CYP2C9 and UGT1A6 Genotypes Modulate the Protective Effect of Aspirin on Colon Adenoma Risk

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Abstract

Regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) has a protective effect on the incidence of colon neoplasia. However, polymorphisms in NSAID-metabolizing enzymes may alter this effect. NSAIDs, particularly aspirin, are glucuronidated by UGT1A6 and some classes of NSAIDs are also metabolized by cytochrome P450 (CYP) 2C9. Both of these enzymes have slow-metabolizing, variant forms. We tested the hypothesis that the slow alleles of these enzymes can modify the inverse association between NSAIDs and colon neoplasia in the Minnesota Cancer Prevention Research Unit (CPRU) adenomatous polyp case-control study. CYP2C9 and UGT1A6 genotypes were determined for 474 adenoma cases and 563 controls. NSAID use was inversely associated with adenoma risk [odds ratio (OR), 0.63; 95% confidence interval (CI), 0.44–0.90 for aspirin; and OR, 0.50; 95% CI, 0.31–0.82 for nonaspirin NSAID]. However, this association was absent in aspirin users who carried the CYP2C9 variant alleles (OR, 0.88; 95% CI, 0.51–1.53) or who were homozygous wild-type UGT1A6 (OR, 0.86; 95% CI, 0.50–1.50). Carriers of both of these alleles who use aspirin were also not at reduced risk of adenomatous polyps (OR, 1.59; 95% CI, 0.68–3.73). The variants of these enzymes did not influence the association between nonaspirin NSAIDs and adenoma risk. These data indicate that the effectiveness of chemopreventive drugs can be modulated by the genotype of metabolizing enzymes.

Introduction

In the United States, ~130,000 new cases of colorectal cancer are diagnosed and up to 55,000 individuals die from the disease every year (1). Colorectal tumors arise as a result of the accumulation of genomic alterations (2). There is evidence that NSAIDs3 decrease the incidence of colorectal cancer and possibly other gastrointestinal tumors in humans (reviewed in Ref. 3). These drugs have been investigated in epidemiological studies as well as in animal models. The majority of epidemiological studies report an inverse association between regular use of NSAIDs (mostly aspirin) and the occurrence of colorectal cancer and adenoma (reviewed in Ref. 3). NSAID users are about one-half as likely to develop colorectal cancer as nonusers. In familial adenomatous polyposis patients, NSAIDs reduce the number and size of intestinal polyps (4, 5). Research on both colorectal cancer and adenoma indicates that any protective effect of NSAIDs on neoplasia requires continued use; infrequent or prior use has generally not been associated with a reduced risk (reviewed in Ref. 3). Consistent with observations in humans, NSAIDs can prevent or reduce chemically induced carcinogenesis in rodents (Refs. 6, 7; reviewed in Ref. 8).

NSAIDs are thought to reduce inflammation by inhibiting the COXs Cox-1 and Cox-2. Aspirin irreversibly inhibits Cox through acetylation, whereas the inhibition by nonaspirin NSAIDs is reversible (9). COXs catalyze the synthesis of prostaglandins from arachidonic acid. Cox-1 is constitutively expressed in many tissues (10), whereas Cox-2 is an inducible enzyme that is expressed predominantly in inflammatory reactions. It is also overexpressed in neoplastic tissue (11–16). Although the precise relationships among Cox expression, prostaglandin production, and colon cancer are not clearly understood, reports suggest that overexpression of Cox-2 leads to prostaglandin-mediated resistance to apoptosis, enhanced expression of bcl-2, and decreased expression of both E-cadherin and the transforming growth factor β2 receptor (17–19).

Metabolism of NSAIDs involves oxidation by CYP enzymes and/or conjugation, particularly glucuronidation by phase II enzymes. In humans, aspirin is rapidly deacetylated to salicylic acid (20), which is further metabolized by glucuronidation, hydroxylation, and glycine conjugation (21). The glucuronide metabolites, salicylacyl glucuronide and salicylphenolic glucuronide, represent a significant proportion of salicylic acid metabolites (21). Gentisic acid, formed through CYP oxidation of salicylic acid, is a minor metabolite (21).

