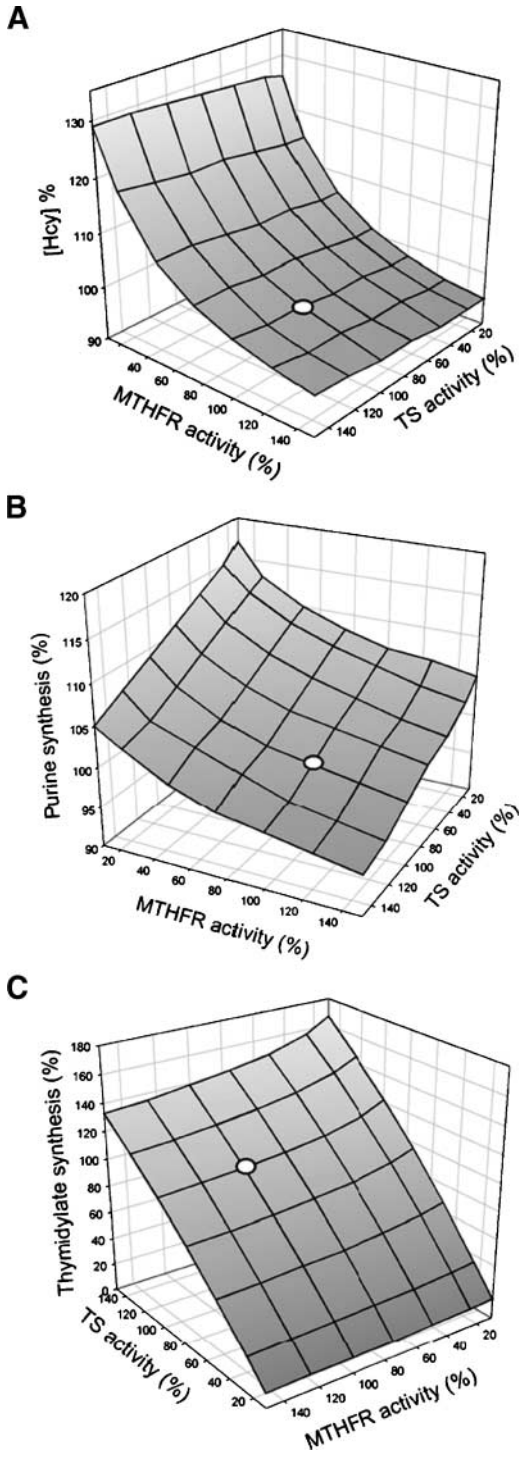

Figure 2. A. Variation in MTHFR and TS activities on homocysteine concentration. **B.** Variation in MTHFR and TS activities on purine synthesis. **C.** Variation in MTHFR and TS activities on thymidylate synthesis. "O" = 100% enzyme activity in both enzymes.

MTCH could play a substantial role in disturbing mechanisms leading to cancer risk. In addition, polymorphisms in the methyltransferases may affect not only methylation rate but also thymidylate and purine synthesis.

We centered our initial investigation of gene-gene interactions on changes in enzyme function that are expected to affect some of the key mechanisms proposed to relate FOCM to cancer risk: thymidylate and purine synthesis (7). Our investigations of *MTHFR* and *TS* polymorphisms suggest that purine synthesis will be clearly affected by variations in both of these key enzymes. Even modest decreases (20%) in MTHFR and TS activity jointly can increase purine synthesis by >15%.

Further, an important consideration is that even very modest changes in, for example, nucleotide synthesis may have major effects if they are present over an individual's entire lifetime. Thus, the general robustness of the pathway needs to be interpreted in that context.

Our results describe the effects of polymorphisms on biomarkers and mechanisms of cancer risk; however, because of the complexity of this pathway, they permit only limited conclusions regarding the effects on cancer risk. For example, the predicted ~50% reduction in thymidylate synthesis associated with the *TSER* 2rpt/2rpt genotype corresponds to a reported 20% to 40% reduction in colorectal cancer risk (36, 37). However, for *MTHFR* C677T, the situation is more complex because this enzyme regulates the diversion of folate metabolites toward DNA methylation and nucleotide synthesis. The TT genotype has been associated with a reduced risk of colorectal tumors under a high-folate status, which may be abrogated or reversed under a low-folate status (5). Our model suggests that TT reduces the methylation rate, whereas it increases purine and thymidylate synthesis modestly. Yet, increased nucleotide synthesis may have opposing effects on carcinogenesis depending on the stage of carcinogenic development (38-40). The role of DNA methylation is currently unclear because both promoter hypermethylation and genomic hypomethylation can occur concurrently during carcinogenesis; in the absence of data on regulatory mechanisms, our model predicts currently ''genomic methylation levels.'' The specific mechanisms linking folate status and folate-related polymorphisms to cancer risk may also differ by tumor type (e.g., hematopoietic versus colorectal cancer), further limiting direct conclusions about cancer risk.

However, more direct links to cancer risk should become evident by using the model-derived predictions to incorporate biological knowledge into the data analysis of epidemiologic studies. We are using the predictions both in the context of both a hierarchical modeling structure and Bayesian averaging techniques. Beyond the ''steady-state'' predictions shown here, we are currently developing methods to derive appropriate estimations of ''variability'' around our predictions of steady state. This will further increase the utility of these predicted variables in more complex epidemiologic data analyses. Our goal with the hierarchical modeling is to derive risk estimates for ''mechanism A'' (e.g., purine synthesis, as measured by the AICART reaction) in relation to a certain disease outcome (e.g., colon cancer). These risk estimates should provide information on which biological mechanism is most strongly linked to the outcome.

