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13. ABSTRACT (Maximum 200 Words)

Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Diets high in vegetables and fruits have been associated with reduced risk of breast cancer in many epidemiologic studies. Minor components of diet such as micronutrients including vitamins may be involved in mediating these associations, but it is not know which micronutrients are involved or how they act. Such knowledge is critical to rationally design and to implement a preventive strategy against breast cancer. Folate, a B vitamin mostly found in vegetables and fruits, may be a protective micronutrient in diet. It is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer. Certain genes involved in these processes differ from one person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to use a multi-disciplinary approach to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer.

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INTRODUCTION

Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Low levels of folate and related B vitamins both in diet and in circulating have been associated with risk of breast cancer in several prospective epidemiologic studies. Folate, a B vitamin mostly found in vegetables and fruits, is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer. Certain genes involved in these processes differ from on person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to utilize resource of the Long Island Breast Cancer Project, a large population-based case-control study, to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer. The significance of this research lies in its potential not only to clarify etiology of breast cancer but also to guide the prevention of breast cancer through dietary modification. Furthermore, because a high proportion of the general population may inherit sub-optimal folate metabolism, attributable risk associated with these genetic factors may be quite significant. Associations between such susceptibility and risk of breast cancer will provide an extremely valuable guide to preventive dietary and other lifestyle modifications in individuals and in the population at large.

BODY

Task 1: To investigate the association of folate intake with risk of breast cancer in the Long Island Breast Cancer Study Project (Month 1-12).

We have computed dietary levels of B vitamins from the dietary questionnaire of the LIBSCP. These micronutrients, folate, vitamin B1, B2, B6, and Niacin, are highly correlated with Pearson Coefficient > 0.8. We have built logistic regression models to assess risk of breast cancer associated B vitamins. Our findings are summarized below:

A. Overall association of dietary B vitamins and risk of breast cancer (Table 1).

Overall, increased dietary intake of B vitamins was associated with reduced risk of breast cancer with ORs < 1; however, the reduction is very modest. For example, compared to those in the lowest quintile of dietary folate intake, individuals in the highest quintile of folate intake had a 12% reduction in breast cancer risk (OR 0.88, 95%CI 0.68-1.14). Similar relations were also observed with other B vitamins.

B. Associations of B vitamins and breast cancer stratified multivitamin use (Table 2).

The most interesting findings so far is that there is a significant dose-relationship between dietary B vitamin intake and breast cancer only among non-multivitamin users (p, trend = 0.01). Among non-multivitamin users, individuals with high dietary B vitamin intakes had significantly reduced risk of breast cancer compared to those with low intakes. The ORs (5^{th} vs. 1^{st} quintile) were 0.57 (95%CI 0.39-0.84), 0.62 (95%CI 0.42-0.90), 0.59 (95%CI 0.41-0.86), 0.65 (95%CI 0.39-0.94) for folate, niacin, B1 and B2, respectively. Multivitamin use was not associated with breast cancer risk (OR=0.96, 95%CI 0.82-1.13). Among the users (~50%), dietary folate was not related to breast cancer risk (p, trend = 0.90).

C. Associations of B vitamins and breast cancer stratified by menopausal status and tumor characteristics (Table 3a, b).
We examined whether the associations of B vitamins and breast cancer were different with menopausal status (pre- vs. post-) and tumor characteristics (invasive vs. *in situ*) by stratified analyses. It appears that these associations were slightly stronger for post-menopausal and invasive breast cancer as shown in the test for trend. But no significant effect modification was reported.

D. Interaction of alcohol consumption and folate intake in relation to breast cancer (Figure 1).

Interactions of alcohol and folate intake in relation to breast cancer has been observed in three large cohort studies, i.e. the Nureses' Health Study, the Canadian National Breast Screen Study and the Iowa Women's Health Study. We observed the significant association between folate intake and breast cancer only among non-alcohol users (p, trend = 0.04). Compared the non-alcohol users, high folate intake (5^{th} quintile) was associated with 34% reduction in risk (OR 0.66, 95%CI 0.49-0.90).

Task 2: To genotype three genetic polymorphisms in folate-metabolizing genes in 1087 breast cancer cases and 1122 population-based controls of the Long Island Breast Cancer Study Project (Month 7-40).

A. DNA isolation

DNA isolation has been completed for all 1087 cases and 1122 controls. Aliquots have been make and sent to the laboratory of Dr. Jia Chen at Mount Sinai School of Medicine, where genotyping is carried out.

B. MTHFR polymorphisms (Table 4).

Genotyping for MTHFR 677C->T polymorphism has been completed and results are summarized in Table 4. The genotype distribution of the control population of the LIBCSP was 47% CC, 39%CT and 14%TT, similar to other US populations. The prevalence of TT genotype is 17% in cases, slightly higher than controls with OR of 1.37 (95%CI 1.02-1.77) (Table 4a). The increased risk was more pronounced in women who are pre-menopausal (Table 4), use multivitamin (Table 4c), drinks alcohol (Table 4d).

We also observed a significant interaction between MTHFR polymorphism and total folate intake (p, interaction = 0.03) (Figure 2). Individuals with TT genotype and low folate intake had a 69% increase in breast cancer risk (OR 1.69, 95%CI 1.07 – 2.66). A significant interaction with alcohol (p, interaction = 0.03) was also apparent; individuals who consume alcohol and have TT genotype had 53% increase in breast cancer risk (OR 1.53, 95%CI 1.05 – 2.23)

In addition, We have also genotyped MTHFR 1298A-> C polymorphisms on 442 individuals.

