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Folate and Vitamin B12 Related Genes and Risk for Omphalocele

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Abstract

Both taking folic acid-containing vitamins around conception and consuming food fortified with folic acid have been reported to reduce omphalocele rates. Genetic factors are etiologically important in omphalocele as well; our pilot study showed a relationship with the folate metabolic enzyme gene methylenetetrahydrofolate reductase (*MTHFR*). We studied 169 non-aneuploid omphalocele cases and 761 unaffected, matched controls from all New York State births occurring between 1998 and 2005 to look for associations with single nucleotide polymorphisms (SNPs) known to be important in folate, vitamin B12, or choline metabolism. In the total study population, variants in the transcobalamin receptor gene (*TCblR*), rs2232775 (Q8R), and the *MTHFR* gene, rs1801131 (1298A>C), were significantly associated with omphalocele. In African-Americans significant associations were found with SNPs in genes for the vitamin B12 transporter (*TCN2*) and the vitamin B12 receptor (*TCblR*). A SNP in the homocysteine-related gene, betaine-homocysteine S-methyltransferase (*BHMT*), rs3733890 (R239Q), was significantly associated with omphalocele in both African-Americans and Asians. Only the TCblR association in the total population remained statistically significant if Bonferroni correction was applied. The finding that transcobalamin receptor (*TCblR*) and transporter (*TCN2*) SNPs and a *BHMT* SNP were associated with omphalocele suggests that disruption of methylation reactions, in which folate, vitamin B12, and homocysteine play critical parts, may be a risk factor for omphalocele. Our data, if confirmed, suggest that supplements containing both folic acid and vitamin B12 may be beneficial in preventing omphaloceles.

Keywords

omphalocele; folate; vitamin B12; homocysteine; transcobalamin; transcobalamin receptor

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Introduction

Omphalocele is a defect of the anterior abdominal wall that ranges in birth prevalence from 0.8 to 3.9 per 10,000 births (Stoll et al. 2001). Genetic factors are known to be etiologically important (OMIM ID 164750 Omphalocele); chromosomal abnormalities and syndromes are responsible for many cases. An autosomal recessive pattern of inheritance has been suggested for isolated cases (Yang 1992) but the etiology of the non-aneuploid cases is largely unknown (Chen 2007). Women who take multivitamins, most of which contain folic acid and vitamin B12 (B12), around the time of conception have been reported to be at reduced risk for having children with omphalocele (Botto et al. 2002). Folic acid is well known to prevent neural tube defects and perhaps other birth defects as well (MRC Vitamin Study Research Group 1991; Czeizel and Dudás 1992), and a variant of a folate enzyme gene, *MTHFR* c.677C>T, has been shown to be a major risk factor for neural tube defects (Kirke et al. 2004). Moreover, low B12 and high total homocysteine blood concentrations have been associated with neural tube defects, but genetic variants in B12 and homocysteine enzyme genes have not been investigated in omphalocele.

Some recent data suggest that the addition of folic acid to fortified cereal grain products in the United States has not only reduced neural tube defect rates, but has significantly reduced omphalocele rates as well (Canfield et al. 2005). These observations have stimulated research into possible folate-related genetic risk factors for omphalocele. Previously, we conducted a small, pilot study of 25 children with euploid omphalocele and 59 matched controls that found an association between omphalocele and the T allele in *MTHFR* c.677 C>T and recommended additional investigation (Mills et al. 2005).

We have now expanded that study by greatly increasing the number of non-syndromic omphalocele cases and the number of single nucleotide polymorphisms (SNPs) that we examined in folate, homocysteine and B12 enzyme genes, and by adding other SNPs that have been associated with other birth defects or shown to be potentially functionally important.

Methods

Subjects

All live births in New York State between 1998 and 2005 formed the cohort for this study (N= 2,023,083). A nested case control study was performed. Cases were live born infants with omphalocele and controls were live born infants without any major malformations. Stratified random sampling was employed to select the control group. A pool of potential controls was randomly selected from the New York State Newborn Screening Program stratifying by year for birth years 1998–2005. To generate controls for this study, subjects from that pool were then frequency matched by race/ethnicity to cases.

