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Maternal Folate-Related Gene Environment Interactions and Congenital Heart Defects

Charlotte A. Hobbs, MD, PhD^{*}, Mario A. Cleves, PhD, Mohammad A. Karim, MD, PhD, Weizhi Zhao, MS, and Stewart L. MacLeod, PhD

University of Arkansas for Medical Sciences, College of Medicine, Department of Pediatrics, Birth Defects Research Section, Arkansas Children's Hospital Research Institute, Little Rock, AR

Abstract

Objective—To investigate whether women with congenital heart defect (CHD)-affected pregnancies were more likely to have functional single nucleotide polymorphisms (SNPs) in genes encoding enzymes in folate-dependent pathways.

Methods—A population-based case-control study of 572 women with CHD-affected pregnancies and 363 control women was conducted. DNA samples were genotyped for SNPs in three genes encoding for folate pathway enzymes. Maternal lifestyle factor information was obtained using standardized interviews.

Results—Case women were 1.5 times more likely to be obese (BMI of 30 or higher) compared to control women. Obese women carrying the *MTHFR* TT genotype were 4.6 times more likely to have an affected pregnancy compared to normal weight women carrying a CC genotype. Obese women carrying one or two copies of the A allele in the *BHMT* polymorphism were 1.8 times more likely to have a CHD-affected pregnancy than normal weight women carrying a *BHMT* GG genotype. Among women who smoked, those carrying a *TCII* CG or GG genotype were 1.8 times more likely to have an affected fetus than women who smoked and carried a CC genotype. Among women who drank alcohol, those carrying a *TCII* CG or GG genotype were 1.7 times more likely to have an affected fetus than women who drank and carried a CC genotype.

Conclusions—Results indicate that functional polymorphisms in folate-related genes increase the risk of having a fetus with CHD when maternal lifestyle factors that alter folate metabolism are present.

INTRODUCTION

Congenital heart defects (CHDs) are the most common birth defects.(1) In the United States approximately 8 to 10 of 1,000 live births are affected by a CHD. The etiology of most non-syndromic CHDs is unknown; the majority are thought to be caused by a complex interaction of multiple factors including genetic and lifestyle factors that alter folate metabolism.(2)

^{*}Address for Correspondence: Arkansas Center for Birth Defects Research and Prevention, 13 Children's Way, Slot 512-40, Little Rock, AR 72202. phone: 501-364-5001, fax: 501-364-5050, hobbsscharlotte@uams.edu.

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Adequate intake of folic acid in the periconceptional period has been shown to be protective against birth defects, including CHDs.(3) Genetic polymorphisms in genes that code for key enzymes in the folate pathway may reduce enzyme activity, leading to disruptions in folate metabolism. A number of functional single nucleotide polymorphisms (SNPs) in critical genes in the folate pathway that affect folate metabolism have been characterized including 1) 677C>T (rs1801133) in the methylenetetrahydrofolate reductase gene (*MTHFR*, NM005957) 2) 742G>A (rs3733890) in the betaine homocysteine methyltransferase gene (*BHMT*, NM0011713), and 3) 776C>G (rs1801198) in the transcobalamin II gene (*TCII*, NM000355).(4–6)

Both fetal and maternal genetic susceptibilities affect the intrauterine environment during the first 8 weeks of pregnancy when the primitive heart is forming and developing. Genetic factors, in conjunction with lifestyle factors such as obesity, cigarette smoking, and ethanol intake, may work in combination to increase the risk of CHDs. In this study, we hypothesized that three functional SNPs in maternal folate-related genes will interact with maternal lifestyle factors to increase the risk of CHDs.