C. TS polymorphism.

A new folate metabolizing gene, thymidylate synthase (TS), has generated attention recently. A tandem repeat polymorphism has been identified in the enhancer region of the TS promoter which contains either triple (TS*3R) or double (TS*2R) repeats of a 28 bp sequence. Individuals homozygous for triple repeats (TS 3R/3R) have 3.6 times higher TS mRNA levels compared to those homozygous for the double repeat (TS 2R/2R) genotype. In a separate study, our laboratory discovered that the TS polymorphism modifies colon cancer risk and survival, as well as plasma folate levels[1].

We decided to investigate whether the TS polymorphism is associated with breast cancer risk in the LIBSCP. We have finished 1200 samples and results are reported in Table 5. The 2R/2R genotype confers a non-significantly increased risk of breast cancer (OR 1.22, 95%CI 0.96 – 1.55). Such association was not modified by menopausal status, multivitamin use, alcohol intake, and tumor characteristics. There appear to be a gene-gene interactions between MTHFR and TS (p, interaction = 0.04).

D. MTRR polymorphism

We have finished genotyping MTRR polymorphisms. We are in the process of analyzing the results.

Task 3: Final analyses and report writing (Month 36-48).

a. Analyze the associations of genetic polymorphisms in folate-metabolizing genes and risk of breast cancer.
b. Assess gene-environment interactions in relation to risk of breast cancer.
c. Investigate possible interactions of folate and alcohol consumption in ration to risk of breast cancer.
d. Prepare final report and manuscripts.

Please see above. We are in the process of manuscript preparation.

KEY RESEARCH ACCOMPLISHMENTS

1. We have performed crude analyses on the dietary B vitamin intake in the LIBCSP.

2. We have isolated DNA from all individuals (1087 cases and 1122 controls).
3. We have finished genotyping on the MTHFR, TS and MTRR polymorphisms.
4. We have set up assays for a new folate-metabolizing gene polymorphism, the TS polymorphism.

REPORTABLE OUTCOMES

We have presented the results of this study at the following meeting:

Chen J, Gammon MD, Chan W, Palomeque C, Kabat GC, Terry MB, Teitelbaum SL, Britton JA, Neugut AI, Santella RM. Folate Metabolism Modifies the Risk of Breast Cancer in the Long Island Breast Cancer Study Project. 91st Annual Meeting of American Association for Cancer Research, Washington DC, 2003.

CONCLUSIONS

- Dietary intake of B vitamins are associated with very modestly reduced risk of breast cancer with OR<1 in general.
- Among non-multivitamin users, dietary intakes of B vitamins are protective against breast cancer in dose-dependent fashion.
- There is a significant association between dietary folate intake and breast cancer risk only among non-alcohol drinkers.
- Genetic polymorphisms in folate metabolism may influence breast cancer risk.

REFERENCES

1. Chen, J., Hunter, D. J., Stampfer, M. J., Kyte, C., Chan, W., Wetmur, J. G., Selhub, J., and Ma, J. A prospective study on relations of a polymorphism in the Thymidylate Synthase Promoter Enhancer Region and the risk and survival of colorectal cancer. Cancer Epidemiol Biomarkers Prev . 2003. Ref Type: In Press

APPENDICES

None

Table 1. Association of daily intake of B vitamins with risk of breast cancer: adjusted OR and 95% CI by quintile or quartile of intake

Nutrient Type	Q1 (lowest intake)	Q2	Q3	Q4	Q5	P for Trend
Folate						
Cases/Controls	330/305	288/304	316/305	273/304	272/304	
OR (95% CI)*	1.00 (Referent)	0.91 (0.70-1.17)	1.00 (0.78-1.29)	0.85 (0.66-1.10)	0.88 (0.68-1.14)	0.20
Vitamin B_1						
Cases/Controls	357/317	311/298	**273/300**	277/309	261/298	
OR (95% CI)*	1.00 (Referent)	0.97 (0.76-1.25)	**0.77 (0.59-0.99)**	0.84 (0.65-1.08)	0.88 (0.68-1.13)	0.10
Vitamin B_2 (Riboflavin)						
Cases/Controls	341/305	312/311	275/298	276/304	275/304	
OR (95% CI)*	1.00 (Referent)	0.91 (0.71-1.17)	0.81 (0.62-1.05)	0.84 (0.65-1.08)	0.89 (0.69-1.14)	0.19
Vitamin B_6						
Cases/Controls	323/310	329/304	279/302	286/306	262/300	
OR (95% CI)*	1.00 (Referent)	1.08 (0.84-1.39)	0.92 (0.71-1.19)	0.94 (0.73-1.21)	0.92 (0.71-1.20)	0.22
Supplemental B_{12}						
Cases/Controls	746/751	40/49	467/468	226/254	--	
OR (95% CI)*	1.00 (Referent)	1.12 (0.70-1.81)	0.96 (0.80-1.16)	0.94 (0.75-1.19)	--	0.51
Niacin						
Cases/Controls	351/305	303/306	264/303	292/304	269/304	
OR (95% CI)*	1.00 (Referent)	0.88 (0.68-1.12)	0.79 (0.61-1.02)	0.90 (0.70-1.16)	0.84(0.65-1.09)	0.18
Total Folate						
Cases/Controls	266/256	256/251	228/251	238/249	240/254	
OR (95% CI)*	1.00 (Referent)	0.98 (0.76-1.26)	0.87 (0.67-1.12)	0.90 (0.69-1.16)	0.90 (0.70-1.17)	0.25

* Adjusted for family history of breast cancer in first-degree relative (yes or no), history of benign breast disease (yes or no), lactation (ever or never), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

Table 2. Association of daily intake of B vitamins with risk of breast cancer: adjusted OR and 95% CI by quintile or quartile of intake, supplemental folate status

