

Supplementary File:

Gene-Metabolites Interaction in the One Carbon Metabolism Pathway: Predictors of Colorectal Cancer in Multi-Ethnic Families

S. Pamela K. Shiao*, James Grayson, Chong Ho Yu

*Corresponding: pshiao@msn.com

Supplementary Table S1. Comparisons on demographic factors across racial groups. Nonparametric test, Posthoc by Wilcoxon test.

		White (n = 10)	Hispanic (n = 9)	Asian (n = 9)	African (n = 2)	p
Gender	male	4 (40%)	2 (22%)	4 (44%)	1 (50%)	0.77
	female	6 (60%)	7 (78%)	5 (56%)	1 (50%)	
Age	years	48 ± 15 (21 – 70)	50 ± 14 (29 – 72)	47 ± 13 (19 – 62)	52 ± 25 (34 – 69)	0.98
BMI		30 ± 8.7 (21 – 51)	28 ± 6.5 (21 – 43)	23 ± 2.1 (21 – 27)	39 ± 11 (31 – 47)	0.036
	posthoc			<White p = 0.03 <African p = 0.045		
Weight	Kg	80 ± 26 (45 – 138)	73 ± 15 (54 – 109)	63 ± 6.3 (52 – 71)	113 ± 38 (86 – 141)	0.035
	posthoc			<African p = 0.045		
Vegetable	cups	2.1 ± 0.2 (1 – 3)	1.8 ± 0.2 (1 – 1)	2.3 ± 0.2 (1 – 3)	1.0 ± 0.5 (1 – 1)	0.093
Fruit	cups	1.2 ± 0.8 (0 – 2)	1.1 ± 0.8 (0 – 2)	1.6 ± 0.5 (1 – 2)	1.5 ± 0.7 (1 – 2)	0.59
Whole grain	oz	2.2 ± 0.6 (1 – 3)	1.6 ± 0.5 (1 – 2)	1.4 ± 0.7 (1 – 3)	1.5 ± 0.7 (1 – 2)	0.071
	posthoc		<White p = 0.029	<White p = 0.038		
Liquid	cups	5.5 ± 1.4 (4 – 8)	6.1 ± 1.8 (4 – 8)	5.4 ± 1.5 (4 – 8)	4.5 ± 0.7 (4 – 5)	0.59

BMI: body mass index.

Supplementary Table S2. Distribution of gene polymorphisms per control and cancer groups across racial groups.

<i>n</i> (%) Genotypes	Control Group				<i>p</i> (HWE)	Allele Frequency#	Cancer Group		
	0	1	2				0	1	2
<i>MTHFR 677</i>	CC	CT	TT			% C/T	CC	CT	TT
Total	7 (47)	6 (40)	2 (13)	NS		75/25	4 (27)	9 (60)	2 (13)
White	0 (0)	5 (83)	1 (17)	NS		53/47	1 (25)	2 (50)	1 (25)
Asian	2 (50)	1 (25)	1 (25)	NS		70/30	3 (60)	1 (20)	1 (20)
Hispanic	1 (25)	3 (75)	0 (0)	NS		55/45	2 (40)	3 (60)	0 (0)
Black	1 (100)	0 (0)	0 (0)	–		91/9	1 (100)	0 (0)	0 (0)
<i>MTHFR 1298</i>	AA	AC	CC			% A/C	AA	AC	CC
Total	9 (60)	6 (40)	0 (0)	NS		75/25	11 (73)	3 (20)	1 (7)
White	5 (83)	1 (17)	0 (0)	NS		85/15	3 (75)	1 (25)	0 (0)
Asian	3 (75)	1 (25)	1 (25)	NS		78/22	3 (60)	2 (40)	0 (0)
Hispanic	2 (50)	1 (25)	1 (25)	NS		84/16	2 (40)	3 (60)	0 (0)
Black	1 (100)	0 (0)	0 (0)	–		85/15	1 (100)	0 (0)	0 (0)
<i>MTR 2756</i>	AA	AG	GG			% A/G	AA	AG	GG
Total	9 (60)	4 (27)	2 (13)	NS			7 (46.7)	7 (46.7)	1 (6.7)
White	2 (33)	3 (50)	1 (17)	NS		84/16	2 (50)	1 (25)	1 (25)
Asian	2 (50)	2 (50)	0 (0)	NS		65-91/9-35	2 (40)	2 (40)	1 (20)
Hispanic	3 (75)	1 (25)	0 (0)	NS		19/81	5 (100)	0 (0)	0 (0)
Black	0 (0)	1 (100)	0 (0)	NS		30-37/63-70	0 (0)	1 (100)	0 (0)
<i>MTRR 66</i>	AA	AG	GG			% A/G	AA	AG	GG
Total	8 (57)	3 (21)	3 (21)	NS		64/36	7 (47)	5 (33)	3 (20)
White	2 (33)	3 (50)	1 (17)	NS		45/55	0 (0)	1 (25)	3 (75)
Asian	2 (50)	1 (25)	1 (25)	NS		74/26	2 (50)	2 (50)	0 (0)
Hispanic	3 (75)	0 (0)	1 (25)	0.0455		72/28	5 (100)	0 (0)	0 (0)
Black	0 (0)	1 (100)	0 (0)	NS		73/27	1 (0)	0 (0)	0 (0)
<i>DHFR 19 bp</i>	II	ID	DD			% I/D	II	ID	DD
Total	6 (40)	6 (40)	3 (20)	NS		50/50	3 (20)	6 (40)	6 (40)
White	1 (17)	3 (50)	2 (33)	NS		45-47/53-55	2 (50)	0 (0)	2 (50)
Asian	0 (0)	2 (50)	2 (50)	NS		63/37	2 (40)	3 (60)	0 (0)
Hispanic	1 (25)	1 (25)	2 (50)	NS		58/42	1 (20)	3 (60)	1 (20)
Black	1 (100)	0 (0)	0 (0)	–		55/45	1 (100)	0 (0)	0 (0)

