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Nicotinamide N-methyl Transferase (*NNMT*) Gene Polymorphisms and Risk for Spina Bifida

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Abstract

Background—Moderate hyperhomocysteinemia is a known risk factor for neural tube defects (NTDs) in a variety of experimental model systems and is believed to be important in humans as well. The enzyme Nicotinamide N-methyl transferase (*NNMT*) was identified in a genome-wide linkage scan as being an important regulator of homocysteine (Hcy) homeostasis in a Spanish population making it an interesting candidate gene for NTDs.

Methods—We evaluated eleven SNPs of the *NNMT* gene in our study population. In this study, 251 cases (infants with spina bifida) and 335 controls (non-malformed infants), born during the periods 1983–1986 in selected counties in California, were genotyped for variants of the *NNMT* gene. Allelic, genotype and haplotype associations with spina bifida risk were evaluated and analyzed.

Results—None of the SNPs studied alone showed allelic or genotypic associations with spina bifida. However, the TCAG haplotype for block 3 (rs2852447, rs2852425, rs4646337 and rs11569688) showed a decreased risk for spina bifida among non-Hispanic whites (OR: 0.4; 95% CI: 0.1–1.0).

Conclusion—No association was found between infant *NNMT* gene variants and risk for spina bifida in our study population. However, small sample sizes for most variant groups and for phase-unknown haplotype data limited the power of the study.

Keywords

Nicotinamide N-methyl transferase (*NNMT*); neural tube defects (NTDs); genotype; haplotype; association study

INTRODUCTION

Maternal hyperhomocysteinemia is considered a risk factor for neural tube defects (Steeegers-Theunissen et al., 1994; Mills et al., 1995, 1996; Rosenquist et al., 2001). Hcy is an intermediate in both sulfur-containing amino acid metabolism and one-carbon

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metabolism. Its remethylation to methionine is catalyzed by the enzyme methionine synthase and serves to link the folate cycle with that of Hcy metabolism. Both epidemiological studies and experimental animal models indicate that folate and other B vitamin deficiencies combined with genetic polymorphisms in pathway-specific genes, such as 5, 10-methylenetetrahydrofolate reductase (MTHFR), 5-methyl-tetrahydrofolate-homocysteine methyltransferase (MTR), 5-methyltetrahydro-folate-homocysteine methyltransferase reductase (MTRR), cystathionine beta-synthase (CBS), thymidylate synthetase (TYMS), contribute to the accumulation of Hcy. Genetic factors among these pathways may contribute to NTDs risk though maternal or fetal genotypes or both (Doolin et al., 2002; Zhu et al., 2003).

In an effort to better understand the genetic basis of Hcy regulation, Souto et al. (2005) conducted a genome-wide linkage scan for genes affecting variation in plasma Hcy concentrations. The strongest linkage signal (LOD score) was observed on chromosome 11q23, where they mapped a gene involved in Hcy metabolism. This gene codes for the enzyme nicotinamide N-methyltransferase, NNMT. It catalyzes the N-methylation of nicotinamide and other pyridines to form pyridinium ions and is one of at least 39 SAM-dependent methyltransferases described in mammals (Clarke and Banfield, 2001). These methyltransferases utilize methyl groups from S-adenosyl-methionine (AdoMet) and produce S-adenosyl-homocysteine (AdoHcy), which are potent inhibitors of transmethylation reactions, which are critical in donating methyl groups for a host of essential reactions, including DNA synthesis (Mato et al., 1997). There is evidence that these methyltransferases could also affect Hcy level, such as phosphatidylethanolamine N-methyltransferase (PEMT) (Noga et al., 2003; Zhang et al., 2006). Souto et al (2005) analyzed 10 SNPs in *NNMT* gene and found that at least one haplotype was associated with increased Hcy concentrations in a Spanish population. Knowing the critical role of NNMT in Hcy metabolism, we investigated the *NNMT* gene to determine whether *NNMT* gene variants increased an infant's risk of spina bifida in a California population.