Omphalocele cases were identified via the New York State Congenital Malformations Registry (CMR). The methods by which cases are identified by the CMR have been reported in detail previously (Sekhobo et al. 2001). In brief, the CMR has been in existence since 1983 and covers the entire state of New York. The CMR relies on reporting of children with major malformations to the CMR as mandated by law, and the CMR staff undertakes several measures to monitor the completeness of reporting. The CMR receives reports on approximately 10,000 children each year. Beginning in 1992, the CMR began to use the British Pediatric Association coding, which allows for greater specificity than coding using the International Classification of Diseases, 9th revision. CMR staff performs the coding based on narrative descriptions of the birth defects reported by hospitals. A capture-recapture analysis showed that the CMR was about 87% complete for major malformations

in general (Honein and Paulozzi 1999). A similar estimate of 89% was recently obtained after matching CMR reports with reports from the active case-ascertainment of the National Birth Defects Prevention Study in New York State (C. Druschel unpublished data -2002).

We identified 199 cases from the CMR of which 7 were excluded for aneuploidy (4 trisomy 21; 1 mosaic trisomy 13; 1 trisomy 13 and 1 trisomy 18) and one for missing race/ethnicity. Next cases with known or suspected causes were dropped: 13 with Beckwith-Wiedemann syndrome, one with OEIS (omphalocele-exstrophy-imperforate anus-spinal defects) complex, one with Pentology of Cantrell plus one infant of a diabetic mother.

Newborn screening is performed on blood samples from heel sticks collected on Guthrie cards for all births in New York State. After testing is completed, residual samples are archived. Samples were retrieved for this study and then de-identified. Samples from omphalocele case and control infants were identified and checked to determine that there was sufficient blood remaining for analysis and that their parents had not requested that the sample not be used for research. Five case infants did not have sufficient blood for analysis and one sample was misidentified; no parents had asked that the samples be excluded. The final study population was 169 cases. There were 764 controls selected. Samples could not be located for two and one was an infant of a diabetic mother, leaving a final study population of 761 controls. The 25 cases from our pilot study were included but different controls were chosen.

Children reported to the CMR are matched to their birth certificate and this data source was used to supplement the information from the CMR report. New York State birth certificates provided data on socio-demographic factors, maternal smoking, and preconceptional maternal diabetes mellitus. Information on exposure to potentially teratogenic drugs was not available. The quality of reporting has been checked for various factors (IMPRO 2001, Roohan et al. 2003). The overall accuracy rate is approximately 95%; however, it is much less accurate for items such as medical conditions and illicit drug use.

Institutional Review Board approval from the New York State Department of Health was obtained for this study. The study was reviewed by the Office of Human Subjects Research at The National Institutes of Health.

Thirteen genes were selected for inclusion based on the following criteria: they were known to affect folate, vitamin B12, homocysteine or choline metabolism; or in the case of *BRCA1*, had been associated with neural tube defects (King et al. 2007). Twenty-three SNPs from these genes were selected for genotyping because they had been shown to cause functional changes, cause changes in highly conserved sequences or to be associated with adverse clinical outcomes.

Laboratory Methods

Genomic DNA was extracted from 3 mm dried blood spot punches using a laboratory-developed protocol for DNA extraction using sodium hydroxide precipitation. At least 30ng of the extracted DNA was whole genome amplified by KBiosciences (Herts, UK) using a primer extension pre-amplification method. For each subject, two separate amplifications were carried out and the amplification products were each genotyped.

SNPs were genotyped by KBiosciences using KASPar technology (proprietary fluorescent-based competitive allelic discrimination assays) after whole genome amplification. There were no discordant genotype results when genotypes from the two independent amplification reactions were compared or when repeat genotyping was performed blinded on 5% of the samples.

One SNP, rs897453, in the *PEMT* gene is triallelic. It was run as a biallelic SNP. All 23 SNPs were successfully called >98% of the time. Tests of Hardy-Weinberg equilibrium were carried out independently for cases and controls by race/ethnic group. Three SNPs were not in Hardy-Weinberg equilibrium using a cut-off of p=0.01. These were *FOLH1* rs61886492 (p=0.000078) and *TCN2* rs9606756 (p=0.008) for African-American controls, and *BRCA1* rs3737559 (p=0.00013) for Asian controls.

Linkage disequilibrium measures were estimated using Haploview (http://www.broadinstitute.org/haploview/haploview) (Barrett et al. 2005) based on the genotypes of control samples.

