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Methylenetetra Hydrofolate Reductase (MTHFR) Gene Polymorphisms (C677T&A1298C) and Risk of Breast Cancer Among Egyptian Women

KEYWORDS

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ABSTRACT *Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme in the metabolism of folatea nutrient which has recently been found to be inversely related to breast cancer. Two common variants in the MTHFR gene (C677T and A1298C) have been associated with a reduced activity of this enzyme, thereby increasing the availability of folate for thymidylate and purine synthesis. We investigated the relationship of these variants with invasive breast cancer in a case-control study of 100 cases and 90 controls within this Study. We found an overall significant, inverse association between breast cancer risk and the 677TT genotype and no association with the 1298C variant. The odds ratio [OR and 95% confidence interval (95% CI)] for the 677CC, 677CT, and 677TT genotypes were 1.00, 1.14 (0.6-2.08), and 5.5(1.5-20.3), respectively. Those for the 1298AA, 1298AC, and 1298CC genotypes were 1.00, 1.5 (0.7-3.01), and 2.005 (0.9-4.5), respectively. Elevation of breast cancer risk was most pronounced among 677TT women who consumed the lowest levels of dietary folate.*

Introduction:
Breast cancer is one of the most prevalent invasive cancers and the second leading global cause of cancer-related deaths among women, in both developed and developing countries, which has become a major public health challenge (Parkin DM, et al.,2002&Smigal C, e t al.,2006)

There are studies suggesting that the effect determined by low-penetrance genes may provide a plausible explanation for breast cancer susceptibility, and in recent years, several common lowpenetrance genes have been identified as potential breast cancer susceptibility genes (Hankinson SE, et al., 2004& Qiu LX, et al.,2010)

As one of the important low-penetrance genes, 5,10-methylenetetrahydrofolate reductase (MTHFR) encodes a critical enzyme for intracellular folate homeostasis and metabolism, which catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to 5-methyltetrahydrofolate (5- methylene-THF), and it is thought to influence DNA methylation and nucleic acid synthesis(Rosenblatt DS, et al.,2001&Lucock M.2004).The MTHFR polymorphisms were considered to be associated with breast cancer susceptibility (Diakite B, et al.,2012&de Cassia Carvalho Barbosa R, et al.,2012).

The C677T (rs1801133, Ala222Val) and A1298C (rs1801131, Glu429Ala) are two common polymorphisms ofMTHFR genes. C677T is in exon 4 at nucleotide 677, which is associated with the decrease of MTHFR activity and increased the level of homocysteine and altered the distribution of folate, while A1298C (rs1801131, Glu429Ala) is in exon 7 at nucleotide 1298, which is also related to the reduction of MTHFR activity but at a lower degree compared to C677T(TaioliE,et al.,2009&De MattiaE,et al.,2009).

Several large prospective epidemiological studies have suggested an importance of folate for breast cancer risk, although the results are not consistent (Feigelson,H.S. etal., 2003 α Lewis,S.J. etal,. 2006).

A number of studies indicate that C677T and A1298C polymorphisms in the MTHFR gene were involved in the etiology of breast cancer (Qi J,et al.,2004&Wu XY,et al.,2012).but not in our Egyption population , So In this study, we intend to explore the possible association between two common variants of the MTHFR gene, C677T and A1298C, and breast cancer risk in Egyptian women.

Materials and Methods
A group of 100 women with breast cancer were randomly selected as the subjects of this study, from department of Nuclear medicine and therapeutic-radiology and Radiotherapy Department, Faculty of Medicine, Mansoura University Hospital, It included 90 subjects healthy controls, who had no personal or family history of breast cancer.

DNA Extraction of samples and purification by Capture columnkit is followed by PCR amplifications. We genotyped two single nucleotide polymorphisms (SNPs) for methylenetetrahydrofolate reductase gene (MTHER) in this case-control study; C677T and A1298C polymorphisms using polymerase chain reaction with sequence-specific primers (SSP-PCR). (Siemianowicz et al., 2003).

The C677T mutation introduces a new HinfI restriction site which results in the digestion of the 198 bp amplicon. into 175 and 23 bp fragments. By abolishing an MboII restriction site, the A1298C mutation results in the digestion of the 163 bp amplicon. into 84, 31, 30, and a8 bpfragments (Mtriaoui et al., 2007). The wild-type 1298AA yields five fragments, and the 84-bp fragment is cut into 56- and 28-bp fragments producing base pair lengths of 56, 31, 30,28 and 18 (Moczulski et al., 2003).