METHODS

Study Subjects

Data and samples of this report are derived from a population-base case-control study. Case and control infants were identified by the California Birth Defects Monitoring Program, an active surveillance system for collecting information on infants and fetuses with congenital malformations. Diagnostic and demographic information was collected by program staff from multiple sources of medical records for all live born, stillborn fetuses (defined as >20 weeks gestation), and pregnancies electively or spontaneously terminated. Overall ascertainment for major malformations has been estimated as 97% complete (Schulman et al., 1993). Because we used the DNA from newborn screening bloodspots for the analyses, only live born infants were eligible for this study. Consequently, 251 cases (infants with spina bifida) and 335 controls (non-malformed infants) born during 1983 and 1986 in selected counties in California were included. Among the cases, 88 (34.9%) were non-Hispanic Whites, 129 (51.2%) were Hispanic Whites, including US and foreign born, and 35 (13.9%) were other ethnic groups. Among controls, 153 (45.7%) were non-Hispanic Whites, 110 (32.8%) were Hispanic Whites, including US and foreign born, and 72 (20.9%) belonged to other ethnic groups. The study protocol was approved by the State of California Health and Welfare Agency Committee for the Protection of Human Subjects. Genomic DNA was extracted from dried newborn screening blood spots on filter paper using the Puregene DNA Extraction kit (Gentra, Minneapolis, MN) and was amplified with Genomiphi DNA amplification kit (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) according to the manufacturers' instructions.

Genotyping analysis

Based on the previous report of *NNMT* gene variants that affect Hcy concentrations (Souto et al., 2005), we selected for genotyping eleven SNPs (rs505978, rs683271, rs694539, rs11214921, rs10891641, rs2852432, rs4646335, rs2852447, rs2852425, rs4646337, and rs11569688). All SNP genotyping was performed with TaqMan Single-Nucleotide-Polymorphism genotyping assays (Assay-on-Demand, Applied Biosystems, Foster City, CA) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Genotyping assays were performed by laboratory technicians who were blinded to case-control status of the samples. Five percent of the samples were duplicates to assess possible genotyping error.

Statistical analysis

The risk for spina bifida was evaluated by using multiple analytical approaches, based upon allelic, genotype and haplotype associations. Genotypic associations were measured by odds ratios (ORs) using logistic regression models (SAS software version 9.1). Allelic and haplotypic associations were evaluated using HaploView version 4.0 software (Daly Lab at the Broad Institute, Cambridge, MA) (Barrett et al., 2005). Among control infants, minor allele frequencies (MAF) were computed and deviations from Hardy-Weinberg Equilibrium (HWE) were evaluated for each SNP. If a *p* value was less than 0.001, data were considered to have deviated from HWE. Pair-wise Linkage Disequilibrium (LD) between markers was measured by D' (defined as the linkage disequilibrium measure, D , divided by the theoretical maximum for observed allele frequencies), and the correlation coefficient r^2 (defined as square of the correlation coefficient between the alleles at two loci, which is related to the power of association in gene mapping). HaploView software predicts haplotype based on an expectation-maximization (EM) algorithm. For each haplotype, counts for case and control association tests were obtained by summing the fractional likelihoods of each individual. For example, if an individual was determined by the EM algorithm to have a 40% likelihood of haplotype A and 60% likelihood of haplotype B, then 0.4 and 0.6 would be added to the counts for A and B respectively. Ninety five percent confidence interval (95% CI) of allelic and haplotype frequencies were calculated using the online program, VassarStats (Lowry, <http://faculty.vassar.edu/lowry/VassarStats.html>).

RESULTS AND DISCUSSION

Eleven SNPs were genotyped, including those near the 5' region of the gene (rs505978, rs683271, rs694539, rs11214921, rs10891641, and rs2852432), those within exon1 (rs4646335), intron2 (rs2852447 and rs2852425), and those at the 3' region (rs4646337 and rs11569688) (Table 1). Genotyping was 100% successful for all SNPs, save for rs505978, which was 99.6% among cases and 99.7% among controls. Missing genotyping results was due to failure of PCR amplification. Table 1 shows the information of all SNPs studied and the results for allelic association analyses. Among controls, non-Hispanic whites and Hispanic whites were in Hardy-Weinberg Equilibrium for all investigated SNPs. Minor allele frequencies in spina bifida infants were not significantly different from those observed in control infants among non-Hispanic whites and Hispanic whites, respectively. Table 2 shows the associations between individual *NNMT* SNP genotypes and spina bifida risk in the overall population, as well as in the two major ethnic subpopulations, non-Hispanic whites and Hispanic whites. Nearly all SNPs had imprecise estimated effects as judged by the confidence intervals. TT genotype of SNP rs4646335 showed rather high odds ratios when stratified by ethnicity group: in non-Hispanic whites: OR=5.7, 95% CI: 1.1–28.5; in Hispanic whites, OR=4.4, 95% CI: 0.9–21, however, these estimates were based on small sample sizes with cell number smaller than 5.