Nutrient Type	Q1 (low)	Q2	Q3	Q4	Q5	P for Trend
Folate			*Supplemental Folate = No*			
Cases/Controls	161/138	122/129	139/126	109/114	82/115	
OR (95% CI)*	1.00 (ref)	0.76 (0.53-1.08)	0.88 (0.62-1.24)	0.79 (0.55-1.14)	**0.57 (0.39-0.84)**	**0.01**
			Supplemental Folate = Yes			
Cases/Controls	109/116	120/122	122/125	114/135	150/141	
OR (95% CI)*	1.00 (ref)	1.00 (0.68 -1.45)	1.06 (0.73-1.54)	0.86 (0.59-1.26)	1.09 (0.76-1.56)	0.90
Niacin			*Supplemental Folate = No*			
Cases/Controls	161/119	135/136	99/116	123/131	95/120	
OR (95% CI)*	1.00 (ref)	0.73 (0.51-1.04)	**0.66 (0.46-0.97)**	**0.67 (0.46-0.96)**	**0.62 (0.42-0.90)**	**0.01**
			Supplemental Folate = Yes			
Cases/Controls	128/132	124/123	114/126	123/120	126/138	
OR (95% CI)*	1.00 (ref)	1.03 (0.72-1.48)	0.95 (0.66-1.37)	1.07 (0.74-1.54)	1.01 (0.71-1.45)	0.90
Vitamin B1			*Supplemental Folate = No*			
Cases/Controls	168/134	143/122	98/120	115/127	89/119	
OR (95% CI)*	1.00 (ref)	0.91 (0.65-1.29)	**0.61 (0.42-0.88)**	0.72 (0.51-1.03)	**0.59 (0.41-0.86)**	**0.002**
			Supplemental Folate = Yes			
Cases/Controls	131/132	122/122	111/126	115/128	136/131	
OR (95% CI)*	1.00 (ref)	1.02 (0.71-1.46)	0.90 (0.62-1.29)	0.89 (0.62-1.28)	1.09 (0.77-1.56)	0.90
Vitamin B2 (Riboflavin)			*Supplemental Folate = No*			
Cases/Controls	161/132	145/131	110/114	96/122	101/123	
OR (95% CI)*	1.00 (ref)	0.83 (0.59-1.18)	0.71 (0.49-1.03)	**0.62 (0.43-0.90)**	**0.65 (0.45-0.94)**	**0.01**
			Supplemental Folate = Yes			
Cases/Controls	121/118	121/130	108/128	134/131	131/132	
OR (95% CI)*	1.00 (ref)	0.89 (0.62-1.29)	0.81 (0.55-1.17)	1.00 (0.70-1.44)	0.99 (0.69-1.43)	0.78

*Odds ratios and 95% CI adjusted for age, education, income, age at menarche, age at first birth, family history of breast cancer, history of benign breast disease (yes or no), lactation (ever or never) and bmi at age 20.

Table 3 Association of daily intake of B vitamins with risk of breast cancer: adjusted OR and 95% CI by quintile or quartile of intake by menopausal status

Nutrient Type	Q1 (low)	Q2	Q3	Q4	Q5	P for Trend
Total Folate			*Pre-menopausal*			
Cases/Controls	90/100	86/86	80/90	75/61	73/95	
OR (95% CI)*	1.00 (ref)	1.06 (0.68-1.65)	1.00 (0.64-1.55)	1.35 (0.84-2.16)	0.84 (0.53-1.31)	0.76
			Post-menopausal			
Cases/Controls	165/144	169/158	144/149	159/175	161/149	
OR (95% CI)*	1.00 (ref)	0.98 (0.71-1.35)	0.87 (0.62-1.21)	0.81 (0.58-1.11)	0.97 (0.69-1.34)	0.47
Folate			*Pre-menopausal*			
Cases/Controls	77/88	84/95	94/79	69/79	80/92	
OR (95% CI)*	1.00 (ref)	0.99 (0.63-1.55)	1.35 (0.85-2.13)	1.10 (0.69-1.76)	0.97 (0.61-1.53)	0.94
			Post-menopausal			
Cases/Controls	182/155	153/145	161/161	154/159	148/155.	
OR (95% CI)*	1.00 (ref)	0.84 (0.61-1.16)	0.84 (0.61-1.15)	0.81 (0.59-1.11)	0.79 (0.58-1.10)	0.17
Niacin			*Pre-menopausal*			
Cases/Controls	64/72	78/79	71/86	99/86	92/110	
OR (95% CI)*	1.00 (ref)	1.05 (0.64-1.70)	0.88 (0.54-1.43)	1.33 (0.83-2.13)	1.06 (0.67-1.69)	0.51
			Post-menopausal			
Cases/Controls	217/170	173/168	137/146	145/154	126/137	
OR (95% CI)*	1.00 (ref)	0.77 (0.57-1.05)	0.76 (0.55-1.04)	**0.73 (0.53-1.00)**	0.77 (0.55-1.06)	0.09
Vitamin B1			*Pre-menopausal*			
Cases/Controls	81/90	82/76	74/79	81/78	86/110	
OR (95% CI)*	1.00 (ref)	1.04 (0.66-1.65)	0.97 (0.61-1.54)	1.19 (0.76-1.89)	0.88 (0.57-1.36)	0.74
			Post-menopausal			
Cases/Controls	207/164	173/159	134/158	149/165	135/129	
OR (95% CI)*	1.00 (ref)	0.89 (0.65-1.21)	**0.67 (0.45-0.91)**	**0.73 (0.53-1.00)**	**0.87 (0.62-1.20)**	0.13
Vitamin B2 (Riboflavin)			*Pre-menopausal*			
Cases/Controls	75/86	85/86	68/72	89/84	87/105	
OR (95% CI)*	1.00 (ref)	0.99 (0.63-1.55)	1.07 (0.66-1.73)	1.30 (0.83-2.05)	0.98 (0.62-1.52)	0.70
			Post-menopausal			
Cases/Controls	202/156	172/163	144/160	138/160	142/136	
OR (95% CI)*	1.00 (ref)	0.82 (0.60-1.12)	**0.68 (0.46-0.94)**	**0.67 (0.49-0.93)**	0.84 (0.60-1.16)	0.12
Supplemental B12			*Pre-menopausal*			
Cases/Controls	212/219	17/19	116/122	59/73	0/0	
OR (95% CI)*	1.00 (ref)	0.84 (0.41-1.74)	0.94 (0.67-1.31)	0.79 (0.52-1.20)	--	0.32
			Post-menopausal			
Cases/Controls	388/377	22/17	258/258	130/123	0/0	
OR (95% CI)*	1.00 (ref)	1.33 (0.68-2.59)	0.98 (0.77-1.23)	1.01 (0.75-1.36)	--	0.93

