BRIEF COMMUNICATION

Genetic Variants in the UGT1A6 Enzyme, Aspirin Use, and the Risk of Colorectal Adenoma

Andrew T. Chan, Gregory J. Tranah, Edward L. Giovannucci, David J. Hunter, Charles S. Fuchs

Genetic variation in the uridine diphosphate glucuronosyltransferase 1A6 (UGT1A6) enzyme is associated with impaired metabolism of aspirin. To determine whether polymorphisms in the UGT1A6 enzyme modulate the protective benefit of regular aspirin use on colorectal adenoma, we conducted a prospective, nested case-control study of 1062 women who provided blood specimens and detailed data on aspirin use before undergoing lower endoscopy. All statistical tests were two sided. Although UGT1A6 genotype was not associated with overall adenoma risk (multivariable odds ratio [OR] = 1.10, 95% confidence interval [CI] = 0.85 to 1.41), functional variant genotypes statistically significantly modified the effect of aspirin on adenoma (Pinteraction = .02). Among the 616 women with variant genotypes, regular use of aspirin (two or more standard tablets per week) was associated with a decreased risk of adenoma (multivariable OR for adenoma = 0.66 [95% CI = 0.45 to 0.95], OR = 0.63 [95% CI = 0.43 to 0.91] for 0.5–7 standard tablets per week and OR = 0.41 [95% CI = 0.24 to 0.71] for more than 7 tablets per week; Prand = .001). In contrast, among women with wild-type genotypes, regular aspirin use was not associated with a reduced risk nor did they obtain any additional benefit with higher doses (Prand = .50). These results were consistent among women with advanced adenomas (Pinteraction = .003). Thus, functional polymorphisms in the UGT1A6 enzyme statistically significantly modify the effect of aspirin on colorectal neoplasia, and certain subsets of the population, defined by genotype, may obtain differential benefit from aspirin chemoprevention. [J Natl Cancer Inst 2005;97:457–60]

Regular aspirin use has been associated with a reduced risk of colorectal adenoma (1–4). However, it remains unclear whether there are specific determinants, including genetic markers, that could identify individuals who may obtain differential benefit from aspirin therapy. Although one study found low-dose, but not standard-dose, aspirin to be effective (1), several lines of evidence (4–7) suggest that the influence of aspirin on adenoma risk may be dose dependent, with the greatest benefit at higher doses. Thus, efficiency of aspirin metabolism may be inversely associated with therapeutic efficacy.

Aspirin is principally glucuronidated by the uridine diphosphate glucuronosyltransferase isoenzyme 1A6 (UGT1A6) (8). In Caucasians, two common variant alleles—a tandem mutation in amino acids 181 (threonine to alanine) and 184 (arginine to serine) (T181A + R184S) and a single mutation in amino acid 184 (R184S)—have allele frequencies of 30% and 2%, respectively. Compared with the wild type, these polymorphisms are associated with 30%–50% lower enzyme activity (9).

A prior case-control study first suggested that the lower risk for adenoma associated with aspirin use was restricted to participants with these functional variant UGT1A6 alleles (10). However, the study lacked a prospective assessment of aspirin intake and did not examine aspirin dose. Using prospectively collected blood specimens and detailed, regularly updated data on aspirin use, we examined these relationships in a nested case-control study of women participating in the Nurses’ Health Study, a cohort of 121701 female nurses who returned a questionnaire in 1976 (11). In 1989–1990, 32826 participants provided a blood specimen; follow-up of this subcohort exceeds 96%. The methods used for follow-up, endpoint ascertainment, and blood collection has been previously detailed (4,12). Eligible women for selection as either an adenoma case participant or control participant were those women who reported a sigmoidoscopy and/or colonoscopy after providing a blood sample and were free from inflammatory bowel disease, a polyposis syndrome, or diagnosed cancer (except nonmelanoma skin). Baseline characteristics of study participants have been previously described (4,13). We defined case participants as women who reported an incident polyp confirmed to be adenomatous through June 1, 1998, after blinded review of medical records. Consistent with other studies (13,14), we defined advanced lesions as adenomas of 1 cm or more in diameter or any size with tubulovillus, villous, or severely dysplastic features. Early lesions were defined as adenomas with tubular histologic characteristics that were less than 1 cm in diameter. Among the case participants, 72% had adenoma localized to the distal colorectum (less than 60 cm from the anus), 38% had advanced adenoma, and 21% had two or more adenomas. After matching one control participant without a polyp to each case participant by age, date of endoscopy, indication for endoscopy, time period of prior endoscopy, date of blood draw, and fasting status, we identified 557 matched pairs. We excluded one case participant whose polyp was later reclassified as hyperplastic, nine with duplicate specimens, six who did not provide aspirin data, and 36 with insufficient sample. The Human Research Committee at the Brigham and Women’s Hospital approved this study.