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Results:

Table (1): comparison between all breast cancer cases and control related to obstetric and gynecologic history:

	Cases n(%)	Control (%)	P
Total cases	100 (100%)	90 (100%)	
Age at first menarche(years)			
<12	66.00(66.00)	12(12.0)	<0.0001
13-14	29.00(29.00)	72(72.0)	<0.0001
>15	5.00(5.00)	6(6.0)	0.76
Parity n			
<3	23.00(23.00)	15(15.0)	0.20
>3			0.20
Abortion			
positive	84.00(84.00)	20(20.0)	<0.0001
negative	16.00(16.00)	70(70.0)	<0.0001
Breast feeding			
positive	82.00(82.00)	85(85.0)	0.05
negative	18.00(18.00)	5(5.0)	o.o5
Menopause			
premenopausal	68.00(68.00)	65(65.0)	0.75
postmenopausal	32.00(32.00)	25(25.0)	0.75
Oral contraceptive			
positive	64.00(64.00)	53(53.0)	0.31
negative	36.00(36.00)	37(37.0)	0.31

N = number of studied cases of breast cancer.
(%) = percentage of studied cases.
P=probability

Table (2): Association of MTHFR C677T and A1298C genotypes with breast cancer risk.

MTHFR677 No		Case(100)		Control(90)		X^2	P value	OR(CI95%)
		%	No	%				
Genotypes	CC	42	42.0%	46	51.1%	-	-	1(Ref)
	CT	43	43.0%	41	45.6%	0.2	0.6	1.14(0.6-2.08)
	TT	15	15.0%	3	3.3%	6.3	0.01*	5.5(1.5-20.3)
	CT+TT	58	58%	44	48.9%	1.5	0.6	1.4(0.8-2.6)
Alleles	C	127	63.5%	133	73.89%	4.7	0.03*	1.6(1.05-2.05)
	T	73	36.5%	47	26.11%			
MTHFR1298 No		Case(100)		Control(90)		X^2	P value	OR(CI95%)
		%	No	%				
Genotypes	AA	19	19.0%	25	27.8%	-	-	1(Ref)
	AC	49	49.0%	44	48.9%	0.7	0.4	1.5(0.7- 3.01)
	CC	32	32.0%	21	23.3%	2.2	0.13	2.005(0.9-4.5)
	AC+CC	81	81%	65	72.2%	2.05	0.15	1.6(0.8-3.2)
Alleles	A	87	43.5%	94	52.2%	2.5	0.11	1.4(0.9-2.1)
	C	113	56.5%	86	47.8%			

X^2: Qui-square test P: Probability OR: odd's rati CI: confidence interval

Table (2):Shows the association of MTHFR C677T and A1298C genotypes with breast cancer risk. There was no significant difference in the frequency of the heterozygous mutant CT genotype of C677T polymorphism as compared to that of control (43% vs. 45.6% & p=0.8) and OR of CT VS. CC (OR=1.14, with 95%CI: (0.6-2.08)).While, there was a significant increase in the frequency of the homozygous mutant TT genotype of C677T polymorphism as compared to that of control (15% vs. 3.3% & p=0.01) and OR of TT vs. CC (OR=5.5, with 95%CI: (1.5-20.3). And therewas a significant difference in the risky value of the Allele contrast (T vs. C) allele of C677T polymorphism in cases as compared to that of control (p=0.03) and (OR=1.6, with 95%CI: (1.05-2.05).As illustrated in table (1) :There was no significant difference in the frequency of the heterozygous mutant AC genotype of A1298C polymorphism in breast cancer cases as compared to that of control (OR=1.5, with 95%CI: (0.7- 3.01)).Also, there was no significant difference in the frequency of the homozygous mutant CC genotype of A1298C polymorphism as compared to that of control (OR=2.005 with 95%CI: (0.9-4.5).

Table (3): Dietary folate intake in all cases of breast cancer regarding their frequency and genotype distribution of C677T and A1298C polymorphisms of MTHF gene.