*Odds ratios and 95% CI adjusted for age, education, income, age at menarche, age at first birth, family history of breast cancer, history of benign breast disease (yes or no), lactation (ever or never) and bmi at age 20.

Table 3b. Association of daily intake of B vitamins with risk of breast cancer: adjusted OR and 95% CI by quintile or quartile of intake, by breast cancer stage

Nutrient Type	Q1 (low)	Q2	Q3	Q4	Q5	P for Trend
Total Folate			*Invasive*			
Cases/Controls	232/256	216/251	190/251	200/249	202/254	
OR (95% CI)*	1.00 (ref)	0.93 (0.71-1.21)	0.83 (0.63-1.08)	0.85 (0.65-1.12)	0.86 (0.66-1.13)	0.21
			In situ			
Cases/Controls	34/256	40/251	38/251	38/249	38/254	
OR (95% CI)*	1.00 (ref)	1.25 (0.75-2.08)	1.13 (0.68-1.90)	1.12 (0.67-1.88)	1.09 (0.65-1.82)	0.93
Folate			*Invasive*			
Cases/Controls	232/254	208/251	215/251	193/249	192/256	
OR (95% CI)*	1.00 (ref)	0.89 (0.68-1.16)	0.96 (0.74-1.25)	0.86 (0.65-1.12)	0.84 (0.64-1.11)	0.22
			In situ			
Cases/Controls	38/254	34/251	46/251	30/249	40/256	
OR (95% CI)*	1.00 (ref)	0.93 (0.56-1.56)	1.21 (0.74-1.96)	0.81 (0.48-1.37)	1.04 (0.63-1.71)	0.94
Niacin			*Invasive*			
Cases/Controls	244/251	221/259	181/242	209/251	185/258	
OR (95% CI)*	1.00 (ref)	0.89 (0.69-1.16)	0.81 (0.62-1.06)	0.92 (0.71-1.21)	0.85 (0.65-1.11)	0.32
			In situ			
Cases/Controls	45/251	38/259	32/242	37/251	36/258	
OR (95% CI)*	1.00 (ref)	0.74 (0.45-1.21)	0.70 (0.42-1.16)	0.86 (0.52-1.41)	0.81 (0.49-1.32)	0.56
Vitamin B1			*Invasive*			
Cases/Controls	264/266	210/244	180/246	195/255	191/250	
OR (95% CI)*	1.00 (ref)	0.87 (0.67-1.13)	**0.75 (0.58-0.98)**	0.81 (0.63-1.06)	0.85 (0.65-1.11)	0.16
			In situ			
Cases/Controls	35/266	55/244	29/246	35/255	34/250	
OR (95% CI)*	1.00 (ref)	1.71 (1.06-2.76)	0.84 (0.49-1.45)	1.11 (0.66-1.85)	1.08 (0.64-1.83)	0.53
Vitamin B2 (Riboflavin)			*Invasive*			
Cases/Controls	246/250	221/261	191/242	190/253	192/255	
OR (95% CI)*	1.00 (ref)	0.87 (0.67-1.13)	0.81 (0.62-1.05)	0.81 (0.62-1.05)	0.84 (0.64-1.10)	0.52
			In situ			
Cases/Controls	36/250	45/261	27/242	40/253	40/255	
OR (95% CI)*	1.00 (ref)	1.24 (0.76-2.03)	0.82 (0.47-1.41)	1.22 (0.73-2.02)	1.23 (0.74-2.04)	0.16

*Odds ratios and 95% CI adjusted for age, education, income, age at menarche, age at first birth, family history of breast cancer, history of benign breast disease (yes or no), lactation (ever or never) and bmi at age 20.

Table 4. **MTHFR1 genotype and risk of breast cancer**

A. OVERALL

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)	P(trend)
Crude	677CC	390 (37.3)	435 (40.0)	1.00 (referent)	**0.03**
	677CT	468 (44.7)	500 (46.0)	1.04 (0.86-1.25)	
	677TT	188 (18.0)	153 (14.1)	**1.37 (1.06-1.77)**	
Adjusted*	677CC	327 (36.8)	372 (39.8)	1.00 (referent)	**0.04**
	677CT	402 (45.2)	430 (46.0)	1.07 (0.87 - 1.31)	
	677TT	160 (18.0)	129 (14.1)	**1.36 (1.02 - 1.81)**	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

B. BY MENOPAUSAL STATUS

Post-menopausal only

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)	P(trend)
Crude	677CC	262 (38.2)	264 (39.1)	1.00 (referent)	0.33
	677CT	304 (44.3)	313 (46.3)	0.98 (0.78-1.24)	
	677TT	120 (17.5)	99 (14.6)	1.22 (0.89-1.68)	
Adjusted*	677CC	214 (37.9)	218 (39.1)	1.00 (referent)	0.45
	677CT	250 (44.3)	257 (46.1)	0.99 (0.76-1.29)	
	677TT	100 (17.7)	83 (14.9)	1.19 (0.83-1.71)	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

