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Prevalence of the C677T substitution of the methylenetetrahydrofolate reductase (*MTHFR*) gene in Wisconsin

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Purpose: The objective of this study was to estimate the prevalence of the C677T substitution of the *MTHFR* gene in the State of Wisconsin. **Methods:** The MTHFR genotypes of 1059 randomly selected newborns were analyzed using PCR amplification, *Hin*fI restriction enzyme digestion, and electrophoresis. The frequency of the substitution was calculated in different genders and statistically analyzed (Chi-square). **Results:** Among 1059 newborn infants, about 59% had a C/C homozygous genotype, 33% had a C/T heterozygous genotype, and the remaining 8% had a T/T homozygous genotype. The frequencies for the C and T alleles were 76% (*p*) and 24% (*q*), respectively. There were no significant differences between males and females. **Conclusion:** The study provides a fair estimate for the prevalence of the C677T substitution of the *MTHFR* gene in the general population in Wisconsin, which will facilitate further investigations of the pathogenic effects of the gene. ***Genet Med* 2003:5(6):458–459.**

Key Words: *5,10-methylenetetrahydrofolate reductase, C677T substitution, genotype, allele frequency, newborn*

5,10-Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in folate metabolism, which catalyzes the 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate reduction. The later provides a methyl group in conversion of homocysteine to methionine that is an important precursor for synthesis of protein and S-adenosyl-L-methionine (SAM), the universal methyl donor for methylation.[1–3] The *MTHFR* gene is located on the short arm of chromosome 1 at 1p36.3, and consists of 11 exons.[1] Several common nucleotide polymorphisms in this gene have been reported, one of which is a C to T substitution at the position 667 (C677T) in the exon 4, resulting in an alanine (Ala) to valine (Val) conversion at the amino acid 225 in the protein product.[3,4] The MTHFR protein coded by the T allele is thermolabile with reduced enzyme activity at 37 °C.[4] Such a deficiency appears to have pathogenic importance, especially with decreased folate intake and other genetic/environmental factors.[1,3,5] The C667T substitution has been associated with elevated plasma homocysteine concentration and genome hypomethylation, as well as increased risk for vascular disease, neural tube defects, diabetes, preeclampsia, and Down syndrome.[1,5–7] In addition, this substitution has been studied for its relation to cancer, longevity, and drug toxicity.[1,8,9] Thus far, the epidemiology information related to this substitution in the general population is limited; for example, to our knowledge no such information is available in the Midwest States of the United States. To study the pathogenic effect of the C677T substitution and its possible impact on related health issues in a geographic region, knowing the prevalence of this substitution in the general population of the region is essential. In this study, we have looked at the frequency of the C677T substitution of the *MTHFR* gene in 1059 randomly selected newborn infants in the State of Wisconsin, which provides an estimate of the prevalence of the substitution in the state.

MATERIALS AND METHODS

A total of 1059 dried blood (Guthrie) spot specimens from infants born in Wisconsin from October 2002 to April 2003 were randomly selected from those received by the newborn screening laboratory at the Wisconsin State Laboratory of Hygiene. DNA samples were extracted by prewashing the spots with phosphate buffered saline and then incubated with TE buffer at 96 °C for 30 minutes. The genotype of each subject was examined using a PCR/restriction digestion-based method.[4] Briefly, about 50 to 80 ng DNA sample was amplified in a final volume of 25 μL containing 1×PCR buffer with 1.5 mmol/L $MgCl_2$, 2 unit *Taq* DNA polymerase (Eppendorf), 100 μmol/L dNTP, and 0.5 μmol/L of each primer (5'-tgaaggagaaggtgtctgcggga-3' and 5'-aggacggtgcggtgagagtg-3'). On a Perkin-Elmer 9600 thermocycler, the DNA sample was first denatured at 94 °C for 3 minutes, and then amplified for 35 cycles of 94 °C 30 seconds, 55 °C 30 seconds, and 72 °C 1 minute, followed by an extension at 72 °C for 10 minutes. The PCR product was digested with *Hin*fI (New England Biolabs) at 37 °C overnight, separated by electrophoresis on 4% 3:1

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Table 1
Prevalence of the C677T substitution of the MTHFR gene in Wisconsin

		Genotype			Allele Frequency	
	No.	C/C	C/T	T/T	C(p)	T(q)
Female	541	316 (58%)	177 (33%)	48 (9%)	75%	25%
Male	518	310 (60%)	170 (33%)	38 (7%)	76%	24%
Total	1059	626 (59%)	347 (33%)	86 (8%)	76%	24%

NuSieve/agarose gel (FMC), and visualized by ethidium bromide staining. A 201-bp PCR product can be seen in a C allele; whereas, C677T substitution creates a *Hin*fI restriction enzyme recognition site within the sequence of the PCR product, resulting in 178-bp and 23-bp fragments. The fragment pattern of each individual was analyzed and recorded for calculation of genotype and allele frequencies. A Chi-square analysis of the frequency differences between males and females was performed.

RESULTS AND DISCUSSION

Our results demonstrated that about 59% of 1059 newborn infants analyzed had a C/C homozygous genotype, 33% had a heterozygous C/T genotype, and the remaining 8% had a homozygous T/T genotype; the frequencies for the wild-type allele C and the deficient allele T were 76% (p) and 24% (q), respectively (Table 1). There were no significant differences of allele frequencies or genotype distributions between males and females, apparently contradicting previous reports that female newborns had a lower proportion of C677T homozygosity than male newborns.[10] Because the frequency of the C677T substitution varies among different ethnic and geographic populations,[3,11] the prevalence of this substitution in Wisconsin may only be accurately estimated using unbiased samples from the general population of the state. According to the US Census Bureau's data, the vast majority of the Wisconsin population are non-Hispanic/Latino origin Caucasians (87.3% in 2000).[12] The newborn screening laboratory at the Wisconsin State Laboratory of Hygiene tests every infant born in Wisconsin. Therefore, randomly selected newborns should be representative of the ethnic distribution in the state, and our data should provide a fair estimate for the prevalence of the C677T substitution in Wisconsin. This estimated prevalence is different from most of those reported in other regions of the United States, but similar to that in a Canadian population.[3,11,13] The information provides a unique and useful reference for further studies of the *MTHFR* gene in the state, such as the genotype-phenotype correlation in different disease and age groups. It is also useful for complementing those obtained through epidemiological studies from other geographic and ethnic groups. In addition, these data may also be valuable for public health agencies and clinical facilities to develop appropriate strategies of prevention and management of diseases that may be associated with the deficiency of the MTHFR gene in the State of Wisconsin.

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