The major enzymes involved in glucuronidation and hydroxylation of NSAIDs are CYP2C9 (Ref. 22; reviewed in Ref. 23) and UGT1A6 (24). Both of these enzymes are polymorphic. The variant alleles CYP2C9*2 (R144C) and *3 (I359L) produce slow-metabolizing enzymes (25–28). In Caucasians, the variant alleles are present at a frequency of ~0.1 and 0.06, respectively (26, 29). The variant alleles retain ~5–30% of the activity of the wild-type allele (28, 30, 31). CYP2C9 is also involved in metabolizing other drugs such as anticoagulants and antibiotics. The CYP2C9 genotype is of clinical importance in patients treated with anticoagulants. Carrying a CYP2C9 variant allele can lead to complications from bleeding, because of the slower elimination of the drugs (25, 28, 31, 32).

There are two known variant alleles for UGT1A6, one having amino acid changes at amino acid 181 (T→A) and amino acid 184 (R→S), and the other having only the change at amino acid 184 (R→S; 24). In Caucasians, the allele frequencies for the variant alleles are 0.3 for T181A+R184S and 0.02 for R184S (33). The two amino acid changes in the variant genes result in an enzyme activity that is reduced to 30–50% of the wild-type allele (24).

Because both CYP2C9 and UGT1A6 play a role in NSAID metabolism and have the known variant alleles, we hypothesized that the CYP2C9 and UGT1A6 genotypes could modify the chemopreventive effect of NSAIDs on colon neoplasia. Because of the major differences in metabolic efficacy between the wild-type and the variant phenotype, it was conceivable that carriers of these alleles would show differences in the inverse association for a given dose compared with wild-type individuals. The association between CYP2C9 and

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3 The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; CYP, cytochrome P450; UGT, UDP-glucuronosyl transferase; OLA, oligonucleotide ligation assay; RFLP, restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval; COX, cyclooxygenase; DH, Digestive Healthcare.
UGT1A6 genotypes and the known chemopreventive effect of NSAIDs was tested in a colon adenoma case-control study.

Materials and Methods

This case-control study was conducted between April 1991 and April 1994 as part of the Minnesota Cancer Prevention Research Unit, a National Cancer Institute-funded program project that combined several units within the University of Minnesota and DH, a large multiclinic private gastroenterology practice. DH conducts colonoscopies in 10 hospitals and, at the time of this study, undertook ~60% of all of the colonoscopies in the Minneapolis metropolitan area. The original study was approved by the internal review boards of the University of Minnesota and each DH endoscopy site. Written informed consent was obtained from each study participant. The study has been described previously (34). Relevant details are described below. The activities associated with the present study were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, Seattle, Washington.

Study Participants. DH staff initiated study recruitment at the time of scheduling colonoscopy appointments. The initial eligibility assessment evaluated whether patients were between 30 and 74 years old, residents of the Minneapolis-St. Paul metropolitan area, English speaking, free of known genetic syndromes associated with predisposition to colonic neoplasia, and with no individual history of ulcerative colitis, Crohn’s disease, adenomatous polyps, and cancer (except nonmelanoma skin cancer). Patients were recruited at all 10 of the DH endoscopy sites.

At the colonoscopy visit, the signed consent form and completed questionnaires were collected, and blood was drawn. The colonoscopists recorded findings on standardized forms. All polyps were removed and examined histologically by a single study pathologist (C.L.) using the diagnostic criteria established for the National Polyp Study (35). If polyps had been removed during a sigmoidoscopy performed prior to the colonoscopy, the relevant slides were also evaluated by the study pathologist.