Pre-menopausal only

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)	P(trend)
Crude	677CC	123 (36.7)	146 (39.7)	1.00 (referent)	0.09
	677CT	149 (44.5)	175 (47.6)	1.01 (0.73-1.40)	
	677TT	63 (18.8)	47 (12.8)	**1.59 (1.02-2.49)**	
Adjusted*	677CC	108 (35.8)	131 (39.2)	1.00 (referent)	**0.04**
	677CT	139 (46.0)	160 (47.9)	1.13 (0.78-1.64)	
	677TT	55 (18.2)	43 (12.9)	**1.79 (1.07 - 2.99)**	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

C. BY SUPPLEMENT USE

Supplement use= Yes

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude	677CC	216 (40.3)	222 (38.5)	1.00 (referent)	0.65
	677CT	221 (41.2)	270 (46.8)	0.84 (0.65-1.09)	
	677TT	99 (18.5)	85 (14.7)	1.20 (0.85-1.69)	
Adjusted*	677CC	184 (40.1)	191 (38.1)	1.00 (referent)	0.73
	677CT	190 (41.4)	233 (46.5)	1.19 (0.81-1.75)	
	677TT	85 (18.5)	77 (15.4)	0.83 (0.62-1.11)	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

Supplement use= No

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude	677CC	174 (34.1)	213 (41.7)	1.00 (referent)	**0.007**
	677CT	247 (48.4)	230 (45.0)	**1.32 (1.01-1.72)**	
	677TT	89 (17.5)	68 (13.3)	**1.60 (1.10-2.33)**	
Adjusted*	677CC	143 (33.3)	181 (41.8)	1.00 (referent)	**0.009**
	677CT	212 (49.3)	196 (45.3)	**1.47 (1.07-2.02)**	
	677TT	75 (17.4)	56 (12.9)	**1.65 (1.06-2.56)**	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

D. ALCOHOL INTAKE

Alcohol = Yes

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude	677CC	179 (35.1)	236 (41.9)	1.00 (referent)	**0.0004**
	677CT	227 (44.5)	260 (46.2)	1.15 (0.88-1.50)	
	677TT	104 (20.4)	67 (11.9)	**2.05 (1.42-2.94)**	
Adjusted*	677CC	153 (34.8)	201 (41.2)	1.00 (referent)	**0.002**
	677CT	196 (44.6)	227 (46.5)	1.16 (0.86-1.57)	
	677TT	91 (20.7)	60 (12.3)	**2.02 (1.34-3.05)**	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

Alcohol = No

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude	677CC	211 (39.4)	199 (37.9)	1.00 (referent)	0.62
	677CT	241 (45.0)	240 (45.7)	0.95 (0.73-1.23)	
	677TT	84 (15.7)	86 (16.4)	0.92 (0.64-1.32)	
Adjusted*	677CC	173 (38.6)	171 (38.3)	1.00 (referent)	0.71
	677CT	206 (46.0)	202 (45.3)	0.96 (0.71-1.30)	
	677TT	69 (15.4)	73 (16.4)	0.93 (0.61-1.41)	

* Adjusted for family history of breast cancer in first-degree relative (yes or no), menopausal status (pre or post), income (8 levels), educational attainment (5 levels) and quintiles of age, age at first birth, and age at menarche

E. BREAST CANCER TYPE

LIBCSP STUDY

Table 3A. TSTR genotype and risk of breast cancer

5

A. OVERALL

	Genotype	No. cases (%)	No. controls (%)	Odds Ratio (95% CI)	P(trend)
Crude*	3R/3R	311 (29.5)	356 (32.4)	1.00 (referent)	0.07
	3R/2R	512 (48.6)	532 (48.4)	1.09 (0.90 - 1.33)	
	2R/2R	230 (21.8)	211 (19.2)	1.22 (0.96 - 1.55)	
Adjusted**	3R/3R	294 (29.4)	340 (32.1)	1.00 (referent)	0.10
	3R/2R	482 (48.2)	512 (48.4)	1.08 (0.88 - 1.32)	
	2R/2R	224 (22.4)	206 (19.5)	1.22 (0.95 - 1.57)	

B. BY MENOPAUSAL STATUS

Post-menopausal only

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)	P(trend)
Crude*	3R/3R	199 (28.9)	216 (31.6)	1.00 (referent)	0.13
	3R/2R	338 (49.1)	337 (49.3)	1.09 (0.85-1.39)	
	2R/2R	152 (22.1)	130 (19.0)	1.22 (0.89-1.65)	
Adjusted**	3R/3R	186 (28.8)	206 (31.4)	1.00 (referent)	0.20
	3R/2R	313 (48.5)	322 (49.1)	1.07 (0.83-1.39)	
	2R/2R	147 (22.8)	128 (19.5)	1.22 (0.89-1.67)	

Pre-menopausal only

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	
Crude*	3R/3R	107 (31.4)	121 (32.6)	1.00 (referent)	0.54
	3R/2R	160 (46.9)	177 (47.7)	1.01 (0.72-1.42)	
	2R/2R	74 (21.7)	73 (19.7)	1.14 (0.75-1.73)	
Adjusted**	3R/3R	103 (50.0)	116 (53.0)	1.00 (referent)	0.57
	3R/2R	154 (47.4)	171 (52.6)	1.02 (0.71-1.46)	
	2R/2R	72 (50.0)	72 (50.0)	1.15 (0.74-1.79)	