MATERIALS AND METHODS

The protocol and provisions for informed consent were reviewed and approved by the Institutional Review Board at the University of Arkansas for Medical Sciences. Cases were mothers of affected infants and were identified and ascertained through the Arkansas Reproductive Health Monitoring System, a statewide birth defects registry. Inclusion criteria for cases were as follows: a) resident of Arkansas at the time of the completion of the index pregnancy and at the time of enrollment in the study; b) pregnancy outcome was a liveborn, stillborn or elective termination; c) pregnancy ended between September 1998 and September 2006; d) a physician diagnosis of a non-syndromic septal, conotruncal, or right or left-sided obstructive heart defect that had been confirmed by prenatal or postnatal echocardiogram, surgical, and/or autopsy report; and e) had completed participation in the National Birth Defects Prevention Study (NBDPS).(7) Cases for whom the pregnancy was also affected by a known single gene disorder, chromosomal abnormality, or syndrome were excluded; only nonsyndromic congenital heart defects were included in this study. Further details regarding the NBDPS have been previously published.(7)

Controls were Arkansas mothers who had live births that were unaffected by any birth defect, were randomly chosen from all birth certificates registered at the Arkansas Department of Health, with birth dates between December 1998 and April 2007, and had completed participation in the NBDPS. Case and control mothers with type I or II diabetes at time of conception were excluded from analyses. By design, the NBDPS protocol has a case:control ratio of 3:1.

Both cases and controls were interviewed using a structured Computer Assisted Telephone Interview that was specifically designed for the NBDPS. In this questionnaire, the respondent was asked about smoking and drinking alcoholic beverages before and after conception, and was also asked during which months. Similarly, questions were also asked regarding the use of multivitamins, the educational level of each participant and household income.

DNA was extracted from maternal buccal cell samples or peripheral blood lymphocytes by use of Pure Gene DNA purification reagents (Gentra Systems, Minneapolis, MN) according to the manufacturer's protocol. Genomic DNA was quantified by use of the Applied Biosystems (ABI, Foster City, CA) TaqMan® RNaseP Detection Reagents using a standard curve of genomic DNA. The standard curve samples as well as the genomic DNA samples

of unknown concentration were subjected to an initial denaturalization at 95°C for 10 minutes followed by 40 polymerase chain reaction (PCR) cycles (95°C, 15 seconds, 60°C 1 minutes) in an ABI PRISM 7900HT real-time PCR instrument. DNA concentrations were calculated from the standard curve using ABI software. Genomic DNA (10 to 15 ng) were used as a template for whole genome amplification (WGA) by use of the Sigma GenomePlex WGA kit and a protocol provided by the manufacturer. WGA product was quantified as above and 10 ng were used for each genotyping assay. PCR of polymorphic alleles was accomplished by the use of TaqMan Assays (ABI) on an Applied Biosystems 7900 Sequence Detection instrument for the following polymorphisms (*BHMT* NM001713 rs3733890; *MTHFR* NM005957 rs1801133; *TCII* NM 000355 rs1801198). PCR conditions were 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, then, 60°C for 1 minute. A post PCR allelic discrimination read was performed using ABI software.

The four periconceptional lifestyle factors (BMI, alcohol use, smoking and folic acid supplementation) were summarized by counts and proportions and were compared using chi-square tests if categorical, and were summarized by means and standard deviations and compared using t-tests if continuous. Deviations from Hardy-Weinberg equilibrium (HWE) in the control mothers stratified by race/ethnicity were tested using an exact HWE test implemented in STATA's GENHW command.(8) Odds ratios and 95% confidence intervals for the association between the occurrence of CHD and allele and genotypes frequencies for each of three SNPs under various genetic models were estimated using linear logistic regression adjusting for maternal race/ethnicity. These associations were further examined using logistic regression models after combining lifestyle factors and genotype and also stratified on lifestyle factor levels. These logistic regression models were adjusted for race/ethnicity and all lifestyle factors.

All eligible cases and controls that met entry criteria were included in the study. With a sample size of 572 cases and 363 controls, assuming an additive genetic model, a minor allele frequency of 35%, a lifestyle factor that is present in 25% of the population, conservative ORs of 1.2 for the main effect of the genetic and environmental factor and a two-sided test with $\alpha=0.05$, the study has over 80% power to detect a minimal odds ratio of 1.85 for a one-way interaction between genotype and lifestyle factor.