On the basis of the colonoscopy and pathology findings, participants were assigned to groups. To be eligible as an adenoma case or a colonoscopy-negative control, the participant must have had a complete colonoscopy reaching the cecum, had all polyps removed, not had a new diagnosis of ulcerative colitis or Crohn’s disease, and had no polyps showing invasive carcinoma. Adenoma cases had at least one adenomatous poly (defined as either adenomatous or mixed pathology). Controls were polyp-free at colonoscopy.

Data Collection. Study participants provided detailed information on demographic characteristics, personal medical history, smoking history, diet, usual physical activity, anthropometric measurements, reproductive history and hormone use (women only), and family history of polyps and cancer. Pack-years of smoking were calculated as years smoked × current or, in the case of former smokers, past daily cigarette use divided by 20. The frequency of current aspirin and nonaspirin NSAID use was assessed as number of pills per week. Duration of drug use was measured as <1 year, 1–2 years, 3–4 years, or >5 years.

Genotyping. Genomic DNA was extracted from peripheral WBCs using the Puregene kit (Gentra Systems, Minneapolis, MN). CYP2C9 genotyping was performed by RFLP (R144C) and by OLA (159SL). A fragment containing the R144C mutation was amplified using primers 5'-GGAGGATGGAAAA-CAGAGAC-3' and 5'-TCCTCCACAAAGCAGCGG-3' (25). The PCR reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM dNTP, 200 mM primers, 50 μg/ml BSA, 100 ng of genomic DNA, and 1 unit of AmpliTaq DNA polymerase (PE Biosystems, Foster City, CA). Cycling was at 94°C for 5 min and 35 cycles of 94°C for 30 s, 61°C for 45 s, and 72°C for 1 min, followed by 72°C for 5 min. The amplified fragment was then digested with AvaII, and the products were separated on a 2% NuSieve agarose gel. The fragment sizes were 223 bp and 38 bp for the wild-type allele and 261 bp for the mutant allele.

For the assessment of the 159SL polymorphism, a fragment containing the mutation was amplified using primers 5'-CCAGAGAAGAGATTGAA-3' and 5'-ATACTATGATTGAGGATCC-3' (25). The PCR reaction mixture was the same as above, except for MgCl2, which was at 2 mM. The cycling conditions were the same as above. The OLA was performed essentially as described previously (36), using the allele-specific primers 5'-Biotin-CACGAGGTCCAGGATAC-3' (wild type) and 5'-Biotin-CACGAGGTCCAGGATAC-3' (mutant) as well as the phosphorylated common primer 5'-TTGACCTTCTCCCTCACGAG-3'. The UGT1A6 genotyping was performed by RFLP (T181A) and OLA (R184S). PCR amplification was performed as described (33). The T181A polymorphism was assessed by restriction digestion with NsiI, and separation of the products on a 2% NuSieve gel. The fragment sizes were 268 bp for the wild-type allele and 174 bp and 94 bp for the mutant allele. The OLA for the R184S polymorphism was performed as described previously (33).

Data Analysis. Standard techniques for case-control studies were used. Unconditional logistic regression models were used to obtain maximum likelihood estimates, OR, and 95% CIs. Regular use of aspirin or nonaspirin NSAIDs was defined as at least one tablet per week for at least 1 year. Study subjects using both aspirin and nonaspirin NSAIDs were included in both groups. Multivariate adjustment included age, sex, smoking, and, among women, ever use of hormone replacement therapy (yes/no). These variables either had been previously shown to be modulators of risk of colorectal polyps in this population (34), or altered some risk estimates by 10%. All tests of statistical significance were two-sided. All of the analyses were performed using SAS Version 6.12 (SAS Institute Inc., Cary, NC).

Results and Discussion

In this study population of 474 adenomatous polyp cases and 563 controls, ~18% of study participants used aspirin on a regular basis, whereas 9% used nonaspirin NSAIDs. As Table 1 shows, aspirin, as well as nonaspirin NSAID, use was inversely associated with adenomatous polyp risk. These observations are consistent with the majority of published reports investigating NSAID effects on colon neoplasia (reviewed in Ref. 3).

