N-acetyltransferase 2 Influences Cancer Prevalence in hMLH1/hMSH2 Mutation Carriers


Research Group Human Genetics, University Hospital, 4031 Basel, and Department of Medical Genetics, University Children’s Hospital, 4058 Basel, Switzerland [K. H., W. W., Hj. M., Z. D.]; Hunter Area Pathology Service, John Hunter Hospital, University of Newcastle, 2305 Australia [R. J. S.]; Unit of Identification of Genetic Predisposition to Cancer, Division of Oncology, University Hospitals, 1211 Geneva, Switzerland [P. C.]; and Laboratoire d’ADN, Institut Central des Hôpitaux Vaudois, 1951 Sion, Switzerland [P. H.]

Abstract

Hereditary nonpolyposis colorectal cancer (HNPCC), an inherited cancer predisposition syndrome, has been associated with germline mutations in DNA mismatch repair (MMR) genes. Because a deficiency in MMR does not predict a specific cancer phenotype, modifying genes may account in part for the variation in disease expression. We determined the N-acetyltransferase 2 (NAT2) genotype in 26 unaffected and 52 cancer-affected hMLH1/hMSH2 mutation carriers coming from 21 Swiss HNPCC families. Slow acetylators were found to be significantly (\(p < 0.03\)) more prevalent in the group of affected mutation carriers. Our results suggest a protective effect of the NAT2 rapid acetylator phenotype, an observation that could have implications for genetic counseling and management of MMR gene mutation carriers.

Introduction

HNPCC\(^3\) is believed to be one of the most common inherited cancer predispositions with an incidence of approximately 1–2% of all CRC cases (1, 2). At least four genes associated with DNA mismatch repair are known to cause susceptibility to HNPCC (hMLH1, hMSH2, hPMS2, and hMSH6), with mutations in hMLH1 and hMSH2 accounting for the majority of cases (3). In addition to CRC, HNPCC encompasses a variety of other cancers including endometrial cancer, ovarian cancer, stomach cancer, cancer of the upper urinary tract, and the hepatobiliary system (4). Because a deficiency in mismatch repair does not predict a specific cancer phenotype, modifying genes may account in part for the variation in disease expression in HNPCC. Up to 80% of human cancers are thought to arise as a consequence of exposure to environmental agents against which xenobiotic metabolizing enzymes such as N-acetyltransferases (NATs) and glutathione S-transferases (GST) represent the first line of defense (5, 6). Therefore, altered enzyme activity is expected to affect susceptibility to cancer and may explain differences in disease expression in HNPCC. To test this hypothesis, 21 Swiss HNPCC families with characterized pathogenic germ-line mutations in either hMLH1 or hMSH2 were screened for specific polymorphisms in the NAT2, GSTM1, and GSTT1 genes, known to enhance, reduce, or abolish the activity of their respective proteins. Enzyme activity was correlated with the age of CRC onset and cancer prevalence.

Materials and Methods

HNPCC Families. Twenty-one unrelated Swiss HNPCC families, each harbouring a pathogenic germline mutation in either hMLH1 or hMSH2 were investigated. Sixty-three individuals from 17 families had a mutation in hMLH1, whereas 15 individuals from 4 families had a mutation in hMSH2. These families comprised 52 patients presenting with 59 cancers (45 CRC, 4 endometrial, 2 stomach, 2 ovarian, and 6 other cancers), 26 unaffected mutation carriers as well as 103 noncarriers. Molecular analyses were carried out only after informed consent was obtained from the individuals. Pedigrees and clinical data on the families under study have been published separately (7–11).

Genomic DNA was isolated from peripheral blood lymphocytes according to the method used by Miller et al. (12). All of the DNA samples were assigned anonymously, and genotyping assays were scored independently by two reviewers (K. H., K. S.).

NAT2 Genotyping. NAT2 genotyping was performed as described by Smith et al. (13), using primers generating a NAT2-specific 547-bp fragment, which was restriction-digested with KpnI, DdeI, TaqI, and BamHI in separate reactions and subsequently analyzed on a 12% non-denaturing polyacrylamide gel (13). The resulting cleavage pattern allowed the distinction between the NAT2*4 allele (confering the rapid acetylator phenotype) and the group of NAT2*5 (including alleles A, B, and C), NAT2*6, and NAT2*7 alleles, all of which confer the slow acetylator phenotype. Homo- or heterozygous carriers of the NAT2*4 allele were phenotypically classified as rapid acetylators (14). Because of ambiguous genotyping results, 6 of 181 individuals analyzed were excluded from further analysis.

GSTM1 Genotyping. GSTM1 genotype status was determined by the method of Fryer et al. (15). Briefly, target DNA was amplified by a common GSTM1-specific upstream primer within intron 6 and different downstream primers within exon 7 that recognize either GSTM1*A or B specifically (3’-A→G substitution), thereby introducing a HaeII restriction site into the amplified DNA. This allows discrimination between different DNA fragment sizes (GSTM1*A 112-bp, GSTM1*B 132-bp product) on a 4%-agarose gel. GSTM1*null homozygotes fail to amplify target DNA. GSTM1 genotype could not be determined in two mutation carriers, who were excluded from further analysis.

