Combined Impact of Polymorphism of Folate Metabolism Genes; Glutamate Carboxypeptidase, Methylene Tetrahydrofolate Reductase and Methionine Synthase Reductase on Breast Cancer Susceptibility in Kashmiri Women

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Abstract:

Background: Folate and methionine play a crucial role in DNA synthesis, repair and the epigenetic profile of cell. Hence, the alterations in the folate metabolism can lead to aberrant proliferation leading to neoplasia. Most of the studies have associated polymorphisms in methylene tetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) genes with reduced risk of cervical and colorectal cancer. However, the association with breast cancer is still controversial. Further, the involvement of Glutamate carboxypeptidase II (GCPII) polymorphism in cancer is not known. In the present study, we analyzed if the individual and combined effects of polymorphisms in folate pathway genes viz., MTHFR 677C > T, MTHFR 1298A > C, MTRR 66A > G and GCP II 1561 C>T, have any role in altering the susceptibility to breast cancer.

Methods: The DNA of 35 female breast cancer patients and 33 healthy individuals, in the Kashmiri population from India, were analyzed using a PCR-RFLP approach for the above mentioned polymorphisms.

Results: Individuals carrying the MTHFR 677CT/TT and GCPII 1561 CT genotype showed a 3.5 (95% CI: 3.1-3.7, P<0.02) and 7.7 (95% CI: 6.7-9.1, P<0.001) fold decreased risk for breast cancer than the wild types (MTHFR 677CC and GCPII 1561 CC). Subjects with MTRR 66 G-allele showed a 4.5 fold decreased risk (OR: 0.22, 95% CI: 0.20, 0.24, P<0.0005) compared to the wild type (MTRR 66A). Further, subjects with combined polymorphisms in MTHFR, GCPII and MTRR loci revealed a significant reduction of breast cancer risk.

Conclusion: This study indicates (i) a protective role of polymorphisms in MTHFR, GCPII, MTRR against breast cancer in the study subjects, and (ii) combined effect of polymorphisms is more pronounced than single genetic polymorphism, thereby emphasizing the role of gene-gene interaction in the susceptibility to breast cancer.

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Introduction

A large number of epidemiological studies point to the involvement of dietary folate and polymorphisms in the enzymes of one carbon metabolism pathway in modulating the risk of cancer. This is because folate and methionine metabolism play an important role in DNA synthesis, repair and methylation. Following folate deficiency, the two major pathways that contribute to neoplasia are: (a) misincorportaion of uracil leading to chromosomal instability, and (b) DNA hypomethylation leading to altered gene expression [1]. There is a number of intermediary steps involved in the folate and methionine metabolism pathway, each catalyzed by a different enzyme (Fig. 1).

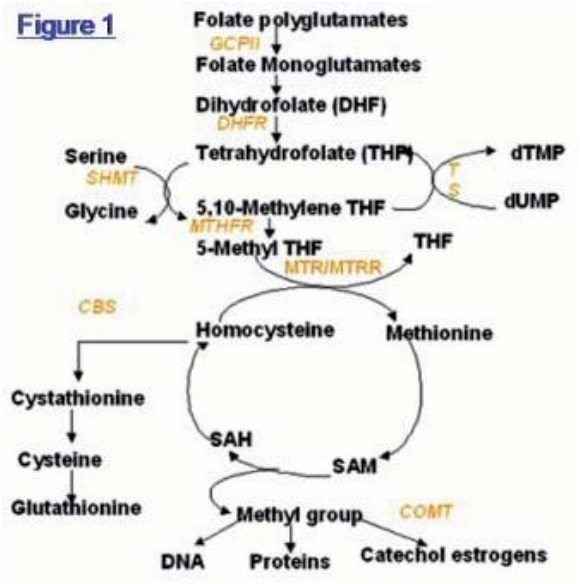

Fig. 1. The schematic representation of intermediary metabolites in folate and methionine metabolism. MTHFR (methylene tetrahydrofolate reductase), MTRR (methionine synthase reductase), GCPII (glutamate carboxypeptidase II), TS (thymidylate synthase), CBS (cystathionine beta synthase), DHFR (dihydrofolate reductase), SHMT (serine hydroxymethyl transferase), COMT (catechol -O- methyl transferase), SAM (S-adenosyl methionine) and SAH (S-adenosyl homocysteine).