Statistical analyses were performed using STATA version 10.1 software (StataCorp LP, College Station, TX).

RESULTS

As presented in Table 1, there were a total of 572 cases and 363 controls included in the current study. The mean maternal age at time of delivery was 26.7 years for cases and 26.4 years for controls ($P=0.48$). Compared to women with normal BMI, significantly more cases than controls were obese ($BMI \geq 30$) prior to pregnancy (26.7% and 21.5%, respectively; $P=0.02$). There were no statistically significant differences between cases and controls for periconceptional alcohol drinking, smoking, or intake of folate supplements. Neither household income nor educational level differed significantly between cases and controls.

No significant deviations from Hardy-Weinberg equilibrium were observed for any SNP within race. There were no significant differences in the distribution of *MTHFR* 677C>T, *BHMT* 742G>A, or the *TCII* 776C>G polymorphisms between women with CHD-affected pregnancies and those with unaffected pregnancies (Table 2). We evaluated allelic, genomic, additive, dominant and recessive inheritance models; none of the maternal genotypes were associated with case-control status.

Multiple logistic regression models were used to estimate if the effect of genotypes was modified by lifestyle factors (Tables 3 and 4) or periconceptional folate intake. The reference group for Table 3 was the major allele homozygous genotype (*MTHFR* 677 CC, *BHMT* GG, and *TCII* CC) and the most favorable lifestyle factor. The effect of maternal obesity on the developing heart was increased among those carrying the *MTHFR* 677 TT genotype and those carrying either one or two copies of the A allele in the *BHMT* 742 polymorphism. Specifically, obese women who carried two copies of the *MTHFR* 677 T allele were 4.62 times (95% CI: 1.54, 13.83) more likely to have a CHD-affected pregnancy than women who were normal weight and had no copies of the T allele (Table 3). Similarly, women who had a BMI ≥ 30 and carried one or two copies of the A allele in the *BHMT* polymorphism were 1.82 times (95% CI: 1.09, 3.03) more likely to have a CHD-affected pregnancy than women who were normal weight and had no copies of the G allele (data not shown). The maternal *TCII* polymorphism did not alter the effect of obesity on the risk of CHDs.

To further understand the association between CHDs and the combined effect of genetic polymorphisms and lifestyle factors, we examined the effect of functional polymorphisms within each stratum of lifestyle factors using as reference groups individuals with the same lifestyle factor who were homozygous for the major allele of each functional polymorphism (Table 4). In this way, we were able to estimate the effect of genotype among those with similar lifestyle choices. For example, among women who were obese, those who carried two copies of the T allele in the *MTHFR* polymorphism were 3.93 times (95% CI: 1.24, 12.50) more likely to have a CHD-affected pregnancy than women who were obese but carried a CC genotype. Among women who smoked, those who carried one or more copies of the G allele in the *TCII* polymorphism were 1.81 times (95% CI: 1.06, 3.11) more likely to have a CHD-affected pregnancy than women who smoked and carried the CC genotype. Similarly, among women who drank alcohol, those who carried the GC or GG genotype in the *TCII* polymorphism were 1.71 times (95% CI: 1.00, 2.92) more likely to have a CHD-affected pregnancy than women who drank alcohol but carried the CC genotype. There was no significant evidence that periconceptional folate supplement use modified the association between CHDs and any of the three candidate polymorphisms.

DISCUSSION

In this case-control study, we evaluated the effect of three folate-related functional polymorphisms combined with maternal periconceptional obesity, smoking, alcohol intake and folate supplement use on the risk of CHDs. Our intent was to determine if the association between CHDs and functional polymorphisms in folate-related genes is modified by lifestyle factors that are known to impair folate metabolism. Several findings should be noted.