No		good		moderate		bad		P
		%	No	%	No	%		
MTHFR677	CC	1	2.4%	14	33.3%	27	64.3%	<0.001**
	CT	1	2.3%	18	41.9%	24	55.8%	<0.001**
	TT	1	6.7%	2	13.3%	12	80.0%	0.007*
MTHFR1298	AA	1	5.3%	7	36.8%	11	57.9%	0.039*
	AC	0	0.0%	17	34.7%	32	65.3%	<0.001**
	CC	2	6.3%	10	31.3%	20	62.5%	0.003*

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P: Probability
Table (3).Comparing between folate intake of breast cancer cases regarding their frequency and genotype distribution. There was highly significant increase in the frequency of the heterozygous mutant CT genotype and the homozygous mutant TT genotype of C677T polymorphism in low folate cases compared to moderate then to high folate respectively (55.8% vs. 41.9% vs. 2.32% & p<0.001) and (80% vs. 13.3% vs. 6.7%& p = 0.007).As illustrated in table (3); There was a highly significant increase in the frequency of the heterozygous mutant AC genotype andthe homozygous mutant CC genotype of A1298C polymorphism in low folate intake cases compared to moderate then to high respectively (65.3% vs. 34.7% vs. 0% & p<0.001) and(62.5% vs. 31.3% vs. 6.3%& p = 0.003).

DISCUSSION

In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) among the Egypt National Cancer Institute (NCI) series of 10 556 patients during the year 2001, with an age-adjusted rate of 49.6 per100 000 population .(Omar S.et al ., 2003).

A number of studies indicate that C677T and A1298C polymorphisms in the MTHFR gene were involved in the etiology of breast cancer (Boccia S et al., 2008& Wu XY etal.,2012) However, the results from those studies remain conflicting. (Hongjie Liang etal., 2014)

Folate plays an essential role in DNA methylation and synthesis, and thus may be involved in the development of breast cancer. High intake of folate, which is plentiful in vegetables, fruits and cereals , has been associated with reduced risk of several cancers. Several large prospective epidemiological studies have suggested an importance of folate for breast cancer risk, although the results are not consistent (Feigelson, H.S. et al., 2003 α Lewis,S.J. et al,. 2006).(Papandreou CN et al ., 2012).

In this work we studied whether there was a relationship between MTHFR gene polymorphisms C677T and/or A1298C and breast cancer susceptibility.

Thus, a cohort sample including 100 cases of women affected with invasive ductal carcinoma of the breast were genotyped and compared to 90 healthy unrelated individuals as controls, who had no personal or family history of breast cancer or any other autoimmune disease in family. The mean age of patient's ± SD was 48.31 ± 11.40 years. Of them 85% had positive consanguinity, 82% had positivefamily history, 20% had positive history of exposure to environmental pollution. According to Socioeconomic standard, were (39%) of a low, (58%) were of moderate and (3%) of high. On the other hand (91%) of cases had low education, (4%) received median and (5%) had a high education level. Regarding nutrition, 3% of cases had good, 62% were of moderate and 35% of bad nutrition.

Regarding to data of breast cancer cases related to obstetric and gynecologic history, 82% of the studied cases had positive abortion, 68% were premenopausal while 32% were postmenopausal disease. And also 64% of cases were using Oral contraceptive.

Regarding to data of breast cancer cases related to examination and investigations, 21% of the studied cases had positive lymph node, 54% had positive estrogen receptors, and 54% had positive progesterone receptors.

The current study found thatThere was no significant difference inthe frequency of the heterozygous mutant CT genotype of C677T polymorphism as compared to that of control OR of CT VS. CC (OR=1.14, with 95%CI: (0.6-2.08)).While, there was a significant increase in the frequency of the homozygous mutant TT genotype of C677T polymorphism as compared to that of control and OR of TT vs. CC (OR=5.5, with 95%CI: (1.5-20.3). And there was a significant difference in the risky value of the Allele contrast (T vs. C) of C677T polymorphism in cases as compared to that of control , T allele had a significantly increased risk of breast cancer, with (OR=1.6, with 95%CI: (1.05-2.05).). Thus the homozygous mutant TT genotype may beconsidered as a genetic risk factor for breast cancer.