GSTT1 Genotyping. GSTT1 genotyping was performed as described by Pembel et al. (16), using a forward primer within exon 4 and a reverse primer within exon 5. The PCR yielded either a 480-bp product (GSTT1 positive/conjugator) or no product (GSTT1*null homozygotes/cononjugator). As an internal control for DNA quality and PCR conditions used for the GSTM1 and GSTT1 amplifications, hMLH1 exon 11 (307-bp product) was coamplified in each reaction. GSTT1 genotype could not be determined in three mutation carriers, subsequently excluded from further analysis.

For statistical analysis, \(\chi^2\) Fisher’s exact test, and Student’s t test were used, with all of the probabilities reported as two-tailed \(p\)s, considering a \(p\) of \(<0.05\) to be significant.

Results and Discussion

In this study, we investigated the influence of NAT2, GSTM1, and GSTT1 geno-phenotype status on both age at diagnosis of CRC and cancer occurrence in 21 Swiss HNPCC families with known germline mutations.
mutations in either hMLH1 or hMSH2. Table 1 depicts the overall frequency of NAT2, GSTM1, and GSTT1 geno-/phenotypes in 181 family members compared with mutation carriers only (n = 78).

**Influence of NAT2, GSTM1, and GSTT1 Genotype Status on Age at Diagnosis of CRC.** As depicted in Table 2, no significant correlation was found between mean age at diagnosis for CRC and particular allelomorphs encoding NAT2, GSTM1, or GSTT1 enzymes. Nevertheless, the five GSTM1*null/GSTT1*null mutation carriers, lacking both enzymes, seemed to have a lower mean age of onset when compared with the 41 nonnull individuals with CRC (38y ± 14.8 SD versus 44y ± 10.6 SD), although this was not statistically significant (P = 0.09). Interestingly, a similar trend has been reported for sporadic colon cancer, which supports the view that the “null-null” genotype may influence the age of onset of CRC (17).

**GSTM1 and GSTT1 Genotype Status and Cancer Prevalence.** The allele frequency of GSTM1 or GSTT1 did not differ between affected (n = 48) and unaffected (n = 26) mutation carriers. We also found no difference when comparing individuals with multiple cancers with unaffected mutation carriers, GSTM1*null/GSTT1*null with nonnull mutation carriers, or GSTM1 and GSTT1 geno-/phenotypes with the anatomical site of CRC.

**NAT2 Acetylator Status and Cancer Prevalence.** Comparison between affected and unaffected mutation carriers revealed a significant difference between the two groups inasmuch as slow acetylators (i.e., no NAT2*2 allele) were more prevalent in individuals with overall cancer (P < 0.03; odds ratio, 3.6; 95% confidence interval, 1.1–12.3). The difference remained statistically significant when subjects were subdivided into mutation carriers with colorectal (P < 0.04; odds ratio, 3.5; 95% confidence interval, 1.0–12.1) or extracolonic cancer (P < 0.05; odds ratio, 4.4; 95% confidence interval, 0.9–23.2) (Tables 3 and 4). When the data were analyzed according to the gene mutated, similar frequencies of NAT2 phenotypes were observed in the affected group (hMLH2: 60% slow, 40% rapid acetylators and hMLH1: 50% slow, 50% rapid acetylators) and the unaffected group (20% slow, 80% rapid acetylators and 24% slow, 76% rapid acetylators, respectively). Still, the latter differences were not statistically significant (P = 0.059 for hMLH1 and P = 0.28 for hMLH2), although this may be a result of our small sample size, particularly for hMLH2 for which only 10 affected and 5 unaffected individuals were available.

A bias due to gender imbalance can be ruled out because in both groups of affected and nonaffected hMLH1/hMSH2 mutation carriers, the proportion of each sex was in fact identical (Table 3). In addition, when excluding the largest HNPCC kindred, which by contributing 21 of the 74 mutation carriers may have led to an over-representation, the original results were confirmed (n = 53; P < 0.03). Because CRC typically develops during the 5th decade (18), young individuals may have been erroneously assigned to the group of unaffected mutation carriers. The group’s mean age at the time of study was 41.9 years (± 12.9 SD). To determine the age-specific acetylator frequency, unaffected individuals (n = 26) were subdivided according to birth date: (a) born before 1940, 50% slow and 50% rapid acetylators (n = 2); (b) born 1940–1949, 25 and 75%, respectively (n = 4); (c) born 1950–1959, 25 and 75%, respectively (n = 8); (d) born 1960–1969, 11 and 89%, respectively (n = 9); and (e) born 1970 or later, 33 and 67%, respectively (n = 3). The rapid acetylator phenotype was more prevalent in all of the subgroups of unaffected mutation carriers, closely resembling the frequency distribution of the overall observation (23% slow and 77% rapid acetylators), except for unaffected individuals born before 1940, of which only two individuals were available for study. Some ascertainment bias in the group of unaffected mutation carriers, however, cannot be formally excluded from our data set.