Any genetic polymorphism affecting the functioning of the enzyme will in turn lead to DNA instability contributing to the risk of cancer. Interestingly, most of the genes involved in the folate metabolic pathway are polymorphic in nature and a particular combination of genetic variants may confer differential susceptibility to cancer. Of all the enzymes involved in this pathway, polymorphism in MTHFR gene is the best-studied aspect for cancer susceptibility. Studies on the association of polymorphisms, in other key members of this pathway viz., methionine synthase reductase (MTRR), thymidylate synthase (TS), cystathionine beta synthase (CBS), glutamate carboxypeptidase II (GCP II), with neoplasia are limited. In different populations, the polymorphisms of folate pathway genes vary greatly in allele frequency, and this in turn may be responsible in differential susceptibility to disease [1, 2]. In view of the fact that studies from the Indian subcontinent are limited on the polymorphic variants and allele frequency distribution of the folate metabolic pathway, the present study was carried out to understand the association of combined polymorphism in three of the candidate genes viz., MTHFR, GCPII and MTRR in breast cancer susceptibility in Indian women.

MTHFR catalyzes the reduction of 5, 10-methy-lenetetrahydrofolate, an intracellular coenzymatic form of folate, to 5-methyltetrahydrofolate, the predominant form of folate in plasma. 5-methyltetrahydrofolate, provides the methyl group for the methionine synthesis and subsequent synthesis of S-adenosylmethionine (SAM), the universal methyl donor of biological methylation. The two common polymorphisms in MTHFR viz. C677T and A1298C reduce the enzymatic activity differentially. In the presence of adequate folate, the C677T mutation confers protection to certain types of cancers such as colorectal cancer due to alteration in the thymidylate pool [3]. Further individuals homozygous (T/T) for MTHFR mutation show DNA hypomethylation, a characteristic of most cancer [4]. There is no conclusive proof regarding the association of cancer susceptibility to A1298C MTHFR polymorphism.

In addition to folate, methionine synthesis requires the enzyme MTRR, a vitamin B12 dependent enzyme. Methionine synthase (MS) is maintained in its active state by MTRR which catalyzes the reductive activation of cob(II)alamin to cob(I)alamin, thus making it available for the remethylation of homocysteine to methionine, which is the precursor of SAM [5]. The A66G MTRR polymorphism results in low methionine biosynthesis, which in turn affects the SAM

production leading to hypomethylation [6]. This polymorphism has been associated with altered susceptibility for colorectal cancer [7], acute leukemia [8], lung cancer [9] and squamous cell carcinoma [10].

GCP II (11p11.2), also known as prostate specific membrane antigen (PSMA), N-acetyl-α-linked acidic dipeptidase I (NAALADase (NLD) I), or folate hydrolase, is a 750-residue type II transmembrane glycoprotein that is highly expressed in prostate cancer cells and in non-prostatic solid tumor neovasculature and expressed at lower levels in other tissues, including healthy prostate, kidney, liver, small intestine and brain [11]. A 50% reduction in enzymatic activity is reported for C1561T GCPII polymorphism, which in turn impairs absorption of dietary folates resulting in low plasma folate levels and hyperhomocysteinemia [12]. While this polymorphism is linked to neural tube defects, there is hardly any report on its susceptibility to cancer. In fact, there is only one report showing non-association of GCPII with colorectal cancer [13].

The genotype frequencies for the folate metabolizing enzymes show marked ethnic and geographic variation, and this in turn can modulate the cancer risk in different geographical regions depending on the micronutrient intake [14]. Most of the studies cited in literature on polymorphisms in the folate metabolizing genes and risk to cancer are in the Caucasian and Chinese populations with major focus on colorectal cancer, with hardly any from the Indian subcontinent. There is only one recent report from India showing an inverse relation of MTHFR 1298CC polymorphism with colorectal cancer [15]. Since the data on genetic polymorphisms in one carbon metabolism pathway for breast cancer susceptibility is inconclusive and conflicting, we carried a pilot study to examine the individual and combined the impact of GCPII, MTHFR and MTRR polymorphisms on the breast cancer susceptibility in the Kashmiri women from India.

Methods

Subjects

The study group comprises 35 patients diagnosed of breast cancer and 33 control women with no history of breast cancer. The patients were registered with the departments of Radiation Oncology and General Surgery of Sher-i-Kashmir Institute of Medical Sciences, Srinagar. Both cases and controls were from Kashmir (India) and were unrelated. Signed consent forms were obtained from each subject and institutional bioethical committee approved the study.

