Letter to the Editor

Smoking Behavior and the C677T Allele of the Methylenetetrahydrofolate Reductase (MTHFR) Gene

To the Editor:

Although little is known about the specific genetic factors that influence cigarette smoking behavior, it is known that genetic factors make a substantial contribution to tobacco use in both humans and animals [Collins, 1990]. For example, in a study of female twin pairs, in-pair correlation for smoking was higher for MZ twins (.797) than DZ twins (.443) [Heath et al., 1999]. In the Virginia Twin Study of Adolescent Behavioral Development, heritabilities for liabilities to lifetime and current tobacco use were estimated to be 84% and 82% respectively [Maes et al., 1999]. The finding that polymorphic variation of the gene coding for the P450 enzyme CYPT2A6 that metabolizes nicotine correlated with smoking behavior raised the possibility that individuals with impaired nicotine metabolism needed fewer cigarettes to maintain constant blood and brain nicotine concentrations and that the unpleasant effects experienced by people learning to smoke lasted longer in people with impaired nicotine metabolism [Pianezza et al., 1998].

Another genetic factor that may influence smoking behavior is a polymorphic variation of the MAOB gene in individuals with Parkinson disease (PD) compared with control individuals [Checkoway et al., 1998]. For unknown reasons, individuals with PD are less likely to smoke than control individuals. The study confirmed the reduced risk of PD among individuals who had ever smoked (adjusted odds ratio [AOR] 0.46, 95% confidence interval [CI] 0.26–0.81, \( P = 0.007 \)). A reduced risk of PD related to pack-years of smoking was found for individuals with the G allele of MAOB (AOR 0.24, 95% CI 0.10–0.55) but an increased risk of PD was found for individuals carrying the A allele (AOR 1.44, 95% CI 0.10–0.55) but an increased risk of PD was found for individuals with the G allele of MAOB (AOR 0.24, 95% CI 0.10–0.55) but an increased risk of PD was found for individuals carrying the A allele (AOR 1.44, 95% CI 0.45–4.68). These findings suggested an unexplained interaction between cigarette smoking and this MAOB polymorphism.

In the course of a study of the methylenetetrahydrofolate reductase (MTHFR) gene C677T allele as a risk factor for low birth weight and preterm delivery, we found that this allele was unequally distributed between smokers and non-smokers.

Data were examined from 232 gravidas who were participants in the Camden Study of Adolescent Pregnancy. All of these individuals had identified themselves as Hispanic and all had given their country of origin of Hispanic descent as Puerto Rico. Hispanics were selected because population stratification has been demonstrated for the C677T allele, which has a lower frequency among individuals of African–American background [Motulsky, 1996; Stevenson et al., 1997]. Individuals of Puerto Rican background are a syncretic blend of three populations (Caribe Native Americans, Africans, and Spanish). DNA was extracted from buffy coat leukocytes of each gravida. Data collected included smoking history (cigarettes per day), parity, ethnicity, circulating folate, and time of blood drawing in gestation. To determine genotypes for the C677T allele of MTHFR, genomic DNA was amplified by the polymerase chain reaction (PCR) using specific primer pairs [Frosst et al., 1995]. HindI-digested PCR products were separated by acrylamide gel electrophoresis. Genotypes were carried out in duplicate and checked for consistency; ambiguous or inconsistent samples were repeated in duplicate. No other gene loci, e.g. those related to nicotine metabolism, were studied since the goal of the study was to determine whether the C677T allele was a major contributor to adverse pregnancy outcome.

Logistic regression was used to examine the influence of homozygosity for the heat-labile allele (genotype T,T) on smoking status controlling for potential confounding variables (age, parity, clinic pay status, inadequate gestational gain). Adjusted odds ratios (AOR’s) and 95% confidence intervals (CIs) were computed from the logistic regression coefficient and the corresponding covariance matrix.

Genotype frequencies for the sample (n = 232) were: genotype C,C (44.8%, n = 104), genotype C,T (47.8%, n = 111), and genotype T,T (7.3%, n = 17). The observed distribution of genotypes was compared with that expected according to the Hardy-Weinberg equilibrium: no difference was found (\( \chi^2 = 1.57 \), 2df, 2-sided \( P = 0.46 \)). Maternal genotype did not significantly influence background and pregnancy characteristics.

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*Correspondence to: William G. Johnson, UBHC D431, 671 Hoes Lane, Piscataway, NJ 08854.
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for the gravidas: age, pregravid BMI (kilos/meter$^2$), parity, medicaid status, adequate gestational gain (kilos/meter$^2$), prior poor outcome (low birth weight or preterm delivery), fetal/neonatal death, low birth weight ($<2500$ grams), or preterm delivery ($<37$ completed weeks from ultrasound modified last menstrual period). However, in univariate analyses, mutation homozygotes more often smoked (58.8%) than heterozygotes (20.7%) or normal homozygotes (33.7%, \( P < 0.005 \) by Chi-Square).

After control for potential confounding variables (age, parity, ethnicity, clinic payment status, inadequate gestational gain) the adjusted odds ratio for smoking was increased more than fourfold among mutation homozygotes (T,T genotype) in comparison to the other genotypes; the 95% confidence intervals did not include unity (Table 1).

This finding of an apparent association between the MTHFR C677T allele and smoking status raised the question of whether homozygosity for the MTHFR C677T allele affected smoking status in pregnant women. MTHFR is an important gene of folate metabolism and the C677T allele, especially in double dose, is associated with hyperhomocysteinemia [Rozen, 1997]. However, a possible mechanism by which the C677T allele might influence smoking behavior is unknown.

The relation of folate status to smoking is well known. For example, smoking is associated with lower circulating folate [Subar et al., 1990; Ortega et al., 1994; Piyathilake et al., 1994]. Intake of both folate and vitamin C (which aids folate absorption) were reported ed lower in smokers compared to non-smokers, and intake of both decreased as smoking increased [Subar et al., 1990]. Deficient folate intake, serum folate, and erythrocyte folate were reported in smokers compared to non-smokers [Ortega et al., 1994; Piyathilake et al., 1994]. Current smokers who smoked within one hr of folate determination had significantly lower serum folate than those who had smoked earlier [Piyathilake et al., 1994]. At the RDA for folate intake, current smokers had 42% lower serum folate than current non-smokers [Subar et al., 1990].

MTHFR is an important gene of folate metabolism and the C677T allele, especially in double dose, is associated with hyperhomocysteinemia [Rozen, 1997]. However, a possible mechanism by which the C677T allele might influence or interact with smoking behavior is unknown.

Evidence that a genetic polymorphism related to folate metabolism might be associated with smoking behavior has not been previously reported to our knowledge. Further studies are required to confirm or deny these observations.

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REFERENCES


Heath AC, Madden PA, Grant JD, Heath AC, Madden PA, Grant JD, Templeton AK, Szklo MC, Eaves LJ. 1990. Genetic inluences on tobacco use: a review of human and animal studies Int J Addict 25:35–55.


TABLE I. Smoking and Mutation Genotype: Adjusted Odds Ratio

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hispanic gravidas (Puerto Rican)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Unadjusted % smokers</td>
<td>Adjusted odds ratioa</td>
<td>95% CI</td>
</tr>
<tr>
<td>T,T</td>
<td>17</td>
<td>58.8</td>
<td>4.03</td>
<td>1.41–11.52</td>
</tr>
<tr>
<td>C,T</td>
<td>111</td>
<td>20.7</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>C,C</td>
<td>104</td>
<td>33.7</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
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aAdjusted for age, parity, inadequate gestational gain and clinic payment status (Medicaid).