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Research Letter

Association Between the NAT1 1095C > A Polymorphism and Homocysteine Concentration

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To the Editor:

N-acetyltransferase 1 [NAT1 (EC 2.3.1.5)] facilitates the activation or deactivation of various arylamine and heterocyclic amines, including several known carcinogens, by catalyzing their O-acetylation and N-acetylation, respectively [Hein et al., 2000; Hein, 2002]. In addition, NAT1, which is expressed in many different tissues [Ward et al., 1995], acetylates the folate catabolite p-aminobenzoylglutamate, to its primary excretory form, N-acetyl-p-aminobenzoylglutamate [Minchin, 1995; Ward et al., 1995]. Several single nucleotide polymorphisms within the NAT1 gene with known functional characterization have been reported to date [Hein et al., 2000].

Due to the role of NAT1 in folate catabolism and the possible feedback inhibition of aspects of folate metabolism by acetylated folate catabolites, it has been suggested that variants of this enzyme may change intracellular folate content, thereby modifying folate/homocysteine metabolism and phenotype [Minchin, 1995; Ward et al., 1995]. The NAT1*10 allele, specified by A nucleotides at positions 1088 and 1095 of the NAT1 mRNA 3′ untranslated region (UTR), has recently been associated with an increased risk of birth defects. Lammer et al. [2004a,b] reported that for pregnancies in which the mother smoked, the offspring NAT1 1088AA and 1095AA genotypes (either when analyzed separately or together) are strongly associated with the risk of orofacial clefts [Lammer et al., 2004a]. Jensen et al. [2005] subsequently published evidence that the risk of spina bifida was influenced by an interaction between the offspring NAT1 1095A allele and maternal smoking.

Some reports suggest that the NAT1*10 allele is associated with a rapid acetylating phenotype [Badawi et al., 1995; Bell et al., 1995; Hein et al., 2000; Yang et al., 2000]. However, others have indicated that it encodes an enzyme with activity similar to that specified by the wild-type NAT1*4 allele [Grant et al., 1997]. There have been no published studies investigating the impact of NAT1*10 on folate/homocysteine phenotype that might underlie the observed association of this NAT1 variant with the above two classes of birth defects. However, the relationship between maternal low folate/high homocysteine and increased risk of spina bifida birth outcome is well established [Mills et al., 1995] and it has been reported that maternal low folate status may contribute to orofacial cleft etiology [Prescott et al., 2002]. We therefore examined a population of healthy adults of reproductive age to determine the association, if any, between the NAT1 1095C > A polymorphism and aspects of folate/homocysteine phenotype. In particular, we assessed such associations in the subset of smokers.

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Study subjects (n = 410) were drawn from the Young Hearts Project, an ongoing prospective study designed to monitor the prevalence of coronary disease risk factors in young adults from Northern Ireland [Boreham et al., 1997, 1999; Kluijtmans et al., 2003]. Ethical approval was granted by the Research Ethics Committee, Queen’s University Belfast, and all subjects provided written informed consent. The biochemical data used for the analyses reported here were acquired at the third screening visit, at which time the subjects were between 20 and 26 years old. At this visit, subjects were also classified as current cigarette smokers or current non-smokers. The 36 former smokers in the population were classified as current non-smokers. One subject with an extremely elevated serum folate concentration (215 nmol/L) was considered to be an outlier and was excluded from our analyses.

Blood samples were collected from fasted subjects for determination of biochemical parameters and for DNA extraction. Homocysteine concentrations were measured by an established high performance liquid chromatography method [Ubbink et al., 1991]. Serum folate concentrations were determined by time-resolved immunofluorescence on an AutoDelfia analyzer (Wallac, Cambridge, UK). Red blood cell (RBC) folate concentrations were determined by a microbiological assay as previously described [Molloy and Scott, 1997]. RBC folate concentrations are expressed as nanomoles per liter of packed RBCs.

NAT1 1095C > A genotypes were determined using a previously published method [Jensen et al., 2005]. Briefly, allelic discrimination was performed using a TaqMan 5’ Nuclease Real-Time PCR assay on a DNA Engine Opticon 2 Continuous Fluorescence Detection System (MJ Research, Waltham, MA). PCR amplification was performed using 2 μl of sample DNA, 1 × TaqMan Universal PCR MasterMix (Applied Biosystems, Foster City, CA), 0.4 μM of each primer (5’-CAGCATTITTAAGAAAGATACACAAACCTTTCTTG-3’ and 5’-CCAACATTITAAAGCTTTTCCTGATACATCACC-3’), 22.5 nM ‘C’-specific probe (6FAM-AATGTCTTTAAGAAGMBNFQ), and 90 nM ‘A’-specific probe (VIC-AATGTATTITTTAAAGAAGMBNFQ). Probes were custom synthesized by Applied Biosystems. PCR was performed with an initial incubation at 50°C for 2 min, then 95°C for 10 min, followed by 60 cycles of denaturation at 95°C for 20 sec and extension/5’ nuclease step at 56°C for 45 sec. Dual fluorescence was detected after each extension 5’ nuclease step. Genotype interpretations were performed using OpticonMonitor Analysis software version 2.02 (MJ Research).