Sample collection

Whole blood samples in EDTA were obtained from all the subjects and genomic DNA was isolated using standard protocols. Tumor DNA was isolated separately from the biopsy specimens and stored for other studies.

Genetic analyses

C677T MTHFR, A1298C MTHFR, C1561T GCP II and A66G MTRR polymorphisms were genotyped using PCR-based RFLP approaches using HinfI, MboII, AccI and NdeI restriction enzymes [12,16-18].

Statistical analyses

Genotype and allele frequencies were calculated and computed as categorical variables. Fisher exact test was done on 2 x 2 contingency tables by segregating the data into cases and controls and also based on the presence or absence of each variable. Odds ratios and 95% confidence intervals were calculated by the application of Vassarstats software (http://faculty.vassar.edu/lowry/VassarStats.html). All statistical tests were based on two-tailed probability and considered significant at $P < 0.05$. Normal, heterozygous and homozygous mutant genotypes were considered as 0, 1 and 2 respectively based on the number of mutated alleles and logistic regression analysis was done to obtain Ptrend. PHWE values were calculated by simple application of Hardy-Weinberg Equilibrium test and Chi2 association test for the observed and expected frequencies. Bivariate interactions were studied initially using Fisher exact test. Later, the haplotype data computed as -/-, -/+, +/-, +/+ in both cases and controls was used for logistic regression analysis. Three-way interactions between C677T MTHFR, C1561T GCPII and A66G MTRR were studied using Fisher exact test and logistic regression analysis similar to Bivariate analysis.

Results

Age association and intake of salted green tea in Kashmiri women

A detailed analysis of age, histopathological

Table 1. Frequency distribution of certain variables in susceptibility to breast cancer patients and control group among Kashmiri women

Parameters	Cancer Cases (N=35)	Control (N=33)	P
Age			
10-20	0	4	0.05
20-30	0	12	<0.0001
30-40	3	8	0.1
41-50	16	6	0.02
51-60	11	2	0.01
61-70	5	1	0.2
Intake of salt tea			
1-3 cups	19	30	<0.001
3-6 cups	13	3	<0.01
6-10 cups	3	0	0.24
Smoking	6/35	6/33	1.00
Grading System			
1	3		
2	14		
3	7		
4	11		

grading of tumors and the salted green tea intake among the women is described in Table 1.

The mean age for control group was 34.4 years, while that of the breast cancer group was 51.3 years. Salted green tea intake more than 3 cups per day was found to be associated with a significant breast cancer risk (P<0.0001).

Genotyping and risk evaluation based on individual polymorphism

We calculated the genotype frequency for the known polymorphisms in MTHFR, MTRR and GCP-II (Table 2) together with the odds ratio for risk. The frequencies of MTHFR 677 C→T mutation in cases and controls were 17.1% (6/35) and 42.4% (14/33) respectively (P value <0.05).

Ptrend values for MTHFR C677T, MTHFR A1298C, GCP II C1561T and MTRR A66G were 0.03, 0.72, 0.0006, <0.0001 respectively. PHWE values for MTHFR C677T, MTHFR A1298C, GCP II C1561T and MTRR A66G in cases were 0.86, 0.22, 0.97 and 0.0023 respectively. The corresponding values in controls were 0.99, 0.016, 0.14 and 0.67 respectively.

Interestingly, in the breast cancer group, the polymorphic 677T mutants showed only the heterozygous genotype. Unlike the cancer patients, in the control group 36.4% (12/33) were heterozygous and 6% (2/33) were homozygous for 677T polymorphism. A lower risk of breast cancer was observed in subjects with the polymorphic heterozygous CàT MTHFR genotype (OR= 0.36,