Statistical Methods

Characteristics of case and control infants were compared using Fisher's exact test except for birth year where the Pearson chi-square test was used. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for comparing genotype distributions between case and control infants. Maternal race/ethnicity (white non-Hispanic, African-American, Hispanic, Asian, other) was included in all regression models. Other potential confounders were identified after reviewing the scientific literature on risk factors for omphalocele. These included maternal age (<20, 20–34, ≥35 years), maternal education (<12 years, 12 years, >12 years), maternal smoking (yes, no), previous live births (yes, no), plurality (singleton, multiple birth), birth year, and conception of index pregnancy using *in vitro* fertilization/assisted reproductive technology (yes, no). An initial model was fitted using race as the only covariate because cases and controls were frequency-matched by race. Covariates were tested for association with omphalocele and those that had a p value <0.1, maternal education and use of *in vitro* fertilization/assisted reproductive technology, were included in the final model. The Bonferroni method was used to adjust for multiple comparisons. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

After the exclusions described above, 169 omphalocele cases and 761 control subjects remained. There were 82 isolated cases and 87 with other major defects. Six cases and 20 controls were multiple births.

Subjects' characteristics are shown in Table 1. Case and control mothers and offspring did not differ significantly on the variables examined, although a greater proportion of the case mothers were ≥35 years of age.

The selected SNPs and their functions are shown on Table 2. In the initial analysis adjusting only for race/ethnicity, the results showed that homozygosity for the minor alleles of two SNPs were associated with omphalocele (uncorrected p values): *MTHFR* 1298 c.A>C (OR: 2.04; 95% CI: 1.06, 3.94; p=0.028) and *TCblR* [*CD320*] p.Q8R (OR: 3.20; 95% CI: 1.55, 6.62; p=0.0017). In the full model, adjusted for maternal race, education and *in vitro* fertilization/assisted reproductive technology, both associations remained significant with almost no change in the OR, CI or p values. After adjustment for multiple comparisons, the association with *TCblR* remained statistically significant.

Race/ethnicity-specific analyses adjusted for the same covariates showed pronounced differences in the associations by race/ethnic group. There were no significant associations in the white non-Hispanic or Hispanic populations (data not shown). In the African-American population homozygosity for *MTHFR c.*1298A>C (p=0.047), *BHMT p.*R239Q (p=0.031) and two SNPs in *TCblR*, rs173665 (p=0.028) and p.Q8R (p=0.024), were

significantly associated with omphalocele (Table 3). Heterozygosity for two SNPs in the transcobalamin 2 gene (*TCN2*), p. I23V (p=0.0024) and p.S348F (p=0.014), were significantly associated with omphalocele. It is possible that the very small number of homozygous cases and controls made it impossible to show an effect in homozygotes (See Table 3). In the Asian population heterozygosity in *MTHFR c.*1298A>C (p=0.034) and *BHMT* p.R239Q (p=0.028) were significantly associated with omphalocele despite there being only 8 cases and 36 controls (Table 3). There were too few homozygotes to examine the effect of homozygosity.

Next, these tests were repeated using only singleton omphalocele cases. The results were similar for all analyses. Examining only isolated omphalocele cases was difficult because of small numbers in each racial/ethnic group. Some findings were no longer statistically significant. Somewhat surprisingly, in white non-Hispanics, homozygosity for *TCN2* p.P259R (OR: 2.92; 95% CI: 1.06, 8.05; p=0.038) and heterozygosity for *FOLH1* p.H475Y (OR: 3.09; 95% CI: 1.16, 8.21; p=0.024) became statistically significant. The findings in these race/ethnic group specific analyses did not remain significant after Bonferroni correction.

Discussion

Our data for the total study population show associations between omphalocele and variants in two genes: the transcobalamin receptor gene (*TCblR*) and the methylenetetrahydrofolate reductase gene (*MTHFR*). Variants in *TCblR* have recently been shown to be a risk factor for neural tube defects (Pangilinan et al. 2010) and the SNP that we found to be associated with omphalocele, (rs2232775) p.Q8R is in linkage disequilibrium with the SNP that was associated with neural tube defects in that study. Despite very conservative correction for multiple testing, this finding remained statistically significant. The *MTHFR* gene has been studied extensively because of its importance in converting the methylene form of tetrahydrofolate to the methyl form. This function is critical to the conversion of homocysteine to methionine, the major source of methyl groups for methylation reactions.

This finding is noteworthy because of the associations we identified in the race/ethnic group specific analysis. Despite modest numbers, we found a number of variants in genes related to homocysteine metabolism in the African-American and Asian groups. *MTHFR* and betaine-homocysteine S-methyltransferase (*BHMT*) SNPs were associated with omphalocele in both groups. The latter gene plays an important role in the generation of methyl groups via the choline pathway. The other associations we identified in African-Americans all related to vitamin B12 transport and receptor functions. Transcobalamin 2 (*TCN2*) and the transcobalamin receptor (*TCblR*) play a critical role in delivery of vitamin B12 into the cell. Vitamin B12 is necessary for the function of the methionine synthase reaction that transports a methyl group from folate to homocysteine to form methionine.