* Adjusted for age

** Adjusted for family history of breast cancer in first-degree relative (yes or no), history of

LIBCSP STUDY
Table 5. TSTR genotype and risk of breast cancer

C. BY SUPPLEMENT USE

Supplement use= Yes

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude*	3R/3R	149 (29.4)	163 (32.1)	1.00 (referent)	0.21
	3R/2R	250 (49.3)	253 (49.8)	1.07 (0.80-1.42)	
	2R/2R	108 (21.3)	92 (18.1)	1.25 (0.87-1.79)	
Adjusted**	3R/3R	152 (29.5)	179 (32.0)	1.00 (referent)	0.24
	3R/2R	244 (47.4)	266 (47.5)	1.09 (0.82-1.45)	
	2R/2R	119 (23.1)	115 (20.5)	1.22 (0.86-1.73)	

Supplement use= No

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	
Crude*	3R/3R	156 (29.4)	186 (32.3)	1.00 (referent)	0.18
	3R/2R	254 (47.9)	274 (47.6)	1.09 (0.83-1.44)	
	2R/2R	120 (22.6)	116 (20.1)	1.21 (0.87-1.70)	
Adjusted**	3R/3R	142 (53.3)	161 (53.1)	1.00 (referent)	0.20
	3R/2R	236 (49.1)	245 (50.9)	1.07 (0.79-1.44)	
	2R/2R	104 (53.3)	91 (46.7)	1.28 (0.88-1.86)	

D. ALCOHOL INTAKE

Alcohol = Yes

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude*	3R/3R	152 (30.0)	174 (31.1)	1.00 (referent)	0.24
	3R/2R	243 (48.0)	286 (51.1)	0.96 (0.72-1.26)	
	2R/2R	111 (21.9)	100 (17.9)	1.24 (0.87-1.77)	
Adjusted**	3R/3R	147 (29.9)	168 (30.8)	1.00 (referent)	0.33
	3R/2R	234 (47.7)	280 (51.3)	0.92 (0.69-1.23)	
	2R/2R	110 (22.4)	98 (17.9)	1.22 (0.85-1.76)	

Alcohol = No

	Genotype				
Crude*	3R/3R	153 (28.8)	175 (33.4)	1.00 (referent)	0.17
	3R/2R	261 (49.2)	241 (46.0)	1.24 (0.93-1.64)	
	2R/2R	117 (22.0)	108 (20.6)	1.22 (0.86-1.72)	
Adjusted**	3R/3R	147 (51.1)	172 (53.9)	1.00 (referent)	0.15
	3R/2R	246 (51.6)	231 (48.4)	1.27 (0.95-1.70)	
	2R/2R	113 (51.1)	108 (48.9)	1.26 (0.88-1.79)	

* Adjusted for age

** Adjusted for family history of breast cancer in first-degree relative (yes or no), history of

LIBCSP STUDY

Table 3. TSTR genotype and risk of breast cancer

E. BREAST CANCER TYPE

In situ

	Genotype	cases (%)	controls (%)	Odds Ratio (95% CI)*	P(trend)
Crude*	3R/3R	55 (29.9)	356 (32.4)	1.00 (referent)	0.28
	3R/2R	87 (47.3)	532 (48.4)	1.06 (0.74 - 1.53)	
	2R/2R	42 (22.8)	211 (19.2)	1.35 (0.87 - 2.09)	
Adjusted**	3R/3R	51 (29.5)	340 (32.2)	1.00 (referent)	0.25
	3R/2R	81 (46.8)	511 (48.3)	1.03 (0.70 - 1.51)	
	2R/2R	41 (23.7)	206 (19.5)	1.34 (0.84 - 2.13)	

Invasive

	Genotype				
Crude*	3R/3R	256 (29.5)	356 (32.4)	1.00 (referent)	0.10
	3R/2R	425 (48.9)	532 (48.4)	1.10 (0.90 - 1.36)	
	2R/2R	188 (21.6)	211 (19.2)	1.20 (0.93 - 1.56)	
Adjusted**	3R/3R	243 (29.4)	340 (32.1)	1.00 (referent)	0.17
	3R/2R	399 (48.4)	511 (48.3)	1.09 (0.88 - 1.35)	
	2R/2R	182 (22.1)	206 (19.5)	1.20 (0.92 - 1.57)	

* Adjusted for age

** Adjusted for family history of breast cancer in first-degree relative (yes or no), history of

Fig. **1. Dietary Folate and Alcohol Interactions: No ETOH intake vs. any ETOH intake**

Alcohol Intake	Dietary Folate levels	OR	Lower limit 95% CI	Upper limit 95% CI	Comment
None	< 159.2	1.00	--	--	1
None	159.2 - 215.8	0.79	0.58	1.08	2
None	215.84 - 279	0.94	0.70	1.27	3
None	279.03 - 356	0.75	0.55	1.03	4
None	**>=356.22**	**0.66**	**0.49**	**0.90**	5
Yes	< 159.2	0.78	0.57	1.06	1
Yes	159.2 - 215.8	0.77	0.57	1.05	2
Yes	215.84 - 279	0.77	0.56	1.05	3
Yes	279.03 - 356	0.73	0.54	1.00	4
Yes	>=356.22	0.83	0.61	1.14	5