HWE: Hardy-Weinberg Equilibrium; *MTHFR*: *methylene tetrahydrofolate reductase*; *MTR*: *methionine synthase*; *MTRR*: *methionine synthase reductase*; *DHFR*: *dihydrofolate reductase*; NS: Not significant; --: cannot be calculated; HWE Calculator: http://www.bioinformatics.org/forums/forum.php?forum_id=3196; #Population Allele Frequencies from: <http://useast.ensembl.org/index.html>; <https://www.cdc.gov/genomics/population/genvar/frequencies/mthfr.htm>.

Supplementary Table S3. Comparisons on metabolites in blood plasma among racial groups.

	White (n = 10)	Hispanic (n = 9)	Asian (n = 9)	African (n = 2)	<i>p</i>
Homocysteine µmol/L	8.3 ± 4.5 (4.2 – 17)	6.6 ± 3 (3.2 – 12)	6.2 ± 3.3 (3.1 – 14)	5.6 ± 0.4 (5.3 – 5.9)	0.72
SAM nmol/L	99 ± 20 (74 – 134)	94 ± 16 (70 – 125)	108 ± 53 (63 – 233)	93 ± 1.2 (92 – 94)	0.96
SAH nmol/L	27 ± 12 (11 – 56)	21 ± 5.6 (12 – 29)	41 ± 39 (16 – 142)	38 ± 14 (27 – 47)	0.17
SAM/SAH Ratio	4.1 ± 1.1 (2.4 – 6.3)	4.7 ± 1.0 (3.4 – 6.3)	3.2 ± 1.0 (1.6 – 4.4)	2.7 ± 1.0 (2.0 – 3.4)	0.031
	Posthoc		< Hispanic <i>p</i> = 0.012		
ADMA nmol/L	531 ± 89 (435 – 754)	626 ± 232 (278 – 917)	534 ± 92 (393 – 697)	574 ± 9.2 (567 – 580)	0.57
SDMA nmol/L, -324	503 ± 96 (401 – 686)	700 ± 523 (349 – 2050)	479 ± 133 (324 – 778)	511 ± 21 (496 – 526)	0.58
Methionine nmol/L	28 ± 5.3 (18 – 35)	29 ± 6.6 (18 – 38)	34 ± 10 (20 – 51)	28 ± 5.1 (24 – 31)	0.49
MMA nmol/L	288 ± 98 (178 – 521)	276 ± 96 (185 – 480)	322 ± 246 (205 – 972)	199 ± 18 (186 – 211)	0.31
Betaine nmol/L	56 ± 10 (37 – 68)	49 ± 12 (36 – 75)	78 ± 20 (47 – 111)	60 ± 1.4 (59 – 61)	0.0098
	Posthoc	<White <i>p</i> = 0.016 < Asian <i>p</i> = 0.0047			
Vitamin B-6 nmol/L	82 ± 40 (29 – 155)	46 ± 30 (14 – 109)	41 ± 20 (5.3 – 67)	22 ± 2.9 (20 – 24)	0.028
	Posthoc	<White, <i>p</i> = 0.025	<White, <i>p</i> = 0.0455	<White, <i>p</i> = 0.041	
5-MTHF nmol/L	45 ± 15 (31 – 78)	40 ± 24 (18 – 97)	34 ± 7.4 (25 – 48)	41 ± 1.6 (40 – 43)	0.24
Choline nmol/L	9.7 ± 3.2 (5.7 – 18)	9.7 ± 1.7 (7.4 – 12)	13 ± 6.6 (6.9 – 27)	9.9 ± 0.8 (9.3 – 10)	0.59

Nonparametric test, Posthoc by Wilcoxon test. SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine; ADMA: Asymmetric dimethylarginine; SDMA: symmetric dimethylarginine; MMA: Methylmalonic acid; 5-MTHF: 5-methyltetrahydrofolate or methylfolate.

Supplementary Table S4. Bootstrap forest analysis of three domains and significant parameters included in the prediction model: (a) gene parameters, (b) metabolites, (c) top demographic and lifestyle parameters, (d) most significant parameters of three domains.

