Ornithine Decarboxylase Polymorphism Modification of Response to Aspirin Treatment for Colorectal Adenoma Prevention

Elizabeth L. R. Barry, John A. Baron, Shubha Bhat, Maria V. Grau, Carol A. Burke, Robert S. Sandler, Dennis J. Ahnen, Robert W. Haile, Thomas G. O’Brien

Background: Previous research suggests that the G315A single-nucleotide polymorphism in the ornithine decarboxylase (ODC) gene may be a genetic marker for risk of colorectal neoplasia and may also modify the association of aspirin use with risk. Methods: We tested these hypotheses among participants in the Aspirin/Folate Polyp Prevention Study who were randomly assigned to placebo or to aspirin treatment (81 or 325 mg daily) and followed for 3 years for the occurrence of new adenomas. Genomic DNA from 973 subjects was analyzed for ODC genotype. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated to test the association between ODC genotype and adenoma occurrence and interactions with aspirin treatment. All statistical tests were two-sided. Results: Of the 973 subjects, 54% were homozygous wild-type (GG), 7% were homozygous variant (AA), and 39% were heterozygous individuals; the allele frequencies varied statistically significantly by race and ethnicity. Among these subjects, the absolute risk of any adenoma was 45% and the risk of an advanced lesion was 10%. Overall, no association was found between ODC genotype and the occurrence of new adenomas, but genotype did modify the effect of aspirin on adenoma risk. Although aspirin treatment had no protective effect among subjects with a GG genotype, among subjects with at least one A allele, it was associated with statistically significant reduced risks of any adenoma (RR = 0.77, 95% CI = 0.63 to 0.95; P = .02, Pinteraction = .04) and of advanced lesions (RR = 0.51, 95% CI = 0.29 to 0.90; P = .02, Pinteraction = .02). Among subjects with at least one A allele, 40.8% who took aspirin versus 52.9% who took placebo developed adenomas; 7.1% versus 14.0% developed advanced lesions. Conclusion: ODC genotype may modify the response to aspirin treatment for colorectal adenoma prevention. [J Natl Cancer Inst 2006;98:1494–1500]

Polyamines are cell-signaling molecules whose regulation is important for cell growth and development and for wound healing (1). Ornithine decarboxylase (ODC) catalyzes the first step in polyamine biosynthesis—the decarboxylation of ornithine to putrescine (2). Several lines of evidence implicate polyamines and ODC in carcinogenesis. Polyamine levels and ODC activity are increased in many epithelial cancers, including cancers of the skin, prostate, and colon (1). Polyamine synthesis and ODC activity appear to be induced, and polyamine catabolism suppressed, by mutations in adenomatous polyposis coli and K-ras, respectively, two genes that are commonly mutated in colon cancer (3–8). Conversely, polyamine catabolism appears to be induced by nonsteroidal anti-inflammatory drugs (NSAIDs), whose use is associated with a decreased risk of several epithelial cancers, including colorectal cancer (9).

In humans, a single-nucleotide polymorphism (SNP) in the ODC gene is thought to reduce its expression (10). The polymorphic site (G315A) is located in a regulatory region in the first intron near the binding sites for transcription factors. Evidence that this ODC polymorphism has functional relevance comes from in vitro studies showing that this region influences the binding of transcription factors (11) and studies of cultured cells showing differential transcriptional regulation of the two alleles (10,12). A previous genetic association study reported that individuals homozygous for the minor A allele were approximately half as likely to have an adenoma occurrence as GG (wild-type) homozygotes, and the risk of adenoma was even lower in AA homozygotes who reported taking aspirin (10). These results suggest that this ODC polymorphism may be a genetic marker for risk of colorectal neoplasia and may also modify the association of aspirin use with risk.

The present analysis was undertaken to examine the relationships among this ODC polymorphism, aspirin treatment, and the risk of colorectal adenoma among subjects at high risk of adenoma who were randomly assigned to placebo or aspirin treatment in the Aspirin/Folate Polyp Prevention Study (13), which showed that low-dose aspirin has a moderate chemopreventative effect on adenomas in the large bowel. We tested the hypotheses that the ODC AA genotype is associated with a reduction in adenoma occurrence and that ODC genotype modifies the reduction in adenoma risk associated with aspirin treatment.