The results of the present study are in agreement with results of(Hongjie Liang et al., 2014) who reported that the association between the MTHFR polymorphism and breast cancer risk was conducted using odds ratios (ORs) and 95 % confidence intervals (95 % CIs). Finally, a total of 22 studies with 6,103 cases and 7,913 controls were included. With regard to C677T polymorphism, significant association was found with breast cancer risk under three models (T vs. C: OR = 1.12, 95 % CI = 1.02–1.23, TT vs. CC: OR = 1.35, 95 % CI=1.10–1.67, TT vs. CC/CT: OR=1.37,95 %CI=1.11–1.70,) during this meta-analysis, they found that MTHFR C677T polymorphism was significantly associated with breast cancer risk in the Chinese population.

The results of the current study are in agreement with results of (Zheng Weiwei et al., 2014) they reported that women with MTHFR 677 TT genotype and T allele had a significantly increased risk of breast cancer, with ORs (95%CI) of 1.8(1.08-2.27) and 1.39(1.02-1.92), respectively. Their study found a significant association between MTHFR C677T polymorphism and breast cancer risk in Chinese females.

The present results are in agreement with results of (Papandreou CN et al., 2012) who performed a single locus analysis for MTHFRpolymorphisms revealed an association only for the C677Tpolymorphism and BC [odds ratio (95% confidence interval), OR=2.05 (1.21-3.48)].

Also these results are in agreement with results of (Diakite B et al., 2012), who investigated if the MTHFR C677Tpolymorphism modulates the risk of developing breast cancer in Moroccan women(96 patients with breast cancer and 117 controls)the heterozygous CT (OR = 2.29 and P = 0.03) was statistically significant in pre-menopausal women. There was a significant association between C677Tpolymorphism and breast cancer risk in both additive (OR = 2.2, 95% CI = 1.24-3.86, p = 0.007) and dominant (OR = 2.10, CI 95% = 1.21-3.64, p = 0.008) models. In addition, the T allele were associated with a high breast cancer risk (OR = 1.59, 95% CI = 1.04-2.44, p = 0.03).In the light of their preliminary study, 677T allele of C677T MTHFR genotype polymorphism may represent a genetic determinant increasing breast cancer risk in Moroccan women.

Thecurrent study results are in agreement with results of(Qi X et al.,2010) they found that With respect to C677Tpolymorphism, significantly elevated breast cancer risk was found in overall analysis (T vs. C: OR = 1.041, 95% CI = 1.009-1.073; TT vs. CC: OR = 1.132, 95% CI = 1.019-1.259; TT vs. CC + CT: OR = 1.119, 95% CI = 1.014-1.236); significantly increased risk was found in East Asian population .It can be concluded that potentially functional MTHFRC677Tpolymorphism may play a role in the development of breast cancer. Also These results are in agreement with results of(Zhang J et al., 2010),they performed a meta-analysis which concluded that the MTHFR T allele is a risk factor for developing breast cancer.

The results of the present study do not agree to results of (Akram M et al., 2012) who found an overall a significant, weak inverse association between breast cancer risk and the 677TT genotype. These results for MTHFRpolymorphism might be population specific in sporadic breast cancer affected patients but manyother factors need to be excluded beforemaking final conclusions including folate intake, popu-

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lation and disease heterogeneity.Also these results are in contrast to results of (Henriquez –Hernandez et al., 2009),(Loï¨c Le Marchand et al., 2004)

The present study showed that there was no significant difference in the frequency of the homozygous mutant CC genotype andthe heterozygous mutant AC genotype of A1298C polymorphism as compared to that of control. We concluded from these results that there is no clear relation betweenA1298C genotype polymorphism and breast cancer.

The current study results agree with (Hongjie Liang et al., 2014), who reported that; There was no significant association found between A1298C polymorphism and breast cancer risk under all genetic models (C vs. A: OR = 0.96, 95 % CI = 0.89-1.03, P = 0.268; CC vs. AA: OR = 0.98, 95 % CI = 0.77-1.26, P = 0.899; AC vs. AA: OR = 0.95, 95 % CI = 0.88-1.02, P = 0.174; CC vs. AC/AA: OR = 1.00, 95 % CI = 0.78-1.28, P = 0.996, CC/AC vs. AA: OR = 0.96, 95 % CI = 0.89-1.02, P = 0.196). During their meta-analysis, they found that MTHFR A1298C polymorphism was not associated with breast cancer risk in the Chinese population.