Maternal genotype was not independently associated with CHD risk for any of the functional polymorphisms. Multiple previous studies have examined the association between CHDs and *MTHFR* 677 C>T.(9–12) Our inability to find evidence of an association with the 677 C>T polymorphism is in agreement with a recent meta-analysis.(13) Among six eligible studies included in the meta-analysis, the overall odds ratio for the association between CHDs and *MTHFR* 677 C>T was 1.2 (95% CI: 0.83, 1.74). As with previous studies, we were unable to find evidence that the *BHMT* 742G>A or the *TCII* 776C>G polymorphisms were associated with CHDs.(11,14)

Our findings indicate that periconceptional maternal obesity, smoking and alcohol use combined with functional polymorphisms may increase the risk of CHD-affected pregnancies. The evidence supporting an association between obesity and CHDs has been

included in a recently published meta-analysis by Stothard and colleagues.(15) Among the 18 studies included in the meta-analysis, the overall odds ratio for the association between CHDs and maternal obesity was 1.30 (95% CI: 1.12, 1.51). Among our population, the odds ratio associated with maternal obesity, independent of underlying genetic susceptibilities, was 1.48 (95% CI: 1.05, 2.09) which was consistent with Stothard's meta-analysis (data not shown). The effect of maternal obesity was markedly accentuated among women who carried either the *MTHFR* 677 C>T polymorphism or the *BHMT* 742G>A, with the risk being highest among those who carried the *MTHFR* TT genotype.

There have been multiple epidemiologic studies examining the association between CHDs and maternal smoking and alcohol intake with inconclusive results.(16–18) We found evidence suggesting that the effect of periconceptional smoking and alcohol intake may depend on underlying genetic susceptibilities. Specifically, the *TCII* 776 CG and GG genotypes when combined with either smoking or alcohol intake increased the risk of CHD-affected pregnancies. This association may be related to lower levels of folate and vitamin B₁₂ in individuals who smoke or drink, coupled with the less efficient transport of B₁₂ into cells and tissue due to the phenotype caused by the *TCII* 776C>G polymorphism.

The current study has several limitations. Only maternal genetic susceptibilities were considered. The genetics of cardiac development is likely a complex interplay between both maternal and fetal genetic susceptibilities. Sample size limitations prevented us from examining individual cardiac phenotypes. It is possible that maternal genetic susceptibilities and lifestyle factors interrupt specific cell types and developmental stages of cardiogenesis. However, it is equally possible that maternal factors that are present throughout the eight weeks of cardiogenesis are just as likely to affect all stages of cardiogenesis, and thus the inclusion of all cardiac phenotypes is justified. Our study was limited to the investigation of only three folate-related polymorphisms, and due to sample size limitations we were unable to examine gene-gene interactions. Information regarding lifestyle factors was obtained through maternal report. We cannot rule out misclassification or residual confounding and interactions with other genetic polymorphisms and/or environmental and lifestyle factors. We were also unable to include information about family history of CHDs or previously affected pregnancies.

In conclusion, the detrimental effect of maternal obesity on the developing heart appears to be greatest among women who carry the *MTHFR* 677C>T or *BHMT* 742G>A polymorphism. Maternal smoking and/or alcohol use in combination with the *TCII* 776C>G polymorphism may increase the risk of CHDs. The current study provides further evidence that the etiology of nonsyndromic CHDs is complex, requiring both genetic susceptibilities and environmental and lifestyle factors to be investigated. Future studies to examine the association between CHDs and maternal and fetal genetic susceptibilities in folate-related genes in association with maternal lifestyle factors are essential to understanding the effect of one-carbon metabolism on the development of CHDs. An improved understanding of CHD etiology will provide evidence upon which personalized primary prevention programs can be built that target the reduction of CHD occurrence. Risk stratification of reproductive-aged women based on knowledge of the impact of genetic susceptibilities, lifestyle factors, and their interaction may play an important role in minimizing adverse pregnancy outcomes, including heart defects and other structural malformations.