As described previously (4,15), we assessed aspirin use on biennial questionnaires. We defined women who reported taking an equivalent of at least two standard 325-mg aspirin tablets per week as regular users and women who reported taking less than two tablets as nonregular.

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We also classified participants according to the number of standard tablets used per week (4,15). Laboratory personnel blinded to case-control status extracted genomic DNA from buffy-coat fractions with QIAmp (Qiagen Inc., Chatsworth, CA) and genotyped UGT1A6 T181A (rs2070959) and R184S (rs4365457) with TaqMan (PE Applied Biosystems, Foster City, CA) primers and probes. We assigned genotypes by use of endpoint fluorescence (Applied Biosystems 7900HT, SDS Software version 1.7a). We inserted quality control samples to validate genotype identification procedures; concordance for blinded samples was 100%.

We used the chi-squared test to assess whether genotypes were in Hardy-Weinberg equilibrium. We used conditional logistic regression to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). We obtained similar results with unconditional or conditional logistic regression adjusting for matching factors. To increase the statistical power in our analyses stratified by genotype, we used unconditional logistic regression models adjusting for age, according to 5-year categories, and multiple known or suspected adenoma risk factors simultaneously. We present unconditional regression models adjusting for adenoma risk factors. As in prior analyses, we used updated data from the most recent questionnaire completed before endoscopy (4).

We did not ask participants to specify colonoscopy or sigmoidoscopy; however, on the basis of secular trends (16), a substantial portion of procedures may have been sigmoidoscopies that only examined the distal colorectum. To minimize misclassification of outcome (e.g., undiagnosed proximal adenoma in control participants), we also performed a secondary analysis in which we restricted our case definition to those participants with adenoma located less than 60 cm from the anus and their matched control participants (4,17).

Consistent with prior studies, we combined participants with one or two variant alleles, because of the low prevalence of homozygous variants (10). We evaluated the interaction of aspirin use and genotype by conducting analyses stratified by genotype and assessed statistical significance by using the Wald test for cross-product terms of variant genotype and aspirin dose in the multivariable regression analyses. All statistical tests were two-sided.

In this study population of 530 case participants with colorectal adenoma and 532 control participants without adenoma, the mean age was 61 years and more than 98% of the cohort was Caucasian. The allele frequencies were consistent with reports in other predominantly Caucasian populations (0.32 for the T181A + R184S allele and 0.03 for the R184S allele (10,18). Compared with the presence of no variant alleles (wild type, the presence of at least one functional variant allele (among 616 participants, 491 had one variant and 125 had two variants) was not statistically significantly associated with an increased risk of colorectal adenoma (multivariable OR = 1.10, 95% CI = 0.85 to 1.41) after adjustment for adenoma risk factors.

Among all women in these nested analyses, we observed that a decreased risk of adenoma was statistically significantly associated with regular aspirin use (OR = 0.76, 95% CI = 0.58 to 0.99) compared with nonregular use, consistent with our prior findings among the larger cohort (4). However, the benefit of regular aspirin use on adenoma risk was largely confined to the group with functional variant UGT1A6 genotypes (OR = 0.66, 95% CI = 0.45 to 0.95, for women who used aspirin regularly compared with women who did not use aspirin regularly). Among women with wild-type genotypes, regular aspirin use was not associated with a statistically significant reduction in the risk of adenoma (OR = 0.93, 95% CI = 0.60 to 1.44, for women who used aspirin regularly compared with women who did not use aspirin regularly; Table 1). Variant genotypes statistically significantly modified the effect of aspirin dose on adenoma risk (Pinteraction = .02). Irrespective of genotype, the effect of aspirin was dose dependent, with the greatest reduction in risk associated with the use of more than seven tablets a week (P trend = .004). However, a statistically significant dose-response relationship was again largely confined to the presence of functional variant UGT1A6 genotypes (for 0.5–7 tablets per week, OR = 0.63 [95% CI = 0.43 to 0.91]; and for more than seven tablets per week, OR = 0.41 [95% CI = 0.24 to 0.71]; P trend = .001). Among participants with wild-type alleles, the influence of aspirin dose was markedly attenuated (P trend = .50; Table 2).