Also these results agree to results of (Akram M et al., 2012) who found an overall inverse association with the 1298C variant. These results for MTHFR polymorphism might be population specific in sporadic breast cancer affected patients but many other factors need to be excluded before making final conclusions including folate intake, population and disease heterogeneity.

Also These results agree to results of (Qi X et al 2010) and (Loï¨c Le Marchand et al., 2004) they found an overall (non significant), no association with the 1298C variant and breast cancer. The odds ratio [OR and 95% confidence interval (95% CI)] for the 1298AA, 1298AC, and 1298CC genotypes were 1.00, 0.93 (0.79-1.08), and 1.20 (0.88-1.65), respectively.

These results are in contrast to results of (Lajin B et al.,2012) who reported that. A total of 245 subjects (119 breast cancer women patients and 126 healthy controls) were genotyped for MTHFR C677T and A1298C polymorphisms. A statistically significant association was found for MTHFR A1298C polymorphism especially under the allele contrast model (odds ratio (OR) = 1.68, 95% confidence interval (CI) (1.16-2.45), P = 0.006) and breast cancer risk.Also These results are in contrast to results of (Jia Chen et al., 2005) The 1298C variant allele was inversely associated with breast cancer risk (P, trend =0.03), and was likely due to the linkage of this allele to the low risk allele of 677CT.

The present study showed that, when comparing between bad, moderate and high folate intake of breast cancer cases regarding their frequency and genotype distribution of C677T and A1298C polymorphism of MTHF gene. There was highly significant increase in the frequency of the heterozygous mutant CT genotypeandthe homozygous mutant TT genotype of C677T polymorphism in low folate cases compared to moderate then to high folate respectively , so may be when low folate intake is in combination with C677T genotype polymorphism the overall risk for breast cancer increase.

Also there was a highly significant increase in the frequency of the heterozygous mutant AC genotype andthe homozygous mutant CC genotype of A1298C polymorphism in low folate intake cases compared to moderate then to high respectively but because we did not found a clear relation between breast cancer and this genotype. These findings need more studies to be reasonable.

The results of the present study was agree with (Zheng weiwei et al., 2014) who reported that Compared to the reference group, women with MTHFR 677 TT genotype and T allele had a significantly increased risk of breast cancer, with ORs (95%CI) of 1.8(1.08-2.27) and 1.39(1.02-1.92), respectively. For those who had folate intake<450 µg/day, MTHFR 667TT genotype was associated with a higher risk of breast cancer (OR=2.45, 95% CI=1.09-5.82, P=0.02). And statistically significant association disappeared among individuals with folate intake≥500 µg/day. A significant interaction was observed between MTHFR C677T polymorphism and folate intake on the risk of breast cancer.The present study results agree with (Maruti SS et al.,2009) who observed a 62% increased risk of breast cancer among postmenopausal women with the TT genotype (OR = 1.62; 95% CI: 1.05 to 2.48). Women with a higher number of variant T alleles had higher risk of breast cancer (P for trend = 0.04). The most pronounced MTHFR-breast cancer risks were observed among women with the lowest intakes of dietary folate (P for interaction = 0.02) with no significant increased risks among women with higher intakes.Alsothe present study results agree with (Yu-Ching, et al.,2006). These resultsprovide support for the important role of folate metabolismin breast cancer.

SUMMARY AND CONCLUSION

We concluded that C677T genotype polymorphism of MTHFR gene, speciallyhomozygous mutant TT and its T allele is a risk factor for developing breast cancer, especially in cases with low folate intake.

We did not found a clear relation between A1298C genotype polymorphism of MTHFR gene and breast cancer in our studied cases.