In this study population, the allele frequencies for the CYP2C9 variants were 0.11 and 0.06 for the CYP2C9*2 and CYP2C9*3 alleles, respectively. The UGT1A6 variant alleles 181A,184S and 184S occurred at frequencies of 0.33 and 0.02, respectively. The allele frequencies for both genes were comparable with other reports for a Caucasian population (26, 29, 33). The genotypes for both enzymes were in Hardy-Weinberg equilibrium. Analysis of the genotype distribution between adenomatous polyp cases and controls showed that, overall, carrying a slow CYP2C9 or UGT1A6 allele (heterozygous or homozygous mutant genotype) did not influence risk of colon adenomas (Table 2).

Variation in genotype, however, did modify risk among aspirin users. An assessment of colon adenoma risk in users and nonusers based on either the CYP2C9 or the UGT1A6 genotype showed an inverse association with aspirin only for individuals who were homozygous wild-type for CYP2C9 or carried a variant UGT1A6 allele (heterozygous or homozygous variant; Table 3). The effect of CYP2C9 and UGT1A6 genotypes on colon adenoma risk was also analyzed for study subjects who were using nonaspirin NSAIDs on a regular basis. The results suggest, contrary to the results obtained for aspirin users, an inverse association between nonaspirin NSAIDs use and colon adenoma risk regardless of genotype (Table 3). Both enzymes, CYP2C9 and UGT1A6, are known to metabolize nonaspirin NSAIDs (23, 24). It was, therefore, expected that the chemopreventive efficacy of these drugs would also be modified by genotype. The absence of effect modification in this study could be explained either by the variety of nonaspirin NSAIDs that were taken by the study participants.

Table 1. Aspirin use and colon polyp risk

<table>
<thead>
<tr>
<th>NSAID use</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>382</td>
<td>363</td>
<td>1.00</td>
</tr>
<tr>
<td>Yes</td>
<td>106</td>
<td>78</td>
<td>0.63 (0.44–0.90)</td>
</tr>
<tr>
<td>Nonaspirin NSAID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>382</td>
<td>363</td>
<td>1</td>
</tr>
<tr>
<td>Yes</td>
<td>69</td>
<td>31</td>
<td>0.50 (0.31–0.82)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, smoking, and hormone-replacement therapy.
subjects or the different metabolic pathways involved in eliminating these drugs.

The fact that the genotypes for both enzymes, CYP2C9 and UGT1A6, modulate the colon adenoma risk in aspirin users led us to assess the effect of aspirin on colon adenomas as a factor of the combined CYP2C9 and UGT1A6 genotypes. On the basis of the influence of the individual genotypes on the inverse association with aspirin, it was expected that individuals homozygous wild type for CYP2C9 and carrying at least one UGT1A6 variant allele would benefit the most from aspirin use. On the other hand, aspirin use would not provide any benefits in individuals homozygous wild type for UGT1A6 and carrying at least one variant allele of CYP2C9. Table 4 shows that the largest inverse association was indeed observed in individuals with a wild-type CYP2C9/variant UGT1A6 genotype. Individuals who carried a variant CYP2C9/wild-type UGT1A6 genotype did not benefit from aspirin use.

Glucuronidation is mainly a detoxification process and glucuronides are not usually biologically active. In vivo, aspirin is rapidly hydrolyzed to salicylic acid, which has a longer half-life than aspirin and reaches significantly higher peak plasma concentrations than aspirin. Aspirin inhibits prostaglandin synthesis by acetylation of the Cox enzymes. However, a reduction of Cox-mediated prostaglandin E2 synthesis has also been reported for salicylate, gentisic acid, and salicyl-CoA, an intermediate product in the formation of salicylic acid (37, 39, 40). Furthermore, salicylate has been shown to inhibit growth in colorectal tumor cell lines (41).