Future research studies investigating the association between CHDs and gene-environment interactions may expand the number of common variants by including haplotype tagging SNPs in candidate pathways and/or in genome-wide association studies. Our study was limited to investigating the association between maternal lifestyle factors and maternal

genotypes. It is likely that fetal genotypes will also be very important and further evaluation of gene-gene interactions (epistasis) are also needed.

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Table 1

Demographic and behavioral characteristics of CHD cases and controls

	All Cases N (%)	Controls N (%)	P value
Total	572	363	
Maternal smoking*			
Yes, smoked	167 (29.2)	100 (27.5)	
No	405 (70.8)	263 (72.4)	0.5867
Maternal alcoholic drinking*			
Yes, drank alcohol	159 (27.8)	114 (31.4)	
No	413 (72.2)	249 (68.6)	0.2370
Maternal folate intake[†]			
Yes	311 (54.4)	215 (59.2)	
No	261 (45.6)	148 (40.8)	0.1445
Maternal Body Mass Index			
≥30	153 (26.7)	78 (21.5)	
18.5 to <25	256 (44.8)	193 (53.2)	0.0200
Maternal race/ethnicity			
White, non-Hispanic	447 (78.1)	281 (77.4)	
African American, non- Hispanic	87 (15.2)	65 (17.9)	
Hispanic	30 (5.2)	12 (3.3)	
Other races	8 (1.4)	5 (1.4)	0.4124
Maternal age			
Mean age (sd)	26.7 (5.9)	26.4 (5.9)	0.4819
<20 years	70 (12.2)	50 (13.8)	
20–24 years	172 (30.1)	107 (29.5)	
25–29 years	173 (30.2)	104 (28.7)	
30–34 years	98 (17.1)	74 (20.4)	
35+ years	59 (10.3)	28 (7.7)	0.4752
Maternal education			
0–11 years	81 (14.2)	47 (12.9)	
High school diploma/GED	204 (35.7)	125 (34.4)	
Some college/Technical	148 (25.9)	95 (26.2)	
4+ years	139 (24.3)	96 (26.4)	0.8618
Household income (annual)			
<\$10,000	104 (19.3)	64 (18.7)	
\$10,000-\$30,000	201 (37.2)	116 (33.9)	
\$30,000-\$50,000	120 (22.2)	76 (22.2)	
\$50,000+	115 (21.3)	86 (25.1)	0.5661
Maternal Body Mass Index			
Mean values (sd)	26.6 (7.0)	25.5 (6.1)	0.0145

CHD, congenital heart defect

* One month before pregnancy through third month of pregnancy

[†] One month before pregnancy through first month of pregnancy

Table 2

Association between polymorphisms and CHD assuming various genetic models*.