Table 2. Genotype frequencies in breast cancer cases and controls

Variable	Cases	Controls	OR	P value
MTHFR C677T				
CC	29 (82.86%)	19 (59.38%)	3.56 (3.06-4.18)	0.06
CT	6 (17.14%)	12 (36.36%)	0.36 (0.31-0.42)	0.10
TT	0 (0%)	2 (6.06%)	ND	0.23
C	64 (91.43%)	50 (75.76%)	3.41 (3.12-3.70)	0.02
T	6 (8.57%)	16 (24.24%)	0.29 (0.27-0.32)	0.02
MTHFR A1298C				
AA	15 (42.86%)	11 (33.33%)	1.5 (1.39-1.63)	0.46
AC	19 (54.29%)	22 (66.67%)	0.59 (0.54-0.65)	0.33
CC	1 (2.86%)	0 (0%)	ND	1.00
A	49 (70%)	44 (66.67%)	1.17 (1.14-1.20)	0.71
C	21 (30%)	22 (33.33%)	0.86 (0.83-0.88)	0.71
GCPII				
CC	32 (91.43%)	16 (48.48%)	11.33 (8.67-15.20)	<0.0001
CT	3 (8.57%)	17 (51.52%)	0.09 (0.07-0.12)	<0.0001
C	67 (95.71%)	49 (74.24%)	7.75 (6.67-9.08)	<0.0005
T	3 (4.29%)	17 (25.76%)	0.13 (0.11-0.15)	<0.0005
MTRR				
AA	1 (2.86%)	0	ND	1.00
AG	27 (77.14%)	9 (27.27%%)	9.00 (8.14-13.25)	<0.0001
GG	7 (20%)	24 (72.73%)	0.10 (0.08-0.12)	<0.0001
A	29 (41.43%)	9(13.64%)	4.48 (3.99-5.05)	<0.0005
G	41 (58.57%)	57 (86.36%)	0.22 (0.20-0.24)	<0.0005

95% CI: 0.31-0.42, P=0.1). The frequencies of MTHFR 1298 A→C mutation in cases and controls were 57.15% (20/35) and 66.67% (22/33) respectively. There was no association seen between the A1298C polymorphism and susceptibility to breast cancer (OR: 0.86, 95% CI: 0.83-0.88, P=0.71). In both cancer and the control subjects, the representation of mutant homozygous genotype (CC) was low with 2.9% in cancer and none in controls.

The frequencies of GCP II 1561 C→T mutation in cases and controls were 8.6% (3/35) and 51.5% (17/33) respectively (OR:0.08, 95% CI: 0.07-0.12, P<0.0001). GCP II CàT polymorphism is associated with significantly reduced risk of breast cancer. Interestingly, the homozygous TT GCP II polymorphism was not detected in the population studied. All the mutants detected in both groups were heterozygous for GCP II polymorphism. The frequencies of MTRR 66 A→G mutation in cases and controls were 97.4% (34/35) and 100% (33/33) respectively. Among the cancer cases 77.1% were heterozygous (77.1% (27/35)) and 20% were homozygous mutant (7/35). In the control group, 27.27% were heterozygous (9/33) and 72.73% were homozygous mutant (24/33). A nine-fold increase was associated with MTRR 66AG heterozygous genotype (OR: 9.0, 95% CI: 7.25-11.36, P<0.0001).

Combined genotypic analysis of different polymorphisms on breast cancer susceptibility

A combined analysis of MTHFR, GCPII and MTRR polymorphism was done by evaluating two or three polymorphisms together to assess the possibility of gene-gene interaction in determining the breast cancer risk.

Two-way analysis:

The impact of the joint effect of genetic polymorphisms on breast cancer risk is shown in Tables 3 and 4, and the logistic regression analysis of two polymorphic loci is shown in Table 4. A combination of variant genotypes of MTHFR C677T x MTHFR 1298 showed an odds ratio of 0.14, 95% CI: 0.10-0.18, which is highly significant (P<0.01). Logistic regression analysis also showed that both the variant alleles act in an additive manner (P<0.01) to lower the risk for breast cancer. However, the other combinations of polymorphic genotypic interactions between the MTHRF 677 and 1298 loci were not significant.

The presence of wild type alleles in the MTHFR 677/GCPII 1561 combination showed a significant higher risk of cancer (OR: 5.06, 95% CI: 4.28-6.05, P<0.005). Odds ratios for the presence of variant allele at either the MTHFR or GCP II were 0.35 (95% CI: 0.29-0.41, P=0.18) and 1.5 (95% CI: 1.36-1.69, P=0.74) respectively, with no significance to cancer risk. None of the cancer subjects in this study carried a variant allele at both GCPII and MTRR loci. However, co-segregation of both mutated alleles was seen

Table 3. Odds ratio (OR) estimates of breast cancer risk for combined effects at two genetic loci