(a) Gene parameters

Term	Number of Splits	G^2		Portion
Total mutation > 4	16	0.34		0.22
<i>DHFR</i> 19bp del	23	0.33		0.21
<i>MTRR</i> A66G	27	0.25		0.16
<i>MTR</i> A2756G	21	0.19		0.12
<i>MTHFR</i> d50	14	0.16		0.11
<i>MTHFR</i> C677T	12	0.16		0.10
<i>MTHFR</i> A1298C	16	0.13		0.08

(b) Metabolites

Term	Number of Splits	G^2	Column Contribution	Portion
Homocysteine 7	25	1.59		0.24
MMA 300	30	1.35		0.20
SAM 90	27	0.75		0.11
Betaine 60	22	0.72		0.11
<i>MTHF</i> 40	19	0.48		0.07
Methionine 30	19	0.44		0.03
SAH 27	19	0.38		0.06
SDMA 400	9	0.29		0.04
Ratio SAM/SAH 4	15	0.23		0.03
Vitamin B6 55	14	0.15		0.02
Cystathionine 290	12	0.14		0.02
Choline 10	14	0.13		0.02
ADMA 400	1	0.03		0.004

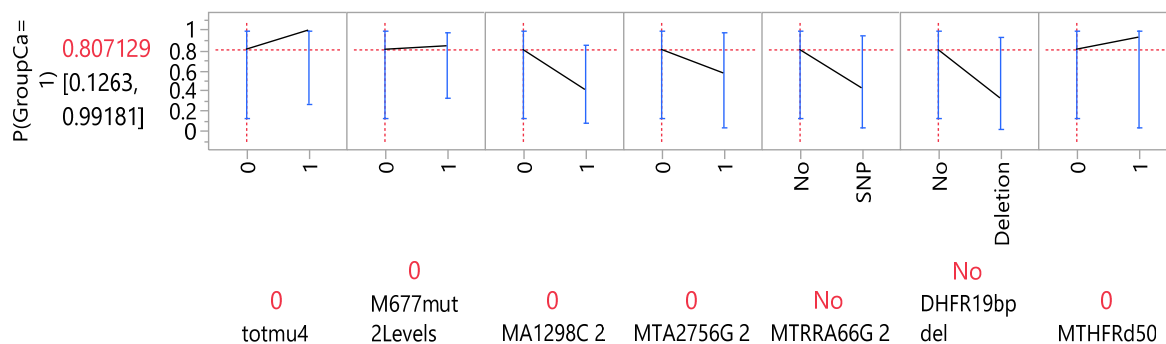
(c) Top demographic and lifestyle parameters

Term	Number of Splits	G^2		Portion
age 51	25	1.53		0.52
fruit intake	30	0.40		0.13
Sex	28	0.39		0.13
BMI overweight	17	0.32		0.11
vegetable intake	16	0.14		0.05
Whole grain intake	16	0.12		0.04
liquid intake	18	0.06		0.02

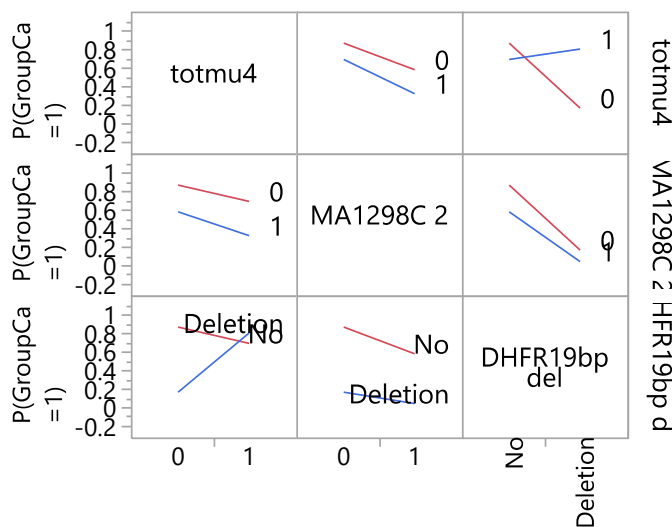
(d) Most significant parameters of three domains

Term	Number of Splits	G^2		Portion
Homocysteine 7	28	1.85		0.35
age 51	26	1.23		0.23
Total mutation > 4	33	0.83		0.16
<i>MTHF</i> 40	32	0.71		0.13
MMA 300	14	0.57		0.11
Vegetable intake	24	0.09		0.02

Prediction Profiler



Interaction Profiles



Supplementary Figure S1. Gene parameters: total gene mutation >4 (totmu4), *MTHFR* (methylenetetrahydrofolate reductase) C677T, *MTHFR* A1298C, *MTR* (methionine synthase) A2756G, *MTRR* (methionine synthase reductase) A66G, *DHFR* (dihydrofolate reductase) 19 bp deletion, and *MTHFR* deficiency >50% calculated from *MTHFR* 677 T and 1298 C alleles: (a). prediction profiler, (b) examples of interaction profiles on *DHFR* 19 bp deletion interacting with total gene mutation > 4 in association with probability of cancer status [$p(\text{GroupCa}=1)$].