It is interesting that an association between low maternal circulating vitamin B12 levels and neural tube defects independent of maternal folate has been reported (Kirke et al. 1993). Omphalocele has been linked to folate via the finding that women who use folic acid-containing vitamins are at reduced risk (Botto et al. 2002). Our data suggest that vitamin B12, another constituent of multivitamins, could be contributing to this protective effect. Our finding that variants in multiple genes involved in homocysteine metabolism are associated with increased risk for omphalocele is noteworthy because women carrying a fetus with a neural tube defect have significantly higher homocysteine levels during pregnancy than women who have unaffected fetuses (Mills et al. 1995). The relationship between homocysteine and omphalocele has not, to our knowledge, been investigated previously except for a study of complex defects of various types in which the mothers

whose fetuses had defects, only 7 (9%) of which were omphaloceles, had 25% higher amniotic fluid homocysteine levels than controls (Brouns et al. 2008). Higher homocysteine levels could be associated with disturbances in one carbon metabolism. This pathway is the source of methyl groups used during DNA methylation.

Because non-syndromic omphalocele cases are rare, little has been published regarding genetic risk factors. Our pilot study (Mills et al. 2005) showed an association between omphalocele and another *MTHFR* variant, 677C>T, in 25 cases and called for additional investigation. The current, much larger study, does not confirm this finding.

Some strengths and limitations of this study should be noted. This study is, to our knowledge, the largest to date; it is population-based; and birth defects have been shown to be well ascertained in the New York system. Samples were available for study on a very large percentage of cases. Thus, neither selection bias, nor or lack of participation, are a concern. Controls are also a representative sample; they were selected from the entire underlying population of births. Limitations include the limited information available on covariates such as diabetes, alcohol and exposure to teratogenic drugs, although these would not be expected to account for many cases. Furthermore, information on family history and details on possible syndromes were limited. Details on syndromes and other clinical information come from hospital reports and the amount of detail varies from case to case. Checks of medical records showed that reporting of omphalocele was accurate. Although many of the associations we found were no longer significant after correction for multiple comparisons, it could be argued that this is overcorrecting because several of the positive findings occurred in genes that were tested as *a priori* hypotheses because of their association with neural tube defects. In any case, these positive findings occurred in race/ethnic group specific analyses where the number of subjects was very small even in this large study. Therefore, they deserve careful follow up.

In summary this study found that numerous SNPs related to homocysteine metabolism via folate, vitamin B12 or choline were associated with omphalocele. These findings suggest that the previously reported protective effect of multivitamin use might have been due to production of methyl groups by converting homocysteine to methionine, a process which would be assisted by several multivitamin constituents including folate and vitamin B12. Our results suggest that the relationship between omphalocele and methylation, and the roles of vitamin B12, and the transcobalamin receptor in particular, merit investigation. Our findings, if confirmed, suggest that both folic acid and vitamin B12 might be useful in preventing omphaloceles.

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Table 1

Characteristics of omphalocele case and control groups.

Characteristic	Cases (N = 169)[a]	Controls (N = 761)[a]	P value[b]
Maternal race/ethnicity			0.91
White, Non-Hispanic	85 (50.30)	380 (49.93)	
African-American	47 (27.81)	195 (25.62)	
Hispanic	27 (15.98)	142 (18.66)	
Asian	8 (4.73)	36 (4.73)	
Other	2 (1.18)	8 (1.05)	
Maternal age (years)			0.38
<20	11 (6.51)	75 (9.86)	
20 – 34	125 (73.96)	552 (72.54)	
≥ 35	33 (19.53)	134 (17.61)	
Maternal education (years)			0.09
<12	23 (13.61)	157 (20.63)	
12	54 (31.95)	232 (30.49)	
≥ 12	91 (53.85)	362 (47.57)	
Missing	1 (0.59)	10 (1.31)	
Maternal smoking			
Yes	19 (11.24)	69 (9.07)	0.38
No	149 (88.17)	691 (90.80)	
Missing	1 (0.59)	1 (0.13)	
Previous live births			
Yes	93 (54.44)	465 (61.10)	0.12
No	77 (45.56)	296 (38.90)	
In vitro fertilization/assisted reproductive technology			
Yes	5 (2.96)	8 (1.05)	0.07
No	164 (97.04)	753 (98.95)	
Plurality			
Multiple birth	6 (3.55)	20 (2.63)	0.45

Hum Genet. Author manuscript; available in PMC 2012 June 13.