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REFERENCE

1. Smigal C, JemalA,Ward E, Cokkinides V, Smith R, Howe HL,(2006) : Trends in breast cancer by race and ethnicity: update 2006. CACancer J Clin.; 56:168–83. | | 2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, (2002):.CA Cancer J Clin. 2005; 55:74–108. | | 3. Hankinson SE, Colditz GA, Willett WC (2004): Towards an integratedmodel for breast cancer etiology: the lifelong interplay of genes, lifestyle, and hormones. Breast Cancer Res.; 6:213–8. | | 4. Qiu LX, Yuan H, Yu KD,Mao C, Chen B, Zhan P, et al(2010). GlutathioneS-transferase M1 polymorphism and breast cancer susceptibility: ameta-analysis involving 46,281 subjects. Breast Cancer Res Treat. 121:703–8. | | 5. Rosenblatt DS. (2001): Methylenetetrahydrofolate reductase. Clin InvestMed.; 24:56–9. | | 6. Lucock M. (2004): Is folic acid the ultimate functional food component for disease prevention? BMJ.; 328:211–4.11. | | 7. Diakite B, Tazzite A, Hamzi K, Jouhadi H, Nadifi S.(2012): | Methylenetetrahy-drofolate reductase C677T polymorphism and | breast cancer risk in moroccan women. Afr Health Sci.; | 12:204–9. | | 8. De Cassia Carvalho Barbosa R, da Costa DM, Cordeiro DE, VieiraAP, Rabenhorst SH. (2012):Interaction ofMTHFR C677Tand A1298C, andMTR A2756G gene polymorphisms in breast cancer risk in a populationin northeast Brazil. Anticancer Res.; 32:4805–11. | | 9. Taioli E, Garza MA, Ahn YO, Bishop DT, Bost J, Budai B, et al.(2009):Meta- and pooled analyses of the methylenetetrahydrofolate reductase(MTHFR) C677T polymorphism and colorectal cancer: aHuGE-GSEC review. Am J Epidemiol.;170:1207–21. | | 10. De Mattia E, Toffoli G.(2009): C677T and A1298C MTHFR polymorphisms,a challenge for antifolate and fluoropyrimidine-based therapypersonalisation. Eur J Cancer.;45:1333–51. | | 11. Feigelson,H.S. et al. (2003): Alcohol, folate, methionine, and risk of incidentbreast cancer in the American Cancer Society Cancer Prevention | Study II Nutrition Cohort. Cancer Epidemiol. Biomarkers Prev., 12, | 161–164. | | 12. Lewis,S.J. et al. (2006): Meta-analyses of observational and genetic associationstudies of folate intakes or levels and breast cancer risk. J. NatlCancer Inst., 98, 1607–1622. | | 13. Qi J, Miao X, Tan W, Yu C-y, Liang G, Lvwen F, et al.(2004):Methylenetetrahydrofolate reductase gene single nucleotide polymorphismsand breast cancer risk. Chin J Oncol.; 26:287–9. | | 14. Wu XY, Ni J, Xu WJ, Zhou T, Wang X.(2012): Interactions betweenMTHFR C677T-A1298C variants and folic acid deficiency affectbreast cancer risk in a Chinese population. Asian Pac J Cancer Prev.; 13:2199–206. | | 15.Siemianowicz K, Gminski J, Garczorz W, Slabiak N, Goss M, | Machalski M, Magiera-Molendowska H. (2003): | Methylenetetrahydrofolate reductase gene C677T and A1298C | polymorphisms in patients with small cell and non-small cell lung | cancer. Oncol. Rep. Sep-Oct; 10(5): 1341-1344. | | 16.Mtiraoui N, Ezzidi I, Chaieb M, Marmouche H, Aouni Z, Chaieb A, Mahjoub T, Vaxillaire M, AlmawiWy. (2007): MTHFR C677T | and A1298C gene polymorphisms and hyperhomocysteinemia as | risk factors of diabetic nephropathy in type 2 diabetes patients. | Diabetes Res. Clin. Pract. Jan; 75(1): 99-106. | | 17.Moczulski D, Fojcik H, Zukowska-Szczechowska E, Szydlowska I, | Grzeszczak W (2003):Effects of the C677T and A1298C | polymorphisms of the MTHFR gene on the genetic predisposition | for diabetic nephropathy. Nephrol Dial Transplant. Aug; 18(8): | 1535-1540. | | 18. S. Omar,1 H. Khaled,1 R. Gaafar,1 A.R. Zekry,1 S. Eissa1 and O. | El-Khatib(.2003):.Breast