Strong linkage disequilibria were observed in both ethnic sub-populations based on calculation of D' and r^2 (Table 3). Fifteen haplotypes for all SNPs were inferred in non-Hispanic white and sixteen among Hispanic whites (Table 4). Haplotype frequency was estimated based on the EM algorithm. The most common haplotype was GCCTGGACTAG, whose estimated frequency was 0.409 in non-Hispanic white controls, and 0.362 in Hispanic white controls. All other haplotypes were calculated compared to the most common haplotype, and no significant association with spina bifida was detected. Three haplotype blocks were defined based on D' and r^2 , including block1, rs505978 and rs683271, block2, rs11214921 and rs10891641, and block3, rs2852447, rs2852425, rs4646337 and rs11569688. The association of these haplotype blocks with spina bifida was investigated. Haplotype TCAG in block 3 showed a reduced risk for spina bifida in non-Hispanic white infants (OR=0.4, 95% CI: 0.1–1.0). To our knowledge, this is the first epidemiologic study to investigate potential associations between *NNMT* gene variants and spina bifida. Despite the biologic plausibility of these gene variants as regulators of Hcy levels, our analyses did not indicate that *NNMT* gene variants contribute to risk of spina bifida.

The primary goal of our study was to evaluate the potential association between embryonic genetic variations of *NNMT* gene and spina bifida. The common haplotype amongst our populations was identical to that described in the Spanish population (Souto et al., 2005). However, allelic, genotypic, and other haplotype association analyses did not reveal substantial changes in risk, nor ones that were precise (statistically significant). One limitation of our study is the small sample size, which effectively reduced the statistical power of our analyses. Another limitation of our study is that the haplotype analyses were based on phase-unknown genotyping data, which is known to be less informative. Larger sample size, and phase-known genotyping data would enhance study power and provide more information in our efforts to discover the causes of human NTDs.

The relationship between genetic variations that are involved in the folate-Hcy metabolic axis and the risk of spina bifida is known to be complicated, especially with the potential involvement of both the maternal and the embryonic genotype in determination of disease risk. Therefore it is important to consider both maternal and embryonic genotypes when evaluating putative susceptibility loci. In this study, we evaluated the *NNMT* gene in infant samples and found that embryonic genotype alone was not sufficient to increase spina bifida risk in our study population. It is necessary to evaluate the *NNMT* gene in case and control mothers in future studies.

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Description of *NNMT* SNP markers and allelic association analyses for spina bifida among non-Hispanic white vs. Hispanic white births in selected counties in California (1983–1986)