Polymorphisms		Number of Cases (%)	Number of Controls (%)	OR (95% CI)
<i>MTHFR</i> 677C>T	C	773 (69.9%)	510 (71.6%)	Reference
	T	333 (30.1%)	202 (28.4%)	1.05 (0.85, 1.30)
	CC	285 (51.5%)	191 (53.7%)	Reference
	CT	203 (36.7%)	128 (36.0%)	1.03 (0.77, 1.38)
	TT	65 (11.8%)	37 (10.4%)	1.10 (0.70, 1.74)
	Additive	553 (100%)	356 (100%)	1.05 (0.85, 1.28)
	T Dominant	268 (48.5%)	165 (46.3%)	1.05 (0.80, 1.38)
	T Recessive	65 (11.8%)	37 (10.4%)	1.09 (0.71, 1.69)
<i>BHMT</i> 742G>A	G	777 (71.9%)	490 (75.2%)	Reference
	A	303 (28.1%)	162 (24.8%)	1.16 (0.93, 1.45)
	GG	290 (53.7%)	189 (58.0%)	Reference
	GA	197 (36.5%)	112 (34.4%)	1.14 (0.84, 1.53)
	AA	53 (9.8%)	25 (7.7%)	1.33 (0.80, 2.23)
	Additive	540 (100%)	326 (100%)	1.15 (0.92, 1.42)
	A Dominant	250 (46.3%)	137 (42.0%)	1.17 (0.88, 1.54)
	A Recessive	53 (9.8%)	25 (7.7%)	1.27 (0.77, 2.10)
<i>TCII</i> 776C>G	C	648 (59.4%)	403 (59.3%)	Reference
	G	442 (40.6%)	277 (40.7%)	0.98 (0.81, 1.20)
	CC	189 (34.7%)	123 (36.2%)	Reference
	CG	270 (49.5%)	157 (46.2%)	1.11 (0.82, 1.51)
	GG	86 (15.8%)	60 (17.6%)	0.93 (0.61, 1.41)
	Additive	545 (100%)	340 (100%)	0.98 (0.80, 1.20)
	G Dominant	356 (65.3%)	217 (63.8%)	1.06 (0.79, 1.41)
	G Recessive	86 (15.8%)	60 (17.6%)	0.86 (0.59, 1.24)

CHD, congenital heart defect, *MTHFR*, methylenetetrahydrofolate reductase gene, *BHMT*, betaine homocysteine methyltransferase gene, *TCII*, transcobalamin II gene

* Models adjusted for race

Table 3

Odds ratios* (OR) and 95% confidence intervals for the associations between lifestyle/SNP genotype combination and CHD.

Exposure/Genotype	BMI>=30 OR (95% CI)	Smoking OR (95% CI)	Drinking OR (95% CI)	Periconceptional folic acid OR (95% CI)
MTHFR				
NO/CC	Reference	Reference	Reference	1.11 (0.75, 1.65)