Haplotypes	Cancer Cases (N=35)	Controls (N=33)	OR	P value
MTHFR 677-1298				
C-A	11 (31.43%)	7 (21.21%)	1.7 (1.55-1.89)	0.42
C-C	18 (51.43%)	12 (36.36%)	1.85 (1.69-2.04)	0.23
T-A	4 (11.43%)	4 (12.12%)	0.94 (0.88-0.97)	1.00
T-C	2 (5.71%)	10 (30.3%)	0.14 (0.10-0.18)	0.01
MTHFR 677-GCPII				
C-C	26 (74.29%)	12 (36.36%)	5.06 (4.28-6.05)	0.003
C-T	3 (8.57%)	7 (21.21%)	0.35 (0.29-0.41)	0.18
T-C	6 (17.14%)	4(12.12%)	1.5 (1.36-1.69)	0.74
T-T	0 (0%)	10(27.27%)	ND	<0.0005
GCPII-MTRR				
C-A	25 (71.43%)	1 (3.03%)	80 (43.67-166.66)	<0.0001
C-G	7 (20%)	15 (45.45%)	0.3 (0.26-0.35)	<0.05
T-A	3 (8.57%)	8 (18.18%)	0.29 (0.24-0.35)	0.11
T-G	0 (0%)	9 (27.27%)	ND	<0.001
MTHFR 677-MTRR				
C-A	22 (62.86%)	4 (12.12%)	12.27 (9.48-16.26)	<0.0001
C-G	7 (20%)	15 (45.45%)	0.3 (0.26-0.35)	<0.05
T-A	6 (17.14%)	5 (15.15%)	1.15 (1.10-1.25)	1.00
T-G	0 (0%)	9(27.27%)	ND	<0.001
MTHFR 1298-GCPII				
A-C	14 (40%)	8 (24.24%)	2.08 (1.87-2.34)	0.2
A-T	1 (2.86%)	3 (9.09%)	0.29 (0.21-0.39)	0.35
C-C	18 (51.43%)	8 (24.24%)	3.31 (2.88-3.84)	<0.05
C-T	2 (5.71%)	14 (42.42%)	0.08 (0.06-0.11)	<0.0005
MTHFR 1298-MTRR				
A-A	15 (42.86%)	3(9.09%)	7.5 (5.94-9.68)	<0.005
A-G	0 (0%)	8 (24.24%)	ND	<0.002
C-A	13 (37.14%)	6(18.18%)	2.66 (2.33-3.08)	0.11
C-G	7 (20%)	16(48.48%)	0.27 (0.23-0.31)	<0.05

Table 4. Logistic regression analysis for two-way gene-gene interactions

Bivariates	Chi2	r2	P value
1. MTHFR 677-MTHFR 1298	5.99	0.78	<0.01
2. MTHFR 677-GCPII	10.56	0.72	<0.001
4 GCPII-MTRR	30.07	0.81	<0.0001
5MTHFR 677-MTRR	16.55	0.74	<0.0001
3. MTHFR 1298-GCPII	4.54	0.33	<0.05
6 MTHFR 1298-MTRR	6.21	0.24	0.013

in the control group, which is highly significant (<0.0005).

The GCP II 1561/MTRR combination with both the wild type alleles confers risk (OR: 80, 95% CI: 43.67-166.66, P<0.05) to breast cancer, which is significant. The presence of MTRR variant allele in the presence of a wild type GCPII allele showed a significant protection (OR: 0.3, 95% CI: 0.26-0.35, P<0.0001) to breast cancer. The co-segregation of both the mutated alleles was observed only in controls.

Subjects with MTHFR 677CC/MTRR 66 AA (wild type) showed higher risk to breast cancer (OR: 12.3, 95% CI: 9.5-16.3, P<0.0001). The presence of MTRR mutated allele alone reduced the risk to cancer (OR: 0.3, 95% CI: 0.26-0.35, P<0.05), whereas the presence of both mutated alleles was detected only in the control group and both these associations were significant with respect to cancer susceptibility.

In the MTHFR1298/GCPII combined analysis, the presence of wild alleles at both the loci confers two-fold increase risk to breast cancer (OR: 2.08, 95% CI, 1.87- 2.34, P<0.2). The presence of GCPII variant allele alone is associated with reduced risk, whereas the presence of MTHFR1298 variant is associated with increased risk (OR 3.31, 95% CI, 2.88-3.84, P<0.05). Mutant alleles at both loci showed a significant reduced risk for cancer (OR 0.08, 95% CI -0.06-0.11, P< 0.0005). Logistic regression analysis showed a significant interaction between these two variant alleles (Table 4).