Our results suggest a protective effect of the NAT2 rapid acetylator phenotype on cancer development, especially CRC, in hMLH1/ hMSH2 mutation carriers. Accordingly to its role in bladder cancer, this effect could be mediated through faster detoxification (N-acetylation) of arylamine substrates, thus, preventing aromatic amine-DNA adduct formation (19). Although recent studies failed to observe any overall association between NAT2 genotype and sporadic CRC risk, results similar to ours have been reported in younger people (<70 years of age), which suggests that slow acetylation may be a risk factor for cancer in this age group (20). Additional studies on larger HNPC collective, preferably combined with information on individual nutritional and life-style habits, are needed to confirm these findings, which may prove critical in establishing genetic risk and, thus, more accurate genetic counseling of hMLH1/hMSH2 mutation carriers.

**Acknowledgments**

We thank Michèle Attenhofer, Alexia Couturier, and Karl Siebold for excellent technical assistance; and the families for participation in the study.

---

Table 1 **NAT2, GSTM1, and GSTT1 geno-/phenotype frequencies in 181 individuals coming from 21 Swiss HNPCC families and compared with hMLH1/hMSH2 mutation carriers only (n = 78)**

<table>
<thead>
<tr>
<th>Geno-/Phenotype</th>
<th>Overall n (%)</th>
<th>Mutation carriers only n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow acetylator</td>
<td>82 (46.9)</td>
<td>31 (41.9)</td>
</tr>
<tr>
<td>Rapid acetylator</td>
<td>93 (53.1)</td>
<td>43 (58.1)</td>
</tr>
<tr>
<td>GSTM1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>83 (46.4)</td>
<td>32 (42.1)</td>
</tr>
<tr>
<td>Homozygous null</td>
<td>96 (53.6)</td>
<td>44 (57.9)</td>
</tr>
<tr>
<td>GSTT1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>147 (82.6)</td>
<td>64 (85.3)</td>
</tr>
<tr>
<td>Homozygous null</td>
<td>31 (17.4)</td>
<td>11 (14.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> NAT2 genotype could not be unambiguously determined in six individuals, of which four were mutation carriers.

<sup>b</sup> GSTM1 genotype could not be determined in two mutation carriers.

<sup>c</sup> GSTT1 genotype could not be determined in three mutation carriers.

---

Table 2 **Mean age at diagnosis of colorectal cancer (n = 43) according to NAT2, GSTM1, and GSTT1 geno-/phenotype status**

<table>
<thead>
<tr>
<th>NAT2 phenotype</th>
<th>AFC</th>
<th>RFC</th>
<th>EC</th>
<th>UMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>43.3</td>
<td>43.9</td>
<td>42.7</td>
<td>43.1</td>
</tr>
<tr>
<td>Rapid</td>
<td>43.5</td>
<td>43.5</td>
<td>42.3</td>
<td>42.3</td>
</tr>
<tr>
<td>SD</td>
<td>±10.9</td>
<td>±12.0</td>
<td>±7.7</td>
<td>±13.0</td>
</tr>
</tbody>
</table>

---

Table 3 **NAT2 allele frequencies in affected and unaffected hMLH1/hMSH2 mutation carriers**

<table>
<thead>
<tr>
<th>Mutation carrier status</th>
<th>NAT2&lt;sup&gt;*,&lt;sup&gt;4&lt;/sup&gt;</th>
<th>NAT2&lt;sup&gt;*,&lt;sup&gt;5&lt;/sup&gt;</th>
<th>NAT2&lt;sup&gt;*,&lt;sup&gt;6&lt;/sup&gt;</th>
<th>NAT2&lt;sup&gt;*,&lt;sup&gt;7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>46</td>
<td>0.29</td>
<td>0.45</td>
<td>0.23</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>0.35</td>
<td>0.46</td>
<td>0.17</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>0.23</td>
<td>0.44</td>
<td>0.29</td>
</tr>
<tr>
<td>Unaffected</td>
<td>26</td>
<td>0.42</td>
<td>0.38</td>
<td>0.19</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>0.46</td>
<td>0.39</td>
<td>0.15</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>0.39</td>
<td>0.38</td>
<td>0.23</td>
</tr>
</tbody>
</table>

---

Table 4 **NAT2 acetylator status in affected and unaffected hMLH1/hMSH2 mutation carriers**

<table>
<thead>
<tr>
<th>NAT2 phenotype</th>
<th>Cancer overall n (%)</th>
<th>CRC n (%)</th>
<th>Extracolonic cancers n (%)</th>
<th>Unaffected mutation carriers n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow acetylator</td>
<td>25 (52.1)</td>
<td>22 (51.2)</td>
<td>8 (57.1)</td>
<td>6 (23.1)</td>
</tr>
<tr>
<td>Rapid acetylator</td>
<td>23 (47.9)</td>
<td>21 (48.8)</td>
<td>6 (42.9)</td>
<td>20 (76.9)</td>
</tr>
<tr>
<td>P &lt; 0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References


N-acetyltransferase 2 Influences Cancer Prevalence in hMLH1/hMSH2 Mutation Carriers

Karl Heinimann, Rodney J. Scott, Pierre Chappuis, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/13/3038

Cited articles
This article cites 16 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/13/3038.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/59/13/3038.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.