ymptotic assumptions to calculate it. In any case, it would seem preferable for analysts to quote one-sided *P* values rather than to have to resort to the average bias-controlling procedure suggested by Province—although this should not be taken to imply that either *P* values or LOD scores are completely satisfactory summary statistics.

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Examinations of Methylenetetrahydrofolate Reductase C677T and A1298C Mutations—and In Utero Viability

To the Editor:

The recently published study by Isotalo et al. (2000) analyzed the methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C mutations in neonatal and fetal groups, to determine whether particular MTHFR genotype combinations are associated with decreased in utero viability. Isotalo et al. (2000) observed all possible genotype combinations in the fetal group, but combined 677CT/1298CC and 677TT/1298CC genotypes were not observed in the neonatal group. Therefore, they hypothesized that decreased viability exists among fetuses carrying the 677CT/1298CC and 677TT/1298CC genotypes, with a possible selection disadvantage in fetuses with an increased number of mutant MTHFR alleles. They also did not observe the 677CT/1298CC and 677TT/1298CC genotypes in a population consisting of healthy adult controls.

We have tested for the MTHFR C677T and A1298C mutations in a Hispanic population of Mexican descent, to determine risk for spina bifida (SB) (Volcik et al. 2000). Although we observed all possible MTHFR 677/1298 ge-

Table 1

Combined MTHFR C677T/A1298C Genotype or Allele Frequencies in a Hispanic Population of Mexican Descent, Composed of Patients with SB, Their Parents, and Controls

Genotype or Allele	Observed Frequency in			
	Patients (n = 302)	Mothers (n = 281)	Fathers (n = 143)	Controls (n = 82)
MTHFR C677T/A1298C genotype:				
CT/CC	.003	.000	.007	.012
TT/AC	.020	.028	.021	.024
TT/CC	.000	.004	.000	.000
MTHFR allele:				
677C	.493	.477	.549	.527
677T	.507	.523	.451	.473
1298A	.854	.856	.815	.811
1298C	.146	.144	.185	.189

Table 2

Combined MTHFR C677T/A1298C Genotype or Allele Frequencies in a U.S. Population of European Descent, Composed of Patients with SB, Their Parents, and Controls

Genotype or Allele	Observed Frequency in			
	Patients (n = 160)	Mothers (n = 149)	Fathers (n = 107)	Controls (n = 66)
MTHFR C677T/A1298C genotype:				
CT/CC	.000	.020	.019	.015
TT/AC	.038	.007	.019	.000
TT/CC	.000	.000	.000	.000
MTHFR allele:				
677C	.578	.601	.551	.682
677T	.422	.409	.449	.318
1298A	.694	.721	.734	.712
1298C	.306	.279	.266	.288

notype combinations in this Hispanic population, the 677TT/1298CC combination was observed only once, in the mother of an affected individual (table 1). We have analyzed the MTHFR C677T and A1298C mutations in a U.S. population of European descent, composed of patients with SB, their parents, and controls, and have observed the 677CT/1298CC genotype combination (table 2). In addition, we have observed the MTHFR C677T and A1298C mutations in a Canadian population of European descent, composed of patients with SB and their parents (table 3). We observed only a single individual, a patient with SB, with the 677TT/1298AC genotype. However, because of the small size of our sample, we expected that only one or two individuals in each of the groups would have the 677TT/1298AC genotype. It is therefore difficult to reach conclusions, on the basis of the absence of this genotype in the small Canadian population that we studied. The presence of these genotypes in healthy parents and controls militates against the hypothesis, proposed by Isotalo et al. (2000), that the absence of the 677CT/1298CC genotype suggests that "additional MTHFR mutations in *cis* are potentially deleterious or lethal" (Isotalo et al. 2000, p. 989). Perhaps it is the combination of two mutant alleles at both sites (677TT/1298CC), reaching a threshold of four, rather than three, mutations that creates a disadvantage. Other groups have also identified individuals with the 677T and 1298C alleles in the *cis* configuration and individuals with the mutations in the *trans* configuration (Weisberg et al. 1998; Friedman et al. 1999).