NO/CT	1.06 (0.70, 1.60)	1.10 (0.78, 1.55)	1.04 (0.73, 1.48)	1.27 (0.81, 1.99)
NO/TT	0.96 (0.48, 1.90)	0.96 (0.55, 1.67)	0.87 (0.52, 1.47)	1.95 (0.90, 4.20)
NO/CT or TT	1.01 (0.68, 1.50)	1.07 (0.77, 1.49)	0.99 (0.71, 1.39)	1.41 (0.92, 2.15)
YES/CC	1.38 (0.87, 2.18)	1.12 (0.74, 1.71)	0.80 (0.53, 1.20)	Reference
YES/CT	1.32 (0.78, 2.24)	0.98 (0.60, 1.60)	0.80 (0.50, 1.30)	0.96 (0.66, 1.41)
YES/TT	4.62 (1.54, 13.83)	1.62 (0.74, 3.55)	1.89 (0.72, 4.95)	0.84 (0.48, 1.49)
YES/CT or TT	1.63 (1.00, 2.66)	1.09 (0.70, 1.71)	0.88 (0.56, 1.39)	0.98 (0.69, 1.41)
BHMT				
NO/GG	Reference	Reference	Reference	1.37 (0.92, 2.02)
NO/GA	0.89 (0.58, 1.38)	1.01 (0.71, 1.45)	0.96 (0.67, 1.37)	1.57 (1.00, 2.47)
NO/AA	1.49 (0.72, 3.07)	1.80 (0.94, 3.47)	1.96 (0.98, 3.91)	1.17 (0.53, 2.61)
NO/GA or AA	0.98 (0.66, 1.47)	1.12 (0.80, 1.56)	1.09 (0.78, 1.53)	1.48 (0.97, 2.27)
YES/GG	1.32 (0.83, 2.09)	1.08 (0.71, 1.66)	0.74 (0.49, 1.12)	Reference
YES/GA	1.60 (0.93, 2.76)	1.45 (0.88, 2.39)	1.14 (0.69, 1.88)	1.07 (0.72, 1.58)
YES/AA	2.88 (0.93, 8.92)	0.84 (0.36, 1.96)	0.56 (0.25, 1.27)	1.78 (0.91, 3.48)
YES/GA or AA	1.82 (1.09, 3.03)	1.27 (0.80, 2.02)	0.92 (0.58, 1.46)	1.19 (0.82, 1.71)
TCII				
NO/CC	Reference	Reference	Reference	1.35 (0.84, 2.17)
NO/CG	0.86 (0.55, 1.32)	0.87 (0.61, 1.26)	0.92 (0.63, 1.32)	1.44 (0.93, 2.24)
NO/GG	0.69 (0.38, 1.25)	0.66 (0.41, 1.08)	0.72 (0.44, 1.18)	1.28 (0.67, 2.44)
NO/CG or GG	0.79 (0.52, 1.20)	0.80 (0.57, 1.14)	0.86 (0.60, 1.22)	1.48 (0.97, 2.27)
YES/CC	1.32 (0.72, 2.40)	0.67 (0.40, 1.11)	0.58 (0.36, 0.96)	Reference
YES/CG	1.21 (0.72, 2.03)	1.21 (0.75, 1.97)	0.96 (0.59, 1.55)	1.12 (0.74, 1.68)
YES/GG	1.31 (0.62, 2.78)	1.29 (0.61, 2.74)	0.86 (0.44, 1.69)	0.88 (0.52, 1.49)
YES/CG or GG	1.25 (0.77, 2.03)	1.27 (0.80, 2.02)	0.95 (0.61, 1.49)	1.19 (0.82, 1.71)