Table 1

dbSNP ID (rs#)	Chromosome position (Chr. 11, NCBI Build 36.2)	Alleles	Gene Region	Observed/Predicted Heterozygosity*				HWE p Value*		Minor Allele Frequency (95% Confidence Interval)				Allelic Association p Value	
				NHW		HW		NHW	HW	control	SB	HW	control	NHW	HW
rs505978	113634267	G/T	5' near gene	0.354 / 0.418	0.433 / 0.436	0.026	0.990	0.305 (0.241,0.377)	0.294 (0.246,0.348)	0.337 (0.282,0.397)	0.303 (0.246,0.367)	0.809	0.423		
rs683271	113634355	C/T	5' near gene	0.361 / 0.421	0.431 / 0.435	0.039	0.966	0.307 (0.243,0.379)	0.297 (0.249,0.351)	0.337 (0.282,0.398)	0.300 (0.243,0.364)	0.828	0.385		
rs694539	113638629	C/T	5' near gene	0.27 / 0.296	0.28 / 0.303	0.238	0.325	0.193 (0.142,0.258)	0.173 (0.135,0.220)	0.190 (0.147,0.242)	0.182 (0.136,0.238)	0.583	0.821		
rs11214921	113642022	T/C	5' near gene	0.324 / 0.361	0.301 / 0.29	0.146	0.739	0.250 (0.192,0.319)	0.229 (0.185,0.280)	0.182 (0.140,0.234)	0.168 (0.125,0.223)	0.597	0.689		
rs10891641	113644291	G/A	5' near gene	0.328 / 0.376	0.318 / 0.316	0.067	1.000	0.256 (0.197,0.325)	0.248 (0.203,0.300)	0.202 (0.157,0.255)	0.191 (0.144,0.248)	0.859	0.771		
rs2852432	113669214	G/A	5' near gene	0.402 / 0.403	0.402 / 0.359	1.000	0.094	0.25 (0.192,0.319)	0.297 (0.249,0.351)	0.244 (0.196,0.300)	0.223 (0.173,0.282)	0.265	0.581		
rs4646335	113672152	A/T	exon 1	0.373 / 0.348	0.41 / 0.376	0.352	0.229	0.233 (0.177,0.301)	0.219 (0.176,0.269)	0.279 (0.228,0.337)	0.218 (0.169,0.277)	0.723	0.126		
rs2852447	113684911	C/T	intron 2	0.349 / 0.386	0.343 / 0.359	0.170	0.587	0.244 (0.187,0.313)	0.271 (0.225,0.324)	0.240 (0.192,0.296)	0.227 (0.177,0.287)	0.517	0.737		
rs2852425	113687717	T/C	intron 2	0.353 / 0.38	0.356 / 0.357	0.324	1.000	0.239 (0.182,0.307)	0.265 (0.218,0.317)	0.240 (0.192,0.297)	0.223 (0.173,0.282)	0.527	0.650		
rs4646337	113688519	A/G	3' near gene	0.307 / 0.295	0.276 / 0.357	0.665	0.413	0.210 (0.157,0.276)	0.193 (0.153,0.241)	0.202 (0.157,0.255)	0.155 (0.113,0.208)	0.645	0.182		
rs11569688	113688653	G/A	3' near gene	0.278 / 0.177	0.163 / 0.357	0.615	0.354	0.199 (0.147,0.264)	0.163 (0.126,0.209)	0.116 (0.083,0.162)	0.077 (0.049,0.120)	0.325	0.153		

NHW: non-Hispanic white; HW: Hispanic white; HWE: Hardy-Weinberg Equilibrium; SB: spina bifida.

* Computed in control group

Table 2

Genotypic association analyses for spina bifida among non-Hispanic white vs. Hispanic white births in selected counties in California (1983–1986), odds ratios estimated using logistic regression.

	No. of Spina Bifida (%)	No. of Controls (%)	Crude OR (95% CI)	NHW OR (95% CI)	HW OR (95% CI)
rs505978					
GG	112 (44.6)	157 (47)	Reference	Reference	Reference
GT	112 (44.6)	133 (39.8)	1.2 (0.8–1.7)	1.5 (0.9–2.7)	0.9 (0.5–1.6)
TT	27 (10.8)	44 (13.2)	0.9 (0.5–1.5)	0.8 (0.3–1.9)	1.8 (0.7–4.6)
rs683271					
CC	118 (46.8)	159 (47.5)	Reference	Reference	Reference
CT	107 (42.5)	135 (40.3)	1.1 (0.8–1.5)	1.5 (0.9–2.7)	0.9 (0.5–1.6)
TT	27 (10.7)	41 (12.2)	0.9 (0.5–1.5)	0.8 (0.3–1.8)	1.9 (0.7–4.7)
rs694539					
CC	167 (66.3)	214 (63.9)	Reference	Reference	Reference
CT	71 (28.2)	105 (31.3)	0.9 (0.6–1.2)	1.1 (0.6–1.9)	0.8 (0.5–1.4)
TT	14 (5.6)	16 (4.8)	1.1 (0.5–2.4)	1.5 (0.4–5.1)	2.2 (0.6–8.6)
rs2852425					
TT	140 (55.6)	191 (57)	Reference	Reference	Reference
CT	92 (36.5)	118 (35.2)	1.1 (0.8–1.5)	1.0 (0.6–1.8)	0.9 (0.5–1.6)
CC	20 (7.9)	26 (7.8)	1.0 (0.6–2.0)	0.6 (0.2–1.8)	1.9 (0.6–6.5)
rs2852432					
GG	134 (53.2)	162 (48.4)	Reference	Reference	Reference
AG	97 (38.5)	139 (41.5)	0.8 (0.6–1.2)	0.7 (0.4–1.1)	1 (0.6–1.7)
AA	21 (8.3)	34 (10.1)	0.7 (0.4–1.3)	0.8 (0.3–2.3)	2.6 (0.5–3.5)
rs2852447					
CC	141 (56)	190 (56.7)	Reference	Reference	Reference
CT	89 (35.3)	114 (34)	1.1 (0.7–1.5)	1.0 (0.6–1.7)	1 (0.6–1.7)
TT	22 (8.7)	31 (9.3)	1.0 (0.5–1.7)	0.7 (0.2–1.8)	1.3 (0.4–3.9)
rs4646335					
AA	142 (56.3)	189 (56.4)	Reference	Reference	Reference
AT	91 (36.1)	129 (38.5)	0.9 (0.7–1.3)	0.7 (0.4–1.2)	1.2 (0.7–2)
TT	19 (7.5)	17 (5.1)	1.5 (0.7–3.0)	5.7 (1.1–28.5)	4.4 (0.9–21)

