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The 1298(A→C) Mutation of Methylenetetrahydrofolate Reductase Should Be Designated to the 1289 Position of the Gene

To the Editor:

van der Put et al. (1998) recently reported that a second common mutation within the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR; E.C. 1.5.1.20) results in significantly reduced catalytic activity. This mutation is important because MTHFR plays a significant role in the metabolism of folate and, ultimately, of homocysteine, which is implicated as a risk factor for neural-tube defects (Copp et al. 1990). van der Put et al. (1998) designated this mutation—MTHFR 1298(A→C)—as changing a glutamate into an alanine residue. A review of the human MTHFR mRNA sequence (GenBank accession number U09806) shows that this mutation is not present in the human sequence at position 1298 but may, in fact, be mutation 1289(A→C). There are two reasons why this may be so. First, the nucleotide at 1298 is a T (fig. 1). Second, the authors report that this mutation, 1298C, destroys an *Mbo*II restriction site. The recognition site for *Mbo*II is GAAGANNNNNNN/N/. There is no *Mbo*II restriction site at 1298; however, there is one at 1289 in the human MTHFR sequence (fig. 1). The enzyme *Mbo*II is expected to cut between nucleotides 1297 and 1298, whereas the mutation they describe is likely to be at position 1289. Although this does not affect the results of their study, it is important to accurately designate mutations to the proper nucleotide position, thereby avoiding errors in subsequent research concerning this important gene.

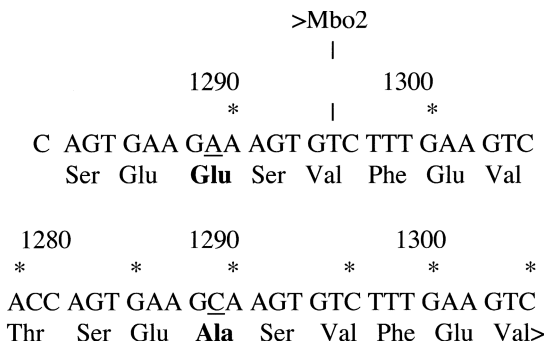

Figure 1 *Mbo*II restriction site on human MTHFR (GenBank accession U09806). *Mbo*II recognizes GAAGA (upper nucleotide sequence). MTHFR 1298C (lower sequence) abolishes the *Mbo*II site. Sequence data were obtained from GenBank (accession number U09806). The lower sequence is identical to the GenBank sequence.

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html (for human MTHFR mRNA sequence [accession number U09806])

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Reply to Donnelly

To the Editor:

Donnelly comments, in his letter, on the confusing numbering used for the nomenclature of the second single-

nucleotide polymorphism (SNP) discovered in the methylenetetrahydrofolate reductase (MTHFR) gene (Van der Put et al. 1998). We agree that the numbering that was used may be confusing for two reasons.

First, we designated this SNP as the "1298(A→C) mutation," by use of the same numbering method used to indicate the 677(C→T) substitution, the first SNP discovered in the MTHFR gene (Frosst et al. 1995). Although this SNP is designated to be at position 677, the actual location of this SNP may be at position 665 of the coding region, if numbering is started at the first ATG site of the reported coding sequence (Goyette et al. 1994). Thus, discrepancies in numbering started before the discovery of the second SNP. We therefore had two options for determining the nomenclature of the second SNP in the MTHFR gene: either to start numbering at the ATG or to use the method of numbering that is in concordance with that used for the first SNP. In our opinion, this last option would be more reasonable, because of the widely accepted nomenclature of the 677(C→T) substitution. Therefore, we designated the second SNP as the "1298(A→C) mutation."

Second, the sequence reported by Goyette et al. (1994) unfortunately contained a C at the 1298 sequence, instead of at the much more common 1298A sequence, of the MTHFR gene. We have no doubt that the mutation is an A→C, rather than a C→A, transition, which is clearly reflected by the prevalence of the 1298 SNP and its effect on MTHFR activity (van der Put et al. 1998).

Additionally, we would like to describe an improved method of PCR/RFLP screening for the 1298 SNP. By use of two PCR primers—forward primer ATGTGGGGGGAGGAGCTGAC and the intronic reverse primer GTCTCCCAACTTACCCTTCTCCC—a 241-bp fragment will be obtained. If the wild-type genotype (1298AA) is present, then the *Mbo*II RFLP results in two fragments—one that is 204 bp and one that is 37 bp. For the homozygous mutated MTHFR genotype (1298CC), only the 241-bp fragment is obtained, and, for the heterozygous genotype, all three fragments are obtained. These RFLP fragments can be easily differentiated by means of agarose-gel electrophoresis.

We examined the possible interference of the 1317(T→C) transition with the proper screening of the 1298 SNP. 1317(T→C) is a silent mutation (Weisberg et al. 1998) that, like the 1298 SNP, results in the formation of an *Mbo*II recognition site and that could thus disturb genotyping of 1298. We performed restriction-enzyme analysis with *Mbo*II and *Bpu*AI—the latter restriction enzyme recognizes only the 1317(T→C) substitution—on the PCR-obtained 241-bp fragment and looked for possible misinterpretation of the 1298 genotype. The 1317CC genotype was not observed among 450 Dutch individuals, and we observed only 3 heterozygous individuals, which resulted in the 1317C allele having a frequency of .003 among the Dutch population. The 1317 SNP did not interfere with the proper genotyping of 1298; in all three cases, the 1317C allele was present on a 1298A allele, which already results in a *Mbo*II recognition site. Possibly, the 1317C substitution arose at the 1298A allele and, thus, will not disturb genotyping of 1298.

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Special Oversight Groups to Add Protections for Population-Based Repository Samples

To the Editor:

The National Institute of General Medical Sciences (NIGMS) supports the Human Genetic Cell Repository through a contract to the Coriell Institute for Medical Research in Camden, NJ. The repository supplies cell lines and DNA samples to investigators worldwide. Al-