We also examined whether these findings were consistent for advanced adenoma (1 cm or more in diameter or any size with tubulovillous, villous, or severely dysplastic features). Among women with Table 1. Risk for colorectal adenoma according to regular aspirin use stratified by UGT1A6 genotype*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nonregular users</th>
<th>Regular users</th>
</tr>
</thead>
<tbody>
<tr>
<td>All UGT1A6 genotypes</td>
<td>373/349</td>
<td>157/183</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.0 (referent)</td>
<td>0.78 (0.60 to 1.02)</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)†</td>
<td>1.0 (referent)</td>
<td>0.76 (0.58 to 0.99)</td>
</tr>
<tr>
<td>Wild-type UGT1A6 genotypes</td>
<td>149/154</td>
<td>68/75</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.0 (referent)</td>
<td>0.90 (0.60 to 1.34)</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)†</td>
<td>1.0 (referent)</td>
<td>0.93 (0.60 to 1.44)</td>
</tr>
<tr>
<td>Variant UGT1A6 genotypes</td>
<td>224/195</td>
<td>89/108</td>
</tr>
<tr>
<td>Age-adjusted OR (95% CI)</td>
<td>1.0 (referent)</td>
<td>0.71 (0.50 to 0.99)</td>
</tr>
<tr>
<td>Multivariable OR (95% CI)†</td>
<td>1.0 (referent)</td>
<td>0.66 (0.45 to 0.95)</td>
</tr>
</tbody>
</table>

*Colorectal adenoma includes all pathology-verified adenoma in the proximal colon, distal colon, and rectum. Regular aspirin use was defined as the consumption of two or more tablets per week, and nonregular use was defined as the consumption of less than two tablets per week, as computed from the questionnaire before endoscopy. Wild-type genotypes include genotypes homozygous for wild-type UGT1A6 alleles (+/+). Variant genotypes include genotypes heterozygous or homozygous for functional variant T181A+R184S or R184S alleles. Odds ratios (ORs) are for regular aspirin use compared with nonregular aspirin use. CI = confidence interval.

†Multivariable ORs are adjusted for age (5-year categories); smoking history (0, 1–15, 16–35, or ≥35 pack-years); body mass index (in quintiles); physical activity (<1.7, 1.7–4.5, 4.6–10.5, 10.6–22.1, or ≥22.1 metabolic equivalent task score per week); colorectal cancer in a parent or sibling (yes or no); postmenopausal hormone use (premenopausal or never, past, or current use); consumption of beef, pork, or lamb as a main dish (≤1 time or less per week, 2–4 times per week, 5–6 times per week, or ≥1 time per day); alcohol consumption (0, 0.1–4.9, 5.0–14.9, or ≥15 g per day); current multivitamin use (yes or no); and energy-adjusted quintiles of folate and calcium intake. All covariates were computed from the questionnaire before endoscopy.
functional variant UGT1A6 genotypes, regular aspirin use was associated with a decreased risk for advanced adenoma (multivariable OR = 0.71 [95% CI = 0.42 to 1.20]); for 0.5–7 standard aspirin tablets per week, OR = 0.74 [95% CI = 0.44 to 1.24]; and for more than 7 tablets per week, OR = 0.20 [95% CI = 0.08 to 0.52]; \( P_{\text{trend}} = .003 \). In contrast, wild-type genotypes were not associated with a reduced risk of advanced adenoma (OR = 1.13, 95% CI = 0.61 to 2.08) or with an additional benefit with higher doses (\( P_{\text{trend}} = .85 \)). Moreover, the functional variant genotypes statistically significantly modified the effect of aspirin dose on risk of advanced adenoma (\( P_{\text{interaction}} = .03 \)). Among women with functional variant genotypes, regular aspirin use was associated with a decreased risk for distal adenoma (OR = 0.61, 95% CI = 0.39 to 0.95), whereas among those with wild-type genotypes, regular aspirin use was not associated with the risk for distal adenoma (OR = 0.90, 95% CI = 0.52 to 1.53). Similarly, a statistically significant influence of aspirin dose was associated with the functional variant genotypes (\( P_{\text{interaction}} = .003 \)) but not with wild-type genotypes (\( P_{\text{trend}} = .53 \); Table 2). Finally, we have previously shown that genetic variation in the cytochrome P450 2C9 (CYP2C9) isoenzyme may be associated with an elevated risk of distal colorectal adenoma in this cohort (13). However, additional adjustment for CYP2C9 genotype did not materially alter these findings (data not shown).