	No. of Spina Bifida (%)	No. of Controls (%)	Crude OR (95% CI)	NHW OR (95% CI)	HW OR (95% CI)
rs4646337					
AA	160 (63.5)	229 (68.4)	Reference	Reference	Reference
AG	79 (31.3)	93 (27.8)	1.2 (0.8–1.7)	1.0 (0.6–1.8)	1.5 (0.8–2.7)
GG	13 (5.2)	13 (3.9)	1.4 (0.6–3.2)	1.5 (0.4–5.1)	1.4 (0.4–5.3)
rs10891641					
GG	144 (57.1)	189 (56.4)	Reference	Reference	Reference
AG	87 (34.5)	116 (34.6)	1.0 (0.7–1.4)	1.2 (0.7–2.1)	1 (0.5–1.6)
AA	21 (8.3)	30 (9)	0.9 (0.5–1.7)	0.9 (0.3–2.4)	1.7 (0.4–7.1)
rs11214921					
TT	156 (61.9)	204 (60.9)	Reference	Reference	Reference
CT	83 (32.9)	106 (31.6)	1.0 (0.7–1.5)	1.3 (0.8–2.3)	1 (0.6–1.8)
CC	13 (5.2)	25 (7.5)	0.7 (0.3–1.4)	1.0 (0.3–2.7)	1.7 (0.3–9.8)
rs11569688					
GG	189 (75)	266 (79.4)	Reference	Reference	Reference
AG	55 (21.8)	62 (18.5)	1.2 (0.8–1.9)	1.1 (0.6–2.0)	1.9 (0.9–3.9)
AA	8 (3.2)	7 (2.1)	1.6 (0.6–4.5)	2.3 (0.6–8.9)	0.9 (0.1–6.8)

OR: odds ratio; CI: confidence interval; NHW: non-Hispanic white; HW: Hispanic white