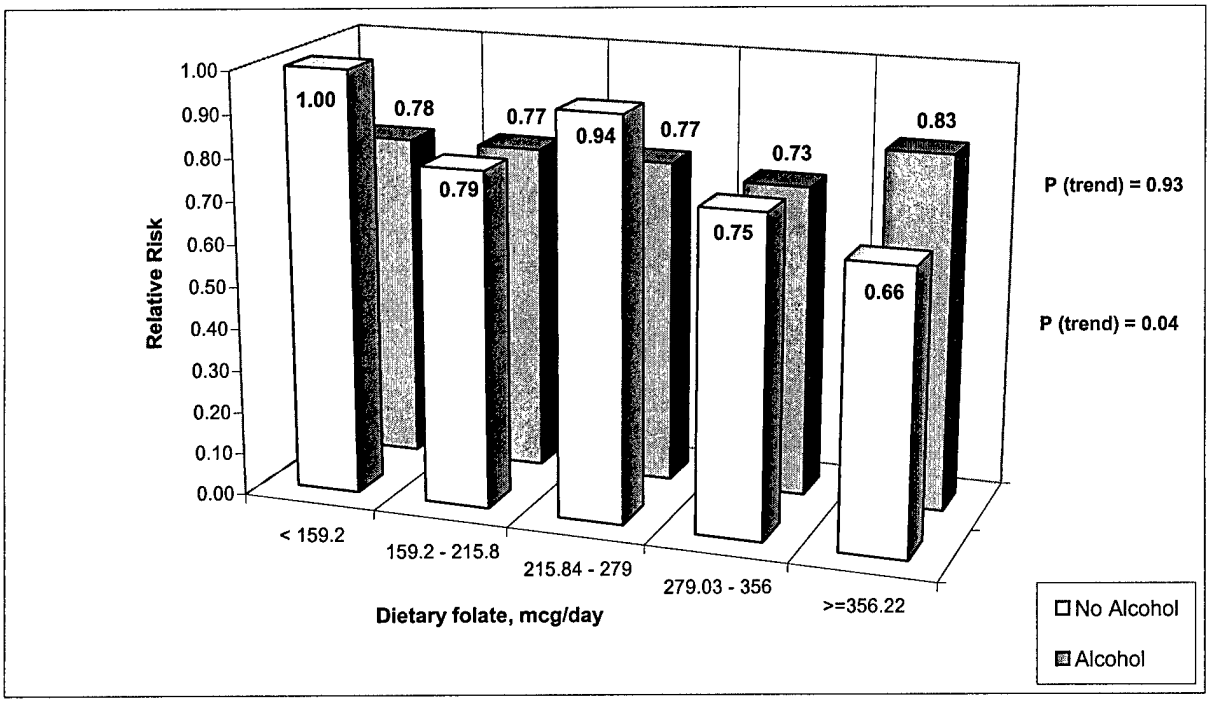

Fig 2. **MTHFR and total folate from diet and supplements (mcg/day) by tertile**

Total Folate (Categorized)	Total Folate Range (mcg/day)	MTHFR	N[a]	OR	Lower 95% CI	Upper 95% CI	Comment
high	606-6259	wt	284	1.00	--	--	
high	606-6259	het	314	0.94	0.68	1.31	
high	606-6259	var	126	1.03	0.67	1.58	
med	284-606	wt	262	0.81	0.57	1.14	
med	284-606	het	303	0.91	0.65	1.27	
med	284-606	var	96	1.30	0.80	2.08	
low	0-283	wt	252	0.98	0.69	1.39	
low	0-283	het	325	1.09	0.79	1.51	
low	0-283	var	112	**1.69**	**1.07**	**2.66**	

[a]Excludes obs with missing adjusted variables that were entered into the model (famhx, age, age at first birth, bmi at 20 yo, edu, hx of benign breast disease, age at menopause, lactation hx)