Characteristic	Cases (N = 169)[a]	Controls (N = 761)[a]	P value[b]
Singleton birth	163 (96.45)	741 (97.37)	
Birth year			0.45
1998	16 (9.47)	109 (14.32)	
1999	14 (8.28)	89 (11.70)	
2000	19 (11.24)	96 (12.61)	
2001	24 (14.20)	96 (12.61)	
2002	24 (14.20)	104 (13.67)	
2003	22 (13.02)	89 (11.70)	
2004	27 (15.98)	94 (12.35)	
2005	23 (13.61)	84 (11.04)	

[a] Values are N (%)

[b] Comparison between case and control groups: Pearson chi-square to compare birth year; Fisher's exact test to compare all other variables.

Table 2

Comparison of genotype distributions between omphalocele cases and controls.

Gene symbol; name	Protein function	SNP	Description	Alleles[a]	Control Genotypes[b]	Case Genotypes[b]	Adjusted odds ratio (95% Confidence Interval)[c]	
							Heterozygous	Homozygous for minor allele
MTHFR; Methylenetetrahydrofolate reductase (NAD(P)H)	Converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate	rs1801131	c.1298A>C	A/C	463/259/37	93/61/15	1.17 (0.81, 1.70)	**2.04 (1.06, 3.94)**
		rs1801133	c.677C>T	C/T	342/316/101	81/76/12	1.02 (0.71, 1.48)	0.52 (0.27, 1.02)
MTR; Methionine synthase	Converts homocysteine and 5-methyltetrahydrofolate to methionine and tetrahydrofolate	rs1805087	p.D919G	A/G	465/250/41	110/43/11	0.73 (0.49, 1.07)	1.10 (0.54, 2.26)
MTRR; Methionine synthase reductase	Regenerates functional methionine synthase via reductive methylation	rs1801394	p.I22M	A/G	288/327/146	60/74/34	1.08 (0.73, 1.58)	1.12 (0.68, 1.82)
		rs1532268	p.S175 L	G/A	378/311/65	82/68/13	0.97 (0.68, 1.40)	0.93 (0.48, 1.78)
BHMT; Betaine-homocysteine S-methyl-transferase	Converts betaine and homocysteine to dimethylglycine and methionine	rs3733890	p.R239Q	G/A	413/282/66	80/71/13	1.34 (0.94, 1.93)	1.02 (0.53, 1.96)
FOLH1 (also known as *GCPII*); Folate hydrolase (folylpoly-γ-glutamate carboxy-peptidase)	Hydrolyzes folate polyglutamates	rs61886492	p.H475Y	C/T	714/40/3	152/14/1	1.58 (0.82, 3.05)	1.56 (0.16, 15.22)
MTHFD1; Methylene tetrahydrofolate dehydrogenase (NADP+ dependent) 1	C1-synthase trifunctional enzyme catalyzing the interconversion of 1-carbon derivatives of tetrahydrofolate	rs2236225	p.R653Q	C/T	282/327/150	68/67/29	0.85 (0.58, 1.26)	0.81 (0.49, 1.34)
PEMT; Phosphatidyl-ethanolamine N-methyl-transferase	Converts phosphatidyl-ethanolamine to phosphatidyl-choline by sequential methylation	rs7946	p.V175M	T/C	271/315/169	57/73/37	1.14 (0.76, 1.70)	1.11 (0.67, 1.85)
		rs897453	c.V58I	G/A	388/278/89	84/61/21	1.01 (0.68, 1.50)	1.16 (0.65, 2.07)
		rs12325817	p.744C>G	C/G	362/301/96	80/68/18	1.04 (0.71, 1.54)	0.87 (0.48, 1.59)
SHMT1; Serine hydroxymethyl-transferase 1 (soluble)	Converts serine and tetrahydrofolate to glycine and 5,10-methylene tetrahydrofolate	rs1979277	p.L474F	G/A	391/304/59	84/71/9	1.05 (0.74, 1.50)	0.64 (0.30, 1.37)
PCFT (also known as SLC46A1); Proton-coupled folate transporter	Transport of folate across cell membranes under specific pH conditions	rs9909629	A>T (5′ near gene)	A/T	615/132/14	142/25/2	0.84 (0.52, 1.34)	0.61 (0.13, 2.80)
		rs11080058	G>A (5′ near gene)	G/A	344/333/83	79/71/17	0.92 (0.64, 1.32)	0.92 (0.51, 1.65)
BRCA1; Breast cancer 1, early onset	Involved in transcription, DNA repair of double-stranded breaks, and recombination; acts as tumor suppressor	rs3737559	G>A (intron)	G/A	673/81/7	146/19/1	1.08 (0.63, 1.84)	-
TCblR (also known as *CD320*); Transcobalamin receptor	Facilitates the uptake of transcobalamin-bound vitamin B12 into tissues	rs173665	C>T (3′ near gene)	G/A	600/153/8	133/29/5	0.87 (0.56, 1.35)	3.10 (0.95, 10.13)

