No evidence for heterozygote advantage at *MTHFR* in patients with lumbosacral myelomeningocele or their relatives

To the Editor:

Weitkamp et al.[1] reported a heterozygote advantage of the *MTHFR* gene in patients with neural tube defects (NTD) and their relatives. A C-to-T substitution at nucleotide 677 that converts an alanine to a valine residue in the 5,10-methylenetetrahydofolate-reductase gene (*MTHFR*) yields a common thermolabile variant (T) with 30% to 50% activity of the allelic form (C). Homozygosity for the C677T allele has been shown to be more prevalent in individuals with NTD and their parents, as compared to controls in several studies.[2–5] Weitkamp et al.[1] used a data sample of 168 families with NTD pregnancy (81 anencephaly, 1 encephalocele, and 86 spina bifida). The families were from a variety of ethnic origins consisting of 92.3% Western European, 7.0% Native American, 0.4% African American, and 0.3% Asian. The study concluded that there was an excess of C/T heterozygosity in NTD affected females and also in fathers and unaffected brothers of NTD patients, demonstrating a C/T heterozygote advantage.

From our series of families collected as part of a national collaborative effort, we have identified 166 families in which the proband has lumbosacral myelomeningocele. Our sample includes 166 probands, 162 mothers, 136 fathers, and 120 unaffected sibs. These families were American Caucasian and had no history of neural tube defects before the birth of the proband. *MTHFR* was genotyped as previously reported.[6] Table 1 shows the number of offspring of each genotype according to sex and diagnosis. In some families, maternal (4 families) or paternal genotypes (30 families) were unavailable. We assigned these families to parental mating types assuming Hardy Weinberg probabilities. Expected values presented in Table 1 were calculated based on Mendelian probabilities. Chi-square tests were used to test for allelic and genotypic transmission distortion from Mendelian expectations in affected and unaffected offspring of heterozygous parents. Deviation from Hardy Weinberg Equilibrium (HWE) in parents of NTD probands was assessed. We also tested for deviation from HWE in NTD male and female offspring.

The allele transmission ratio from C/T heterozygous parents was 84.93C: 86.67T to offspring with NTD ($\chi^2 = 0.018$, $P = 0.8932$) and 55.27C: 65.7T to unaffected offspring ($\chi^2 = 0.891$, $P = 0.3451$). Additionally, the number of offspring of each genotype (C/C, C/T, or T/T) among the 225.3 offspring of C/T heterozygous parents did not differ from Mendelian expectations ($\chi^2 = 1.14$, $P = 0.5655$, 2df). This was the case in the 129.5 affected offspring ($\chi^2 = 1.0$, $P = 0.61$, 2df), and the 95.76 unaffected offspring ($\chi^2 = 0.65$, $P = 0.72$, 2df). Therefore, there is neither an excess of heterozygotes (107.22 observed, 112.65 expected) nor a deficiency of C/C (74.52 observed, 74.84 expected) or T/T homozygotes (43.56 observed, 37.82 expected).

There was no departure from Hardy-Weinberg equilibrium in mother's genotypes (65 C/C, 82 C/T, 20 T/T observed; 67.05 C/C, 76.90 C/T, 22.05 T/T expected; ($\chi^2 = 0.71$, $P = 0.70$, 2df) or father's genotypes (63.91 C/C, 84.84 C/T, 17.25 T/T observed; 68.11 C/C, 76.44 C/T, 21.45 T/T expected; $\chi^2 = 2.0$, $P = 0.3678$, 2df). Weitkamp et al.[1] found increased heterozygosity in affected female offspring compared to affected male

Table 1
Number of offspring of each genotype according to sex and diagnosis

Mating type		Estimated # of matings	Number of offspring with genotype[a]											
			Affected						Unaffected					
			Male			Female			Male			Female		
MAT	PAT		CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT
CC	TT	6.08		1.36			4.72		2				1	
TT	CC	5.98		4.62	1					2.2	0.54		1	0.82
CC	CT	29.76	10.9 (8.5)	6.1 (8.5)		10.3 (8.8)	7.3 (8.8)		12.4 (12.7)	13 (12.7)		5.5 (5.7)	6 (5.7)	
CT	CC	29.23	5.1 (5.8)	6.6 (5.8)		9.1 (8.8)	8.5 (8.8)		4 (3.2)	2.4 (3.2)		4 (4.5)	5 (4.5)	
CT	CT	42.06	3.4 (4.8)	10.7 (9.5)	5 (4.8)	5.9 (5.8)	9.3 (11.5)	7.8 (5.8)	3 (2.9)	4.5 (5.7)	4 (2.9)	1 (3.4)	8.9 (6.9)	3.8 (3.4)
CC	CC	28.7	11.5	0.54		11.7			6.6			10.5		
CT	TT	10.63		1.4 (2.7)	4 (2.7)		2.3 (2.7)	3.2 (2.7)		1 (2)	3 (2)		1.1 (2.5)	4 (2.5)
TT	CT	13.02		3.4 (3.6)	3.8 (3.6)		4 (2.8)	1.6 (2.8)		2 (2.2)	2.5 (2.2)		4 (2.4)	0.82 (2.4)
TT	TT	0.54			0.18		0.36							0.36
Total		166	30.9 (19.1)	34.6 (30.2)	14 (11)	37 (23.3)	36 (34.6)	13 (11.3)	28 (18.7)	25 (25.8)	10 (7.1)	21 (13.7)	27 (22)	9.8 (8.4)