While MTHFR1298/MTRR wild alleles together confer 7.5 folds risk (95% CI: 5.94-9.68, P<0.005), the presence of both variant alleles confers significant protection (0.27 (0.23-0.31), P<0.05). MTRR variant allele alone was protective, whereas MTHFR 1298 variant allele was risky (Table 2). Logistic regression analysis showed that all bivariate interactions were statistically significant.

Three-way analysis:

We also analyzed by logistic regression the polymorphic combinations of the three genetic loci (MTHFR, MTRR and GCPII) for breast cancer risk (Table 5).

For MTHFR we considered only the C677T genotype. A combination of homozygous wild type genotypes at the three loci together showed increased risk (OR: 38, 95% CI: 23.58-66.23), where as the presence of variant alleles at MTHFR, GCPII and MTRR loci reduces the risk in a dose dependent manner.

Discussion

The breast cancer samples studied in the present study represent the ethnic population from the Kashmir valley in the northern part of India. While the Kashmir region is known for a very high incidence of esophageal cancer, there is a rise in the breast cancer incidence from this region [19-21]. The traditional Kashmiri salt tea brewed with sodium bicarbonate is a source of mutagenic nitrosamines, a well-known cause to high risk of cancer [22]. While processed red meat and the heterocyclic amines formed in cooking have been correlated to breast cancer, there are no reports on the association between salt

Table 5. Tri-variate interactions.

MTHFR 677	GCPII	MTRR	Cases	Controls	OR	P
C	C	G	7 (20%)	11 (33.3%)	0.5 (0.44-0.56)	0.27
T	C	G	0	4 (12.1%)	ND	0.05 B
C	T	G	0	4 (12.1%)	ND	0.05 B
C	C	A	19 (54.3%)	1 (3%)	38 (23.58-66.23)	<0.0001 H
T	T	G	0	5 (15.2%)	ND	0.023
T	C	A	6 (17.1%)	0	ND	0.025
C	T	A	3 (8.6%)	3 (9.1%)	0.94 (0.87-0.98)	1.0
T	T	A	0	5 (15.2%)	ND	0.023
Total			35	33		

P value for three-way interaction>0.05.

tea and mammary cancer [23]. In this pilot study, we found a strong correlation between the high consumption of salted tea intake with breast cancer. However, detailed analysis is needed to fully confirm the role of nitrates in salt tea for conferring breast cancer risk.

Epidemiological studies indicate an inverse correlation between dietary and blood folate levels to breast cancer risk and consequently the genes involved in folate and homocysteine play a crucial role in cancer progression. This is because folate pathways are intricately linked to DNA synthesis and methylation process. Of the many genes involved in the folate pathways, three genes viz. (MTHFR, GCPII and MTRR) play an important role in maintaining the levels of SAM. The polymorphic nature of the genes involved in the folate pathway can affect the functioning of the enzyme and hence the availability of folate leading to the instability of DNA. MTHFR is a crucial enzyme responsible for maintaining the balance between 5, 10-methylene tetrahydrofolate and 5-methyl tetrahydrofolate, which are substrates for thymidylate and methionine synthesis respectively. Lesser availability of 5, 10-methylene tetrahydrofolate leads to decreased thymidylate synthesis, increased misincorporation of uracil in DNA and results in disruption of DNA integrity [24, 25]. Lesser availability of 5-methyl tetrahydrofolate results in impaired remethylation of homocysteine to methionine. MTRR is responsible for the functional availability of cyanacobalamin for this remethylation step. MTRR mutation blocks this remethylation process resulting in methionine depletion and reduced synthesis of SAM. Hypomethylation as a result of low SAM may affect the expression of both oncogenes and tumor suppressor genes [26]. As MTRR mutation was reported to be associated with increased DNA damage [27], this can promote neoplastic transformation of normal cells. Most of the naturally occurring dietary folate occurs in form folylpoly-γ-glutamate, which is hydrolyzed by folylpoly-γ-glutamate carboxypeptidase encoded by GCP-II. Polymorphism in GCP-II leads to lower hydrolytic cleavage of the dietary folate leading to impaired absorption and low levels of serum folate. However, the reports on GCPII polymorphism and folate levels are inconsistent [11].