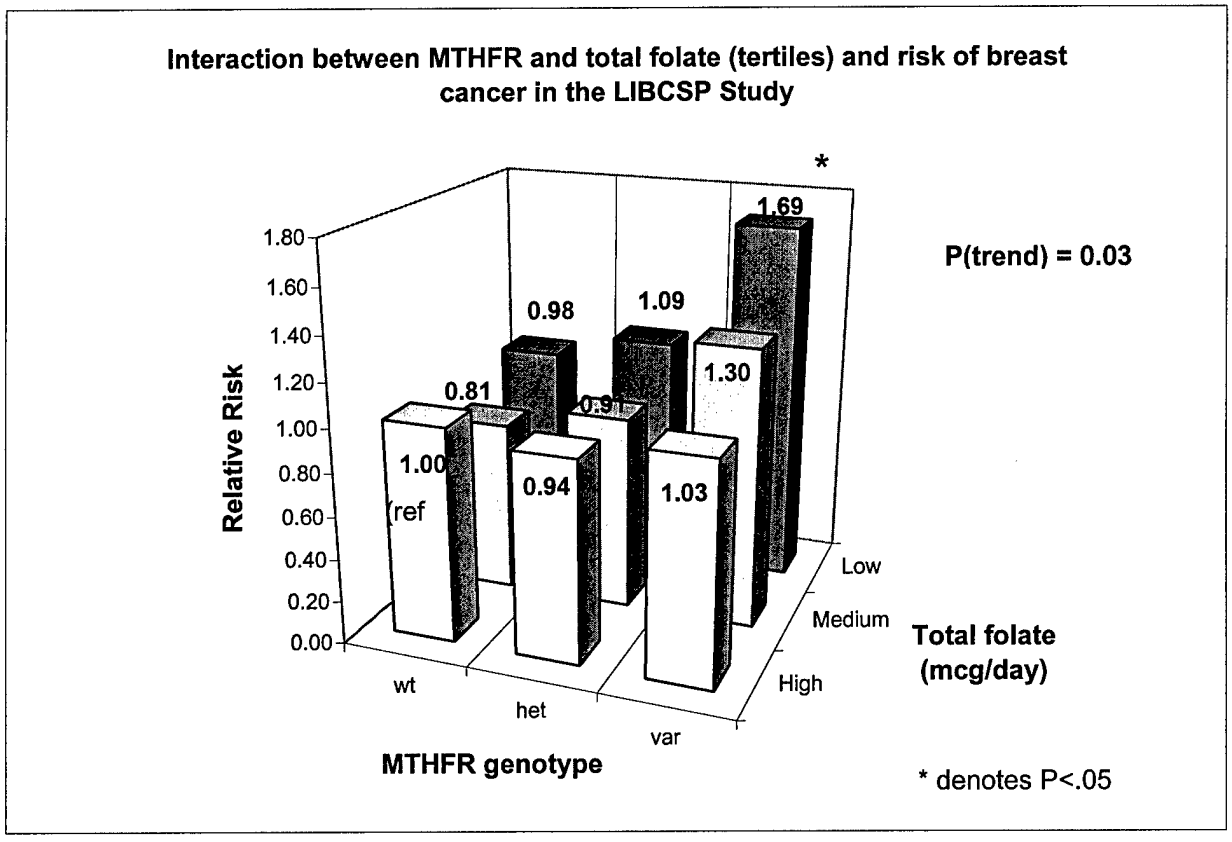

Fig **3. MTHFR and alcohol intake**

Alcohol (Categorized)	Alcohol Range (mcg/day)	MTHFR	N[a]	OR	Lower 95% CI	Upper 95% CI	Comment
None	0	wt		1.00	--	--	
None	0	het		0.96	0.73	1.27	
None	0	var		0.97	0.67	1.40	
Any	> 0	wt		0.75	0.57	1.01	
Any	> 0	het		0.88	0.67	1.16	
Any	> 0	var		**1.53**	**1.05**	**2.23**	

[a]Excludes obs with missing adjusted variables that were entered into the model (famhx, age, age at first birth, bmi at 20 yo, edu, hx of benign breast disease, age at menopause, lactation hx)

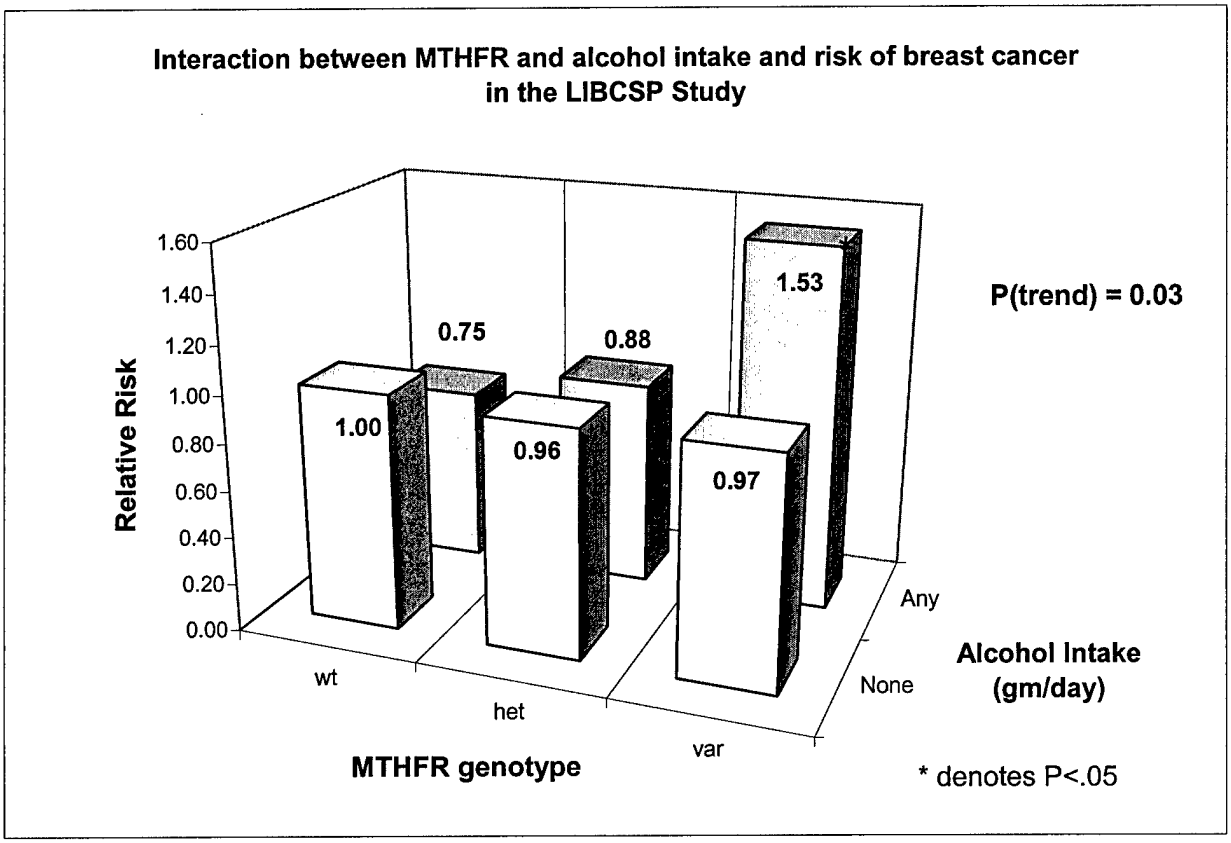

Fig 4

LIBCSP STUDY

Table .

Interaction between MTHFR and TS and variables categorized by tertile and risk of breast cancer

1. MTHFR1 and TSTR by tertile and risk of breast cancer

MTHFR	TSTR	N[a]	OR	Lower 95% CI	Upper 95% CI	Dietary Folate (mcg/day)	Comment
wt	3R/3R	261	1.00	--	--	284.19	1
het	3R/3R	277	1.17	0.82	1.65	267.11	2
var	**3R/3R**	**96**	**2.24**	**1.38**	**3.64**	263.15	3
wt	3R/2R	372	1.26	0.91	1.75	269.91	4
het	3R/2R	463	1.24	0.91	1.70	258.74	5
var	**3R/2R**	**156**	**1.64**	**1.09**	**2.46**	261.51	6
wt	**2R/2R**	**156**	**1.50**	**1.00**	**2.25**	255.47	7
het	**2R/2R**	**194**	**1.54**	**1.05**	**2.26**	268.65	8
var	2R/2R	79	1.39	0.83	2.33	266.01	9