Table 3

Combined MTHFR C677T/A1298C Genotype or Allele Frequencies in a Canadian Population of European Descent, Composed of Patients with SB and Their Parents

Genotype or Allele	Observed Frequency in		
	Patients (n = 46)	Mothers (n = 45)	Fathers (n = 30)
MTHFR C677T/A1298C genotype:			
CT/CC	.022	.067	.033
TT/AC	.022	.000	.000
TT/CC	.000	.000	.000
MTHFR allele:			
677C	.641	.722	.683
677T	.359	.278	.317
1298A	.696	.589	.667
1298C	.304	.411	.333

An additional concern is that Isotalo et al. (2000) fail to indicate the ethnicity of the population that they studied. Therefore, we have provided data from three ethnic groups (Hispanics of Mexican descent, U.S. individuals of European descent, and Canadians of European descent), to compare genotype and allele frequencies. If the population studied by Isotalo et al. (2000) was Canadian, it is notable that they did not observe the 677CT/1298CC genotype in their neonatal group, whereas we observed this genotype in 2%–6% of the Canadian population, of patients with SB and their parents, that we studied. Our data support the conclusion of Isotalo et al. (2000) concerning decreased viability among fetuses with the 677TT/1298CC genotype, because we did not observe this genotype in the U.S. and Canadian populations that we studied. Because we observed, in three different populations, the 677CT/1298CC genotype at frequencies nearing those expected, we conclude that this genotype does not result in a significant selective disadvantage.

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Reply to Volcik et al.

To the Editor:

Volcik et al. (2001 [in this issue]) provide supporting evidence that methylenetetrahydrofolate reductase (MTHFR) 677 and 1298 alleles have crossed over, as we have demonstrated in fetal tissue and in antenatal subjects (Isotalo et al. 2000). Their observations also support the findings of Hanson et al. (2001), who demonstrated, in a study of adults, a genotype frequency of 0.2% for both MTHFR 677CT/1298CC and MTHFR 677TT/1298AC. The only MTHFR crossover combination that we observed in an antenatal control group was 677TT/1298AC (Isotalo et. al 2000). We hypothesized that the allelic combinations 677TT/1298CC and 677CT/1298CC are potentially deleterious or lethal in utero; however, the existence of 677CT/1298CC in both adults and children with spina bifida (SB) casts some doubt on our hypothesis.

A major consideration not addressed by Volcik et al. (2001 [in this issue]), concerning their study group, is that their study focused on children with SB and on their parents. Children with SB have survived a fetal defect—one that is known to cause stillbirth and miscarriage. Without medical intervention, postgestational mortality in infants with SB is high. Therefore, the MTHFR genotype combinations and frequencies observed in the nonviable group that we studied may overlap with those in groups with SB.

Additionally, development of SB, as well as of other neural-tube defects, can be reduced by folate sufficiency and therefore is affected by diet and, possibly, by other defects within the folate-delivery and metabolic pathways. Folate status has been shown to affect the contribution of the MTHFR 677 genotype to SB (Christensen et al. 1999). Nutritional-status differences between the study groups may influence the survival of specific genotypes. The contribution that the mother makes to SB or to fetal demise may be, in part, genetic—as was possibly the case in the U.S. 677TT/1298AC representation—and most certainly is in part due to maternal folate sufficiency. The distribution of 677TT/1298AC combined genotypes was well represented in all children with SB and in Hispanic parents but not in U.S. parents. Notably, the only groups with a strong representation of 677CT/1298CC, relative to the 677TT/1298AC genotype, were the U.S. and Canadian mothers of children with SB. This is in contrast to the lack of 677CT/1298CC in our Canadian antenatal control group. Of 148 control subjects, Volcik et al. found 2 individuals with the 677CT/1298CC genotype.

Volcik et al.'s Canadian study group consisted of affected families with SB and did not contain controls; therefore, we find it difficult, in relation to our control group, to draw conclusions concerning their findings of the 677CT/1298CC combinations. Our Canadian group was predominantly of European (Celtic) descent, although some Canadians of other derivations were included. It is interesting to note that Weisberg et al. (1998) also studied a Canadian population of children with SB and their mothers and did not identify any individuals with the 677CT/1298CC or 677TT/1298CC genotypes.

We still hypothesize that combined common polymorphisms of MTHFR play a role in fetal demise and in the development of neural-tube defects. Maternal MTHFR is in the position to affect the quantity and form of folate delivered to the fetus, whereas fetal MTHFR may subsequently affect the utilization and distribution of the supplied folate. The most important determinant for the development of neural-tube defects, however, is likely the initial folate sufficiency of the mother. It is unfortunate that Volcik et al. did not examine patterns between the parental—in particular, the maternal—MTHFR genotypes and the genotypes of the children with SB that they studied. Perhaps there are specific maternal/fetal combinations of MTHFR that have a relationship with either fetal demise or the development of SB.

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