cancer in Egypt: a review ofdisease | presentationanddetectionstrategies La Revue de Santé de la | Méditerranéeorientale, Vol. 9, NO 3, 2003 | | 19. Boccia S, Hung R, Ricciardi G, Gianfagna F, Ebert MP, Fang JY, et al. (2008): Meta- and pooled analyses of the methylenetetrahydrofolatereductase C677T and A1298C polymorphisms and gastric cancerrisk: a huge-GSEC review. Am J Epidemiol.; 167:505–16. | | 20.Hongjie Liang, Yulan Yan, Taijie Li, Ruolin Li, Meng Li, Shan Li, | and Xue Qin(2014):Methylenetetrahydrofolate reductase | polymorphisms and breast cancer risk in Chinese population: a | meta-analysis of 22 case–control studies Tumour Biology | Tumour Biol. Feb; 35(2): 1695–1701. | | 21.Papandreou CN1, Doxani C, Zdoukopoulos N, Vlachostergios PJ, | Hatzidaki E, Bakalos G, Ziogas DC, Koufakis T, Zintzaras E.(2012): Evidence of association between ethylenetetra- hydrofolate | reductase gene and susceptibility to breast cancer: a candidate-gene | association study in a South-eastern European population. DNA | Cell Biol. Feb; 31(2):193-8. | | 22.Zheng Weiwei, Chen Liping, and Li Dequan(2014):Association | between dietary intake of folate, vitamin B6, B12& MTHFR, MTR | Genotype and breast cancer risk Pak J Med Sci. Jan-Feb; | 30(1): 106–110. | | 23.Qi X1, Ma X, Yang X, Fan L, Zhang Y, Zhang F, Chen L, Zhou Y, | Jiang.(2010):Methylenetetrahydrofolate reductase polymorphisms | and breast cancer risk: a meta-analysis from 41 studies with | 16,480 cases and 22,388 controls. Breast Cancer Res Treat. | Sep; 123(2): 499-506. | | 24. Zhang J, Qiu LX, Wang ZH, Wu XH, Liu XJ, Wang BY, Hu XC.(2010):MTHFRC677Tpolymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls. Sep; 123(2):549-55. | | 25. Akram M1, Malik FA, Kayani MA. (2012): Mutational analysis of the MTHFR gene in breast cancer patients of Pakistani population. Asian Pac J .Cancer Prev.; 13(4):1599-603. | | 26.Henríquez-Hernández LA1, Murias-Rosales A, Hernández González A, Cabrera De León A, Díaz-Chico BN, Mori De Santiago M, Fernández Pérez L.(2009):Gene polymorphisms in TYMS, MTHFR,p53 and MDR1 as risk factors for breast cancer: a case-control study.Oncol Rep2009 Dec;22(6):1425-33 | | 27. Loï¨c Le Marchand,1 Christopher A. Haiman,2 Lynne R. Wilkens,1 Laurence N. Kolonel,1 and Brian E. Henderson2.(2004):MTHFR Polymorphisms, Diet, HRT, and Breast Cancer Risk: The | Multiethnic Cohort Study Cancer Epidemiol Biomarkers Prev | ;13(12). December 2004. | | 28.Lajin B1, AlhajSakur A, Ghabreau L, Alachkar A.(2012): Associationof polymorphisms in one-carbon metabolizing genes with breast cancer risk in Syrian women. Tumour Biol.Aug ; 33(4) : 1133-9. doi: 10.1007/s13277-012-0354-y. Epub 2012 Feb 29. | | 29.Jia Chen,1Marilie D. Gammon,3 Wendy Chan,1 Caroline | Palomeque,1James G. Wetmur,2Geoffrey C. Kabat,4 Susan L. | Teitelbaum,1 Julie A. Britton,1 Mary Beth Terry,5 Alfred I. | Neugut,5 and Regina M. Santella6(2005):One-Carbon Metabolism, MTHFR Polymorphisms, and Risk of Breast Cancer, Columbia University, New York, New York Cancer Res ;65:(4).February 15, 2005 | | 30. Maruti SS, Ulrich CM, Jupe ER, White E. (2009): MTHFR C677T and postmenopausal breast cancer risk by intakes of one-carbon Metabolism nutrients: a nested case-control study. Breast Cancer Res.;11(6):R91. doi: 10.1186/bcr2462. Epub 2009 Dec 23. | | 31. YU-Ching. Meei-Shyuan Lee. Mei-Hsuan WU. Hsiu-Lan Shih. Tsan Yang. Cheng- Ping YU.Jyh- Cherng YU.Chien – An Sun. (2007): Plasma homocystiene as a metabolic risk factor for breast cancer. Findings from a case – control study in Taiwan. Beast cancer Res. Treat. (2007)101:199-205. |