On the basis of these reports, we hypothesize that slow glucuronidation in carriers of the UGT1A6 variant alleles may lead to increased salicylate levels and/or increased elimination through the gentisic acid and salicylic acid pathways. This results in increased levels of gentisic acid as well as salicyl-CoA, the intermediate product in the formation of salicylic acid, both of which can inhibit prostaglandin synthesis (40). Lower levels of prostaglandins could eventually lower the risk of colon neoplasia. In carriers of the CYP2C9 variant alleles, the pathway to gentisic acid is impaired, resulting in a reduced inhibition of prostaglandin synthesis and absence of risk reduction for colon neoplasia.

Recently, a new class of drugs has been developed to inhibit Cox-2 specifically. These drugs are used for the treatment of chronic diseases such as osteoarthritis and rheumatoid arthritis (42, 43). In vitro experiments have shown that CYP2C9 can hydroxylate the Cox-2 inhibitor celecoxib (44); however, the activity of variant CYP2C9 forms on this drug has not been tested. It is conceivable that the CYP2C9 variant alleles show different kinetics from the wild type in metabolizing this drug and, thus, may affect its efficacy. With the increasing use of Cox-2 inhibitors, the effect of CYP2C9 genotypes may play an important role.

### Table 2 CYP2C9 and UGT1A6 genotype and colon polyp risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>389</td>
<td>327</td>
<td>1.00</td>
</tr>
<tr>
<td>Variant</td>
<td>174</td>
<td>147</td>
<td>1.10 (0.83–1.46)</td>
</tr>
<tr>
<td>UGT1A6 Wild-type</td>
<td>240</td>
<td>200</td>
<td>1.00</td>
</tr>
<tr>
<td>Variant</td>
<td>323</td>
<td>274</td>
<td>0.97 (0.74–1.26)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, smoking, and hormone replacement therapy.

### Table 3 CYP2C9 and UGT1A6 genotypes, NSAID use, and colon polyp risk

<table>
<thead>
<tr>
<th>NSAID use</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls/Cases</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Aspirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Wild-type</td>
<td>256/257</td>
</tr>
<tr>
<td></td>
<td>Variant</td>
<td>126/106</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Wild-type</td>
<td>165/151</td>
</tr>
<tr>
<td></td>
<td>Variant</td>
<td>217/212</td>
</tr>
<tr>
<td>Nonaspirin NSAID</td>
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<td></td>
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<tr>
<td>CYP2C9</td>
<td>Wild-type</td>
<td>256/257</td>
</tr>
<tr>
<td></td>
<td>Variant</td>
<td>126/106</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Wild-type</td>
<td>165/151</td>
</tr>
<tr>
<td></td>
<td>Variant</td>
<td>217/212</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, smoking, and hormone replacement therapy.

### Table 4 CYP2C9 + UGT1A6 genotype, aspirin use, and colon polyp risk

<table>
<thead>
<tr>
<th>Aspirin use</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls/Cases</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>CYP2C9wt/UGT1A6wt†</td>
<td>103/104</td>
<td>1.00</td>
</tr>
<tr>
<td>CYP2C9var/UGT1A6var</td>
<td>64/59</td>
<td>0.94 (0.58–1.51)</td>
</tr>
<tr>
<td>CYP2C9wt/UGT1A6var</td>
<td>153/153</td>
<td>1.08 (0.74–1.58)</td>
</tr>
<tr>
<td>CYP2C9var/UGT1A6wt</td>
<td>62/47</td>
<td>0.99 (0.60–1.63)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, smoking, and hormone replacement therapy.
† wt, wild type; var, variant.
Our findings show that the presence of variants of drug-metabolizing enzymes can influence the effectiveness of chemotherapeutic agents. Although the relatively large size of this case-control study did allow the investigation of gene-environment interactions, the sample size was limited for exploring the combined effects of two genotypes, or interactions between genotypes and specific NSAIDs. This emphasizes the need for large epidemiological studies to investigate such associations; however, additional approaches to exploring these gene-drug interactions are also warranted, particularly to clarify whether some agents are not effective in sizable subsets of the population.

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References

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