Because participants in the study may have initially undergone either a sigmoidoscopy or a colonoscopy (16), we considered the possibility that the differential use of colonoscopy among case participants and control participants could have biased our findings. We therefore repeated our analyses after excluding case participants with adenomas beyond the splenic flexure and their matched control participants. Among 384 case participants with distal (less than 60 cm from the anus) adenoma and their 383 matched control participants, the effect of aspirin remained statistically significantly modified by variant genotypes (\( P_{\text{interaction}} = .03 \)). Among women with functional variant genotypes, regular aspirin use was associated with a decreased risk for distal adenoma (OR = 0.61, 95% CI = 0.39 to 0.95), whereas among those with wild-type genotypes, regular aspirin use was not associated with the risk for distal adenoma (OR = 0.90, 95% CI = 0.52 to 1.53). Similarly, a statistically significant influence of aspirin dose was associated with the functional variant genotypes (\( P_{\text{interaction}} = .003 \)) but not with wild-type genotypes (\( P_{\text{trend}} = .53 \); Table 2). Finally, we have previously shown that genetic variation in the cytochrome P450 2C9 (CYP2C9) isoenzyme may be associated with an elevated risk of distal colorectal adenoma in this cohort (13). However, additional adjustment for CYP2C9 genotype did not materially alter these findings (data not shown).

In this large, prospective nested case-control study, genetic variation in the UGT1A6 enzyme statistically significantly modified the effect of aspirin use on risk of colorectal adenoma. Specifically, functional variant genotypes associated with impaired aspirin metabolism were in turn associated with a greater benefit observed with regular aspirin use and increasing aspirin dose. Controlling for other known or purported risk factors for colorectal adenoma and cancer did not alter these findings.

A growing body of data that the chemopreventive mechanism of aspirin is dose dependent supports the biological plausibility of our results (4–6). Although low-dose aspirin sufficiently inhibits cyclooxygenase 1 in anucleated platelets, higher doses are needed to inhibit cyclooxygenase 2, which is overexpressed in adenoma and carcinoma tissues (19,20). Moreover, experimental models have described cyclooxygenase-independent mechanisms of aspirin maximized at higher doses (21–23). Finally, consistent with our findings, in a recent cross-sectional study, Bigler et al. (10) found that the inverse association between aspirin use and adenoma risk was restricted to participants with variant UGT1A6 genotypes.

The strengths of our study include its relatively large size, prospective design, repeated assessments of aspirin use, detailed data on potential confounders, and high follow-up rate. In particular, we had a unique ability to examine a wide range of dose categories and interaction with genotype. Because participants were nurses, the accuracy of self-reported use is likely to be high; information on many exposures, including endoscopy, has been previously validated (24–27); and other associations, including aspirin use and colorectal neoplasia, have been well-corroborated (1–4,15). Moreover, because our study was nested within a larger, well-defined cohort, control participants were sampled from the same population as case participants. Thus, our results are unlikely to be influenced by population stratification or selection bias (28).

We acknowledge several limitations. First, our study was limited primarily to Caucasian women. However, to our knowledge, there has been no sex difference noted in UGT1A6 genotype prevalence or function (9,10,18), and a prior study that included men reported consistent results.


Notes

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