[a]Expected values in parenthesis were based on Mendelian probabilities for gene transmission from heterozygous parents (or from all parents in the totals). Expected values in brackets were calculated assuming Hardy-Weinberg equilibrium for each group (spina bifida male, spina bifida female, unaffected male, unaffected female). Chi-square analysis of the totals for each group compared with expectation based on Mendelian gene transmission from heterozygous parents or Hardy-Weinberg equilibrium.

offspring, suggesting a sex difference. Because male fetuses may have a higher spontaneous abortion rate than females, this result may suggest that the *MTHFR* heterozygous genotype may reduce fetal survival in males. In our study, we failed to find any such evidence in affected females (37 C/C, 36 C/T, 13 T/T observed; 35.2 C/C, 39.7 C/T, 11.2 T/T expected; $\chi^2 = 0.72$, $P = 0.70$, 2df) or in affected males (30.92 C/C, 34.64 C/T, 14 T/T observed; 29.2 C/C, 38 C/T, 12.3 T/T expected; $\chi^2 = 0.632$, $P = 0.73$, 2df), or in the fathers (68.11 C/C, 76.44 C/T, 21.45 T/T expected; $\chi^2 = 2.0$, $P = 0.37$, 2df)

There are several potential explanations for our failure to replicate the finding of Weitkamp et al.[1] Firstly, it is well established that population stratification, known or unknown, can adversely impact the conclusions regarding allelic associations; in other words, the results found in the previous study may have been due purely to effects of mixing of different ethnic groups whereas our sample was limited to American Caucasians. Additionally, in several previous studies, an excess of females was generally reported among children with anencephaly, but no major difference was seen among spina bifida patients.[7–9] Perhaps *MTHFR* heterozygous males may have a selective disadvantage in upper level NTDs, including anencephaly and not in lower level NTDs. This may have been the case in our sample, which consisted primarily of lumbosacral myelomeningocele NTDs.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from NS39818 and NS26630.

References

1. Weitkamp LR, Tackels DC, Hunter AG, Holmes LB, Schwartz CE. Heterozygote advantage of the *MTHFR* gene in patients with neural-tube defect and their relatives. *Lancet* 1998;351:1554–1555.
2. van der Put NM, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* 1995;346:1070–1071.
3. Whitehead AS, Gallagherm P, Mills JL, Kirke P, Burke H, Molloy AM et al. A genetic defect in 5,10 methylenetetrahydrofolate reductase in neural tube defects. *Quart J Med* 1995;88:763–766.
4. Ou CY, Stevenson RE, Brown VK, Schwartz CE, Allen WP, Khoury MJ et al. 5,10 Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. *Am J Med Genet* 1996;63:610–614.
5. Christensen B, Arbour L, Tran P, Leclerc D, Sabbaghian N, Platt R et al. Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. *Am J Med Genet* 1999;84:151–157.
6. Rampersaud E, Melvin EC, Siegel D, Mehltretter L, Dickerson ME, George TM et al; NTD Collaborative. Updated investigations of the role of *MTHFR* in human neural tube defects. *Clin Genet* 2003;63:210–214.
7. Bale PM. Sacrococcygeal developmental abnormalities and tumors in children. *Perspect Pediatr Pathol* 1984;8:9–56.
8. Martinez Frias ML, Parralo JA, Salvador J, Frias JL. Sex ratios in neural tube defects. *Lancet* 1986;2:871–872.
9. Hall JG. Neural tube defects, sex ratios, and X inactivation. *Lancet* 1986;1334–1335:

E. Rampersaud, MSPH
C. Brusato, BS
E.C. Melvin, MS, CGC
M.C. Speer, PhD
and the NTD Collaborative Group
Center for Human Genetics
Duke University Medical Center
Durham, North Carolina
K. Metcalf
NC School of Science and Math
Durham, North Carolina