It has to be noted that most of the studies on association with gene polymorphism and cancer are limited to studying single candidate genes with very few reports on gene-gene interactions. Since there is a number of genes involved in 1-carbon folate metabolism pathway, confining studies to single gene polymorphisms for evaluating cancer risk susceptibility may be confounding. It has been suggested, at least for folate metabolizing genes, that risk for disease is much better assessed when multi-locus comparison are performed [10-28]. Since all the steps in the folate metabolic pathway are interconnected, the presence of different polymorphic variant enzymes will ultimately determine the availability of the folate which plays

an integral role in DNA repair, synthesis and methylation status and any alteration in these pathways would confer risk to cancer. Thus, it is clear that studying polymorphisms in genes involved in the folate pathway will help in assessing the risk/protection they confer for neoplastic development. Further, the ultimate outcome would be determined by the right combination of the various polymorphic variants.

The present pilot study, therefore, evaluates the susceptibility to breast cancer by analyzing individual and combined impact of polymorphisms in three genes viz. (GCPII, MTHFR and MTR) involved in the transfer of 1-carbon groups.

The present investigation, therefore, evaluates the susceptibility to breast cancer by analyzing individual and combined impact of polymorphisms in three genes and is suggestive of an epistatic interaction of MTHFR 677 T-variant allele with GCP II 1561T-variant allele and MTRR 66G-allele in the Kashmiri subjects. We observed an inverse association between breast cancer and the MTHFR 677 T, GCP II 1561T and MTRR 66G variant alleles, with a synergetic effect. Interestingly, MTHFR homozygous TT genotype is very rare at least in the south Indian population [10-29]. No statistically significant association was observed between breast cancer with MTHFR 1298 C-allele, although it appears to be weakly protective and to act additively with MTHFR 677 T-allele. The MTHFR 677T-variant allele was particularly protective among subjects with MTRR 66G- allele. The GCP II 1561T-variant allele was protective irrespective of the nature of MTRR allele. MTHFR 677T-variant allele acts additively along with the GCPII 1561T-variant allele in conferring protection against the MTRR 66G-variant allele. In the absence of MTHFR 677T and GCPII 1561T-variant alleles, MTRR 66A-allele was associated with high risk for breast cancer.

We found MTHFR is protective in conjunction with GCPII and MTRR mutated alleles. The protection by MTHFR variant allele (677T) could be due to the increased availability of 5,10-methylene tetrahydrofolate, which prevents uracil misincorporation by aiding in thymidylate synthesis. We report that combined MTHFR 677T and MTRR 66G confer protection against breast cancer, which is in agreement with [8], who reported similar protective effect for acute lymphobalstic leukemia.

Folate metabolism remains elusive and interestingly mice deficient for MTHFR exhibit tissue specific methylation capacity [30]. Therefore, it needs to be explored further if combined polymorphisms in genes involved in folate pathway confer differential susceptibility to different cancer types. There are controversial reports about the association of MTHFR with breast cancer with some showing lack of association, some showing increased risk and some showing decreased risk [31-35]. These discrepancies in the results could arise because of the multitude of factors such as differences in the allele frequencies due to ethnic variation, nutritional status, population size studied, etc. [36]. Studies on association of MTRR variants in cancer are limited, and the findings are not conclusive. MTRR is associated with the risk of colorectal cancer and squamous cell carcinoma [10-37], while no association is seen in ALL, non-Hodgkins lymphoma, gastric and breast cancer [38-40]. There is only one study where GCPII polymorphism does not alter the risk of colon cancer [13].

Conclusion

This pilot study suggests that MTHFR 677T-variant allele can decrease the susceptibility of breast cancer whether it is in conjunction with MTRR 66G-variant allele or GCP II 1561 T-variant allele. Further, we report a novel protective effect of GCPII variant allele, which has to be confirmed in a larger population. Although a small sample size remains a limitation to our study, nevertheless it underscores the importance of gene-gene interactions among folate pathway genes in modulating the susceptibility to breast cancer. As significant genetic complexities underlie the folate metabolic pathways, future studies have to be directed not only to identify genetic loci that interact epistatically, but also to study the gene-nutrient interactions to get a more precise risk assessment for cancer.

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