SNP, single nucleotide polymorphisms, CHD, congenital heart defect, *MTHFR*, methylenetetrahydrofolate reductase gene, *BHMT*, betaine homocysteine methyltransferase gene, *TCII*, transcobalamin II gene

* Odds ratios adjusted for maternal ethnicity and all other exposures in table.

Table 4

Odds ratios* (OR) and 95% confidence intervals for the associations between SNP genotype and CHD stratified by levels of lifestyle factors.

	Heterozygous [†]			Homozygous rare [‡]			Dominant			Recessive			Additive		
	Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval	
BMI ≥ 18.5 & BMI <25															
MTHFR	1.03	(0.68, 1.56)		0.94	(0.47, 1.89)		1.01	(0.68, 1.50)		0.93	(0.47, 1.82)		0.99	(0.74, 1.34)	
BHMT	0.88	(0.57, 1.36)		1.46	(0.71, 3.02)		0.98	(0.66, 1.47)		1.53	(0.76, 3.10)		1.08	(0.80, 1.45)	
TCII	0.84	(0.54, 1.30)		0.67	(0.37, 1.21)		0.79	(0.52, 1.20)		0.74	(0.44, 1.27)		0.82	(0.62, 1.10)	
BMI ≥ 30															
MTHFR	1.01	(0.55, 1.86)		3.93	(1.24, 12.50)		1.33	(0.74, 2.37)		3.91	(1.27, 12.02)		1.52	(0.98, 2.34)	
BHMT	1.29	(0.70, 2.40)		2.30	(0.71, 7.47)		1.43	(0.79, 2.57)		2.06	(0.65, 6.52)		1.41	(0.89, 2.24)	
TCII	0.98	(0.51, 1.88)		1.09	(0.46, 2.62)		1.01	(0.54, 1.88)		1.11	(0.52, 2.39)		1.04	(0.68, 1.58)	
SMOKE = NO															
MTHFR	1.10	(0.78, 1.55)		0.98	(0.56, 1.70)		1.07	(0.77, 1.49)		0.93	(0.55, 1.58)		1.02	(0.80, 1.31)	
BHMT	1.00	(0.70, 1.43)		1.80	(0.94, 3.47)		1.12	(0.80, 1.56)		1.80	(0.95, 3.41)		1.18	(0.91, 1.54)	
TCII	0.86	(0.60, 1.25)		0.64	(0.39, 1.05)		0.80	(0.57, 1.14)		0.70	(0.46, 1.09)		0.81	(0.64, 1.03)	
SMOKE = YES															
MTHFR	0.91	(0.51, 1.62)		1.52	(0.65, 3.54)		1.04	(0.61, 1.78)		1.58	(0.71, 3.56)		1.13	(0.78, 1.66)	
BHMT	1.15	(0.64, 2.07)		0.81	(0.33, 1.98)		1.06	(0.62, 1.82)		0.77	(0.32, 1.83)		0.98	(0.66, 1.45)	
TCII	1.78	(1.01, 3.16)		1.91	(0.84, 4.34)		1.81	(1.06, 3.11)		1.40	(0.65, 2.99)		1.48	(1.00, 2.19)	
DRINKING = NO															
MTHFR	1.03	(0.72, 1.48)		0.88	(0.52, 1.48)		0.99	(0.71, 1.39)		0.86	(0.52, 1.42)		0.96	(0.76, 1.23)	
BHMT	0.96	(0.67, 1.37)		2.01	(1.01, 4.01)		1.09	(0.78, 1.53)		2.05	(1.04, 4.02)		1.19	(0.91, 1.55)	
TCII	0.91	(0.63, 1.31)		0.70	(0.43, 1.17)		0.86	(0.60, 1.22)		0.75	(0.48, 1.17)		0.85	(0.67, 1.09)	
DRINKING = YES															
MTHFR	0.94	(0.54, 1.62)		2.27	(0.83, 6.18)		1.11	(0.67, 1.87)		2.33	(0.87, 6.20)		1.24	(0.84, 1.83)	
BHMT	1.49	(0.84, 2.62)		0.73	(0.31, 1.73)		1.26	(0.75, 2.12)		0.62	(0.27, 1.44)		1.03	(0.70, 1.51)	
TCII	1.76	(0.99, 3.10)		1.58	(0.74, 3.37)		1.71	(1.00, 2.92)		1.15	(0.58, 2.28)		1.34	(0.93, 1.94)	
FOLATE = NO															
MTHFR	1.11	(0.69, 1.80)		1.58	(0.71, 3.50)		1.20	(0.76, 1.88)		1.51	(0.70, 3.27)		1.20	(0.86, 1.68)	
BHMT	1.05	(0.64, 1.72)		0.83	(0.36, 1.91)		1.00	(0.63, 1.60)		0.81	(0.36, 1.83)		0.96	(0.67, 1.38)	

	Heterozygous [†]			Homozygous rare [‡]			Dominant			Recessive			Additive		
	Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval		Odds Ratio	95% Conf. Interval	
TCII	1.03	(0.65, 1.65)		0.96	(0.48, 1.92)		1.02	(0.65, 1.60)		0.94	(0.50, 1.77)		0.99	(0.71, 1.38)	
FOLATE = YES															
MTHFR	0.99	(0.68, 1.45)		0.95	(0.53, 1.70)		0.98	(0.69, 1.41)		0.96	(0.55, 1.66)		0.98	(0.76, 1.27)	
BHMT	1.05	(0.71, 1.55)		1.88	(0.96, 3.68)		1.19	(0.82, 1.71)		1.84	(0.96, 3.55)		1.24	(0.94, 1.63)	
TCII	1.09	(0.73, 1.65)		0.85	(0.50, 1.44)		1.02	(0.69, 1.50)		0.81	(0.51, 1.28)		0.94	(0.73, 1.22)	

SNP, single nucleotide polymorphisms, CHD, congenital heart defect, BMI, body mass index, *MTHFR*, methylenetetrahydrofolate reductase gene, *BHMT*, betaine homocysteine methyltransferase gene, *TCII*, transcobalamin II gene

* Odds ratios adjusted for maternal ethnicity and all other exposures in table.

[†] Compare to Heterozygous wild.