Table 3

Pairwise LD of NNMT SNP markers in non-Hispanic white vs. Hispanic white

Non-Hispanic White*													
$r^2 \setminus D'$	rs505978	rs683271	rs694539	rs11214921	rs10891641	rs2852432	rs4646335	rs2852447	rs2852425	rs4646337	rs11569688		
rs505978	---	1.000	0.831	0.823	0.796	0.354	0.161	0.004	0.019	0.110	0.240		
rs683271	0.990	---	0.832	0.837	0.810	0.366	0.153	0.010	0.017	0.124	0.250		
rs694539	0.355	0.354	---	0.871	0.917	0.782	0.387	0.095	0.099	0.727	0.688		
rs11214921	0.491	0.505	0.540	---	0.952	0.576	0.189	0.086	0.061	0.204	0.363		
rs10891641	0.498	0.511	0.553	0.838	---	0.622	0.227	0.124	0.102	0.120	0.294		
rs2852432	0.114	0.121	0.347	0.264	0.334	---	0.649	0.158	0.139	0.235	0.573		
rs4646335	0.018	0.016	0.114	0.033	0.044	0.312	---	0.618	0.599	0.766	0.752		
rs2852447	0.000	0.000	0.001	0.006	0.015	0.023	0.039	---	1.000	1.000	1.000		
rs2852425	0.000	0.000	0.001	0.003	0.010	0.017	0.035	0.968	---	1.000	1.000		
rs4646337	0.001	0.002	0.029	0.003	0.001	0.005	0.042	0.703	0.726	---	1.000		
rs11569688	0.005	0.006	0.022	0.009	0.006	0.027	0.035	0.605	0.625	0.861	---		
Hispanic White*													
$r^2 \setminus D'$	rs505978	rs683271	rs694539	rs11214921	rs10891641	rs2852432	rs4646335	rs2852447	rs2852425	rs4646337	rs11569688		
rs505978	---	0.990	0.883	0.872	0.751	0.184	0.072	0.084	0.087	0.053	0.132		
rs683271	0.971	---	0.885	0.853	0.701	0.156	0.053	0.074	0.076	0.041	0.135		
rs694539	0.373	0.381	---	0.491	0.416	0.382	0.057	0.159	0.057	0.437	0.761		
rs11214921	0.339	0.329	0.225	---	0.939	0.447	0.048	0.242	0.246	0.113	0.145		
rs10891641	0.285	0.255	0.162	0.768	---	0.437	0.009	0.287	0.291	0.197	0.094		
rs2852432	0.022	0.016	0.109	0.139	0.153	---	0.225	0.275	0.295	0.064	0.510		
rs4646335	0.001	0.000	0.002	0.001	0.000	0.046	---	0.921	0.919	0.856	0.775		
rs2852447	0.005	0.004	0.002	0.041	0.066	0.076	0.087	---	0.988	0.984	1.000		
rs2852425	0.005	0.004	0.000	0.043	0.068	0.086	0.086	0.965	---	0.984	1.000		
rs4646337	0.001	0.001	0.010	0.013	0.035	0.003	0.054	0.694	0.702	---	1.000		
rs11569688	0.004	0.004	0.014	0.011	0.004	0.009	0.022	0.356	0.361	0.497	---		

* D' is above the diagonal, and r^2 is below the diagonal.

Table 4

Haplotype association analyses for spina bifida among non-Hispanic white vs. Hispanic white births in selected counties in California (1983–1986),