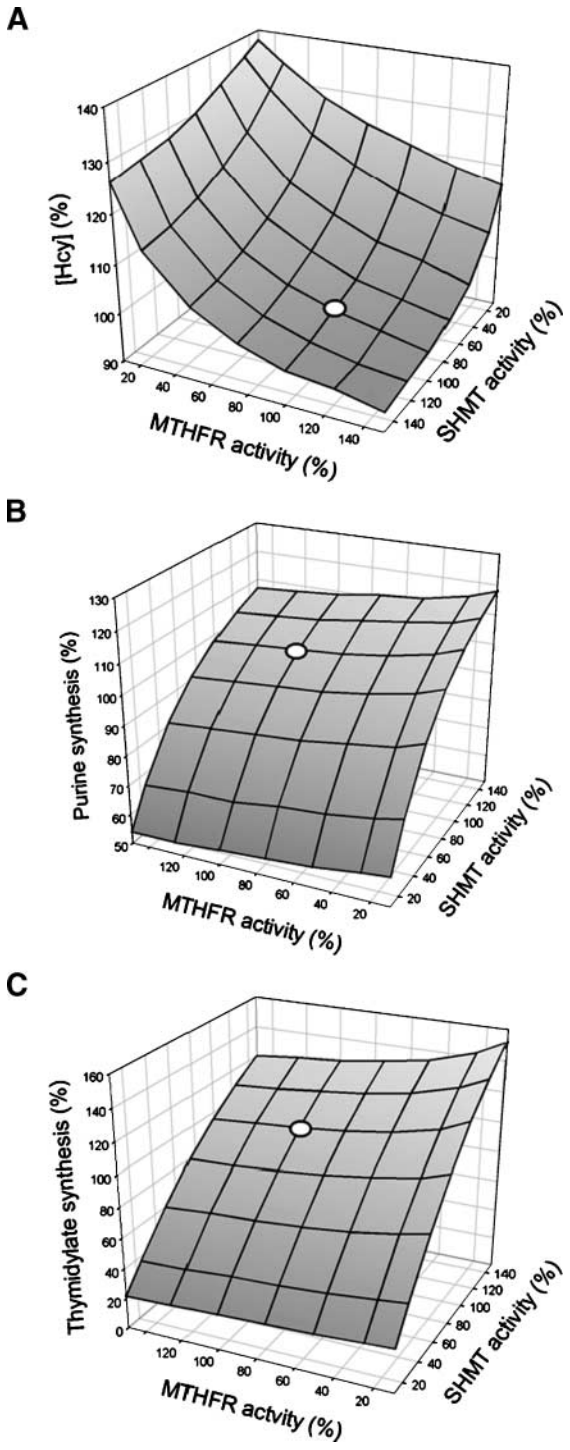

Figure 3. A. Variation in MTHFR and SHMT activities on homocysteine concentration. **B.** Variation in MTHFR and SHMT activities on purine synthesis. **C.** Variation in MTHFR and SHMT activities on thymidylate synthesis. "O" = 100% enzyme activity in both enzymes.

We have modeled the effects of mutations on enzyme activity by changing the V_{max} of the relevant kinetic equation. This can be thought of as corresponding to changes in the overall activity, enzyme availability, or expression level of the enzyme, which is relevant for many polymorphisms (e.g., *MTHFR* C677T and *TS* promoter). Effects of variants on variables such as K_m and K_{cat} could also be modeled if such specific effects of polymorphisms were known. Additionally, folate metabolism may be compartmentalized in the cell through substrate channeling or nuclear localization of enzymes; therefore, the overall cellular folate concentrations of our current model may not fully predict the function of a given pathway, such as thymidine synthesis. We are in the process of expanding our model to incorporate these more complex processes and have already modeled mitochondrial folate metabolism (41).

This study has several strengths. Our mathematical simulation model of FOCM has been shown to replicate central properties of FOCM and make valid predictions (22). A key advantage of these *in silico* simulations is that they are rapid and inexpensive and allow for an easy investigation of variation in multiple inputs (e.g., gene-gene or gene-diet interactions). There is no limit to the number of variables that can be varied simultaneously, although we have only presented data on two-way interactions here. The software for this work (*in silico* metabolism, ISM1) will be made shortly available for public licensing and we expect that it will be used both (*a*) to guide experimental and epidemiologic studies and (*b*) to aid in the interpretation of epidemiologic findings, particularly for interactions.

However, our modeling also certainly has limitations. For example, regulatory mechanisms such as metabolic switches that involve regulation of gene expression with subsequent alterations in protein expression are not taken into account. Further, there may be other potential mechanisms connecting FOCM with carcinogenesis, including links to gluconeogenesis and energy balance that we have not modeled or described as output here. We plan to expand our model of ISM into those new areas.

In conclusion, our mathematical modeling of FOCM provides a new tool for investigating *in silico* the effects of genetic variation in folate enzymes on biomarkers and mechanisms relevant to cancer risk. With this approach, one can rapidly and inexpensively explore the effects of gene-gene and gene-diet interactions, including three-way and multiway interactions. We anticipate that the model will help address challenges to approaching complex pathways in cancer epidemiology and provide information that is of relevance to both study design and our biological understanding.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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