Gene symbol; name	Protein function	SNP	Description	Alleles[a]	Control Genotypes[b]	Case Genotypes[b]	Adjusted odds ratio (95% Confidence Interval)[c]	
							Heterozygous	Homozygous for minor allele
		rs2227288	G>C (5′ near gene)	G/C	536/207/17	122/38/8	0.83 (0.56, 1.25)	2.06 (0.85, 4.95)
		rs2336573	p.G220R	C/T	593/145/22	127/31/9	0.98 (0.62, 1.55)	1.99 (0.84, 4.74)
		rs2232775	p.Q8R	A/G	544/180/33	112/35/18	1.02 (0.64, 1.61)	**3.20 (1.55, 6.62)**
RFC1 (also known as *SLC19A1*); Reduced folate carrier	Facilitates the transport of reduced folates into cells	rs1051266	p.H27R	G/A	216/359/184	40/76/49	1.16 (0.76, 1.78)	1.43 (0.89, 2.31)
TCN2; Transcobalamin II	Binds cobalamin and mediates its transport into cells	rs9606756	p.I23V	A/G	571/173/17	117/46/4	1.32 (0.90, 1.94)	1.23 (0.40, 3.76)
		rs1801198	p.P259R	C/G	315/340/101	62/77/26	1.16 (0.79, 1.70)	1.35 (0.79, 2.30)
		rs9621049	p.S348F	C/T	574/172/14	118/46/2	1.30 (0.89, 1.92)	0.72 (0.16, 3.21)

[a] Major allele is listed first

[b] Genotypes values are numbers of individuals with homozygous major allele/heterozygous/homozygous minor allele

[c] Homozygous for major allele was reference category; adjustment for maternal race, maternal education, and *in vitro* fertilization/assisted reproductive technique

Table 3

Single nucleotide polymorphisms associated with omphalocele based on logistic regression analyses stratified by race/ethnicity.

Race/Ethnicity	Gene	SNP	Description	Alleles[a]	Control Genotypes[b]	Case Genotypes[b]	Adjusted odds ratio (95% Confidence Interval)[c]	
							Heterozygous	Homozygous for minor allele
African-American	*MTHFR*	rs1801131	c.1298A>C	A/C	148/44/2	32/12/3	1.34 (0.63, 2.83)	**6.44 (1.03, 40.41)**
	BHMT	rs3733890	p.R239Q	G/A	138/52/5	23/18/4	**2.05 (1.02, 4.12)**	**4.62 (1.15, 18.52)**
	TCblR	rs173665	C>T (3′ near gene)	G/A	142/52/1	31/11/3	0.98 (0.46, 2.10)	**13.27 (1.33, 132.35)**
	TCblR	rs2232775	p.Q8R	A/G	69/94/32	12/18/15	1.09 (0.49, 2.42)	**2.74 (1.14, 6.57)**
	TCN2	rs9606756	p.I23V	A/G	146/40/9	24/20/2	**2.92 (1.46, 5.83)**	1.40 (0.28, 6.90)
	TCN2	rs9621049	p.S348F	C/T	147/42/6	26/18/1	**2.38 (1.19, 4.77)**	0.96 (0.11, 8.30)
Asian	*MTHFR*	rs1801131	c.1298A>C	A/C	20/13/3	1/6/1	**13.54 (1.22, 150.88)**	4.62 (0.20, 106.66)
	BHMT	rs3733890	p.R239Q	G/A	21/13/2	1/6/1	**14.30 (1.34, 152.78)**	18.25 (0.56, 591.86)

[a] Major allele is listed first

[b] Genotype values are numbers of individuals in the race/ethnic group with homozygous major allele/heterozygous/homozygous minor allele

[c] Homozygous for major allele was reference category; adjustment for maternal education and *in vitro* fertilization/assisted reproductive technique

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