The distributions of RBC folate, serum folate, vitamin B12, and homocysteine concentrations were all skewed; therefore, all analyses were performed using logarithmically transformed ranked data. Differences in RBC folate, serum folate, vitamin B12, and homocysteine among the different genotype subgroups were assessed by the Wilcoxon Rank Sum test. P-values of ≤ 0.05 were considered statistically significant. The Hardy–Weinberg equilibrium for NAT1 1095C > A genotype was assessed by χ² analysis. All statistical analyses were carried out using SAS version 9.1.

Population characteristics, including concentrations of RBC folate, serum folate, vitamin B12, and homocysteine for the population as a whole and after stratification by smoking status are presented in Table I. Median RBC folate, serum folate, and vitamin B12 concentrations were all significantly higher in non-smokers than in smokers. Median homocysteine concentrations were lower in non-smokers than in smokers but the difference was not statistically significant and similar to those reported previously [Brown et al., 2004]. NAT1 1095C > A genotype frequencies are also listed in Table I. NAT1 1095C > A genotypes in the population as a whole and after stratification by smoking status were in Hardy–Weinberg equilibrium (entire population P = 0.3; non-smokers P = 0.3; smokers P = 0.1).

Biochemical parameters by NAT1 1095C > A genotype for the entire population and after stratification by smoking status are presented in Table II. After such stratification smokers who are A allele carriers (the NAT1 1095AA and 1095CA genotypes) were considered to be an outlier and were excluded from our analyses.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (%)</th>
<th>Non-smokers (%)</th>
<th>Smokers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 (pmol/L) (n)</td>
<td>267.0 [106.0–344.5] (352)</td>
<td>280.0 [201.0–359.0] (218)</td>
<td>254.0 [179.0–319.0] (131)</td>
</tr>
<tr>
<td>RBC folate (nmol/L RBCs) (n)</td>
<td>642.7 [478.8–833.6] (363)</td>
<td>666.3 [507.8–861.9] (229)</td>
<td>587.1 [461.1–793.9] (131)</td>
</tr>
<tr>
<td>Homocysteine (μmol/L) (n)</td>
<td>9.0 [7.5–11.1] (398)</td>
<td>8.7 [7.5–10.6] (245)</td>
<td>9.5 [7.5–11.6] (150)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>188 (45.8)</td>
<td>115 (61.2)</td>
<td>73 (38.8)</td>
</tr>
<tr>
<td>Men (%)</td>
<td>221 (54)</td>
<td>138 (62.4)</td>
<td>81 (36.6)</td>
</tr>
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</tr>
<tr>
<td>Folate (nmol/L) (n)</td>
<td>129 [94–181.0] (351)</td>
<td>139 [99–201.0] (218)</td>
<td>112 [87–157.0] (130)</td>
</tr>
<tr>
<td>NAT1 1095C &gt; A genotypes</td>
<td></td>
<td></td>
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<tr>
<td>CC (%)</td>
<td>254 (61.9)</td>
<td>151 (59.4)</td>
<td>101 (39.8)</td>
</tr>
<tr>
<td>CA (%)</td>
<td>131 (31.9)</td>
<td>83 (63.4)</td>
<td>47 (35.9)</td>
</tr>
<tr>
<td>AA (%)</td>
<td>25 (6.1)</td>
<td>19 (76)</td>
<td>6 (24)</td>
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n, number of individuals, RBCs, red blood cells.

Sex was missing for one subject; smoking status was missing for three subjects.

Homocysteine, RBC folate, Folate and Vitamin B12 levels given as median [interquartile range].
had significantly higher median homocysteine concentrations than the NAT1 1095CC homozygotes (10.3 μmol/L vs. 9.3 μmol/L, P = 0.015). No such association was observed between these genotype classes in non-smokers.

Although no statistically significant differences in folate or vitamin B12 concentrations were observed between genotypes in different subsets, there was a modest trend among smokers towards lower median serum folate concentrations in the NAT1 1095A carriers versus the NAT1 1095CC homozygotes. If real, such difference may contribute to higher levels of homocysteine among smoking NAT1 1095A allele carriers but this possibility needs to be tested in a larger study population.

The mechanism by which NAT1 variants might influence homocysteine levels in smokers is not known. However, the ability of NAT1 to N-acetylate p-aminobenzoylglutamate [Minchin, 1995; Ward et al., 1995] suggests a possible mechanism. The putative feedback of acetylated folate catabolites to modify intracellular folate distribution might be more prominent in NAT1 1095A carriers, thereby predisposing them to elevated homocysteine concentrations. If so, this predisposition might be manifested in smokers who are known to have generally lower folate status [Nygard et al., 1995]. Furthermore, this may also explain, at least in part, the higher risk of orofacial clefts in the NAT1 1095AA offspring of mothers who smoked during the first trimester of pregnancy [Lammer et al., 2004].

In conclusion, we have demonstrated that the NAT1 1095A allele is significantly associated with increased homocysteine levels in smokers only. As smokers are already established to have higher homocysteine concentrations than non-smokers [Nygard et al., 1995], the even higher homocysteine levels associated with smoking NAT1 1095A allele carriers may be especially damaging and may have important consequences for pathologies as diverse as spina bifida and cardiovascular disease.

ACKNOWLEDGMENTS
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REFERENCES