Haplotype	Frequency (95% CI)*	Estimated Haplotype Counts		OR (95% CI)
		SB	Control	
Non-Hispanic white				
GCCTGGACTAG	0.409 (0.355, 0.465)	69.0	124.9	Reference
GCCTGGATCGA	0.109 (0.079, 0.149)	24.4	33.3	1.3 (0.7, 2.4)
TTTCAATCTAG	0.066 (0.043, 0.100)	14.4	20.2	1.3 (0.6, 2.7)
GCCTGATCTAG	0.056 (0.035, 0.088)	9.2	17.1	1.0 (0.4, 2.3)
TTCTGGACTAG	0.051 (0.031, 0.081)	8.2	15.4	1.0 (0.4, 2.4)
GCCTGGTCTAG	0.037 (0.021, 0.065)	9.0	11.3	1.4 (0.6, 3.6)
TTCCAGACTAG	0.034 (0.019, 0.061)	4.4	10.5	0.8 (0.2, 2.4)
TTTCAAACCTAG	0.027 (0.014, 0.052)	5.1	8.3	1.1 (0.4, 3.5)
GCCTGAATCAG	0.029 (0.015, 0.054)	0.1	8.7	---
TTTCAAATCAG	0.017 (0.007, 0.038)	2.9	5.1	---
TTCCAGATCGA	0.015 (0.006, 0.036)	2.8	4.6	---
TTCCAAATCGG	0.015 (0.006, 0.036)	2.0	4.7	---
TTCTGGATCG	0.013 (0.005, 0.033)	1.8	3.9	---
GCCTGATTCGA	0.011 (0.004, 0.030)	1.8	3.4	---
TTTCAGACTAG	0.007 (0.002, 0.025)	2.9	2.2	---
Hispanic white				
GCCTGGACTAG	0.362 (0.292, 0.418)	80.3	77.3	Reference
GCCTGGTCTAG	0.107 (0.073, 0.155)	35.0	23.5	1.4 (0.8, 2.6)
TTTTGGACTAG	0.071 (0.044, 0.112)	18.9	15.5	1.2 (0.6, 2.5)
GCCTGGATCGA	0.056 (0.032, 0.094)	12.7	12.2	1.0 (0.4, 2.3)
TTTCAATCTAG	0.036 (0.018, 0.070)	16.9	7.9	2.1 (0.8, 5.1)
GCCTGATCTAG	0.043 (0.023, 0.078)	11.9	9.4	1.2 (0.5, 3.0)
TTCTGGACTAG	0.035 (0.018, 0.069)	9.3	7.7	1.2 (0.4, 3.2)
TTCCAGACTAG	0.047 (0.026, 0.084)	5.8	10.4	0.5 (0.2, 1.6)
TTCTGGTCTAG	0.028 (0.013, 0.059)	6.8	6.1	1.1 (0.3, 3.3)
GCCTGAATCAG	0.024 (0.010, 0.054)	6.5	5.2	1.2 (0.4, 4.0)
TTCCAGATCGA	0.009 (0.003, 0.033)	8.2	2.0	---
GCCTGAATCGG	0.016 (0.006, 0.043)	6.5	3.6	---
GCCTGGATCGG	0.015 (0.005, 0.041)	4.9	3.3	---
TTTCAAATCAG	0.024 (0.010, 0.054)	2.0	5.2	---
GCCTGAACTAG	0.010 (0.003, 0.034)	3.1	2.2	---
GCCTAGATCGG	0.010 (0.003, 0.034)	3.0	2.2	---

Haplotype	Frequency (95% CI)*	Estimated Haplotype Counts		OR (95% CI)
		SB	Control	
Non-Hispanic white				
Block1: rs505978-rs683271				

Haplotype	Frequency (95% CI)*	Estimated Haplotype Counts		OR (95% CI)
		SB	Control	
GC	0.703 (0.649, 0.751)	122.0	215.0	Reference
TT	0.294 (0.246, 0.348)	54.0	90.0	1.1 (0.7, 1.6)
Block2: rs11214921-rs10891641				
TG	0.748 (0.697, 0.794)	128.0	229.0	Reference
CA	0.225 (0.182, 0.276)	41.0	69.0	1.1 (0.7, 1.7)
TA	0.023 (0.011, 0.047)	4.0	7.0	---
Block3: rs2852447-rs2852425-rs4646337-rs11569688				
CTAG	0.729 (0.676, 0.776)	133.0	223.0	Reference
TCGA	0.163 (0.126, 0.209)	35.0	50.0	1.2 (0.7, 1.9)
TCAG	0.072 (0.048, 0.107)	5.0	22.0	0.4 (0.1, 1.0)
TCGG	0.029 (0.016, 0.055)	2.0	9.0	---
Hispanic white				
Block1: rs505978-rs683271				
GC	0.691 (0.627, 0.748)	171.0	152.0	Reference
TT	0.295(0.239, 0.359)	87.0	65.0	1.2 (0.8, 1.8)
Block2: rs11214921-rs10891641				
TG	0.795 (0.737, 0.843)	204.9	174.9	Reference
CA	0.154 (0.112, 0.208)	45.9	33.9	1.2 (0.7, 1.9)
TA	0.037 (0.019, 0.071)	6.1	8.1	0.6 (0.2, 1.9)
Block3: rs2852447-rs2852425-rs4646337-rs11569688				
CTAG	0.768 (0.708, 0.819)	195.0	169.0	Reference
TCGA	0.077 (0.049, 0.120)	30.0	17.0	1.5 (0.8, 2.8)
TCAG	0.077 (0.049, 0.120)	21.0	17.0	1.1 (0.5, 2.1)
TCGG	0.064 (0.038, 0.104)	11.0	14.0	1.1 (0.5, 2.2)

* Estimated haplotype frequencies and their 95% confidence intervals in control group CI: confidence interval; SB: spina bifida; OR: odds ratio.