SUBJECTS AND METHODS

Study Population

The subjects were participants in the Aspirin/Folate Polyp Prevention Study, a double-blinded placebo-controlled clinical trial of aspirin and folate as chemopreventive agents against the occurrence of new colorectal adenomas (Fig. 1, clinical trial registration no. NCT00272324) (13). All subjects had a recent history of a histologically confirmed colorectal adenoma and a complete colonoscopy within 3 months before enrollment (qualifying examination), with no known polyps left in the bowel. A total of 1121 subjects were randomly assigned to one of three study arms.

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See “Notes” following “References.”

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between September 25, 1994, and July 15, 1998, at recruitment sites associated with nine clinical centers in North America. Subjects gave written informed consent, and all research (including this analysis) was approved by the research ethics committees of the participating institutions.

Subjects were randomly assigned to placebo, 81 mg of aspirin (lower dose), or 325 mg of aspirin (higher dose) daily. Subjects were also independently randomly assigned to placebo or 1 mg of folate daily in a factorial design. At enrollment, subjects agreed to avoid the use of NSAIDs outside the study while they were receiving their randomly assigned treatment; NSAID use was monitored by questionnaires every 4 months. By protocol, follow-up colonoscopies were to be scheduled 3 years after the qualifying examination. A total of 1084 subjects (97%) completed a follow-up colonoscopy during the period from at least 1 year after random assignment through the anticipated 3-year follow-up (mean intervention period ± standard deviation, 32.7 ± 3.8 months) and were considered suitable for inclusion in the present analysis. Of them, 471 (43%) were diagnosed with at least one adenoma. Findings from interim endoscopic examinations, which were performed after random assignment but before the follow-up examination in approximately 5% of the subjects, were included in the analysis. A single study pathologist (D.C.S.) reviewed all clinical samples removed from the large bowel after random assignment and categorized the lesions as nonneoplastic or neoplastic (i.e., adenomatous). Adenomas were characterized as to degree of dysplasia and architecture: tubular, tubulovillous (25%–75% villous component), or villous (>75% villosity).

Genotyping

Of the 1084 subjects who completed a follow-up colonoscopy, ODC genotyping was performed on 973 (90%). The remaining 111 subjects (10%) could not be included because of lack of consent. Genomic DNA was isolated from whole blood cells using proteinase K digestion and phenol–chloroform extraction. ODC was genotyped using a TaqMan-based allelic discrimination assay as described (12). Briefly, a 172-bp fragment containing the polymorphic site (SNP accession no. rs2302615) was amplified using the polymerase chain reaction, and the SNP was detected with allele-specific probes containing different 5′ fluorescent labels. All DNA samples were analyzed in duplicate or triplicate. Of the samples analyzed by the TaqMan assay, 7% were also analyzed by a restriction fragment length polymorphism–polymerase chain reaction assay based on the polymorphic Pst1 site (12) with identical results.

Race and Ethnicity

At enrollment, subjects were asked to complete a questionnaire on which they identified their “race or ethnic background” by selecting one of the following (Table 1): 1) white, not of Hispanic origin; 2) black, not of Hispanic origin; 3) Hispanic; 4) American Indian or Alaskan Native; 5) Asian or Pacific Islander; 6) other; 7) uncertain. For multivariable analysis, a bivariate race/ethnicity variable was created: self-reported “non-Hispanic whites” (answer 1, N = 860) and all others (answers 2–6 above, N = 113). There were no “uncertain” responses.

Statistical Analysis

Comparisons of subject characteristics by ODC genotype at SNP rs2302615 (Tables 1 and 2) were performed using Pearson chi-square tests for categorical variables and analysis of variance or nonparametric Kruskal–Wallis tests for continuous variables. Deviations from Hardy–Weinberg equilibrium were assessed by the likelihood ratio chi-square test.
The principal outcome of the trial was the occurrence of one or more adenomas during randomized treatment. We also evaluated the occurrence of advanced lesions: tubulovillous adenomas, villous adenomas, large adenomas ($\geq$1 cm in diameter), adenomas with severe dysplasia, and invasive cancer. Risk ratios were calculated using an overdispersed generalized linear model for the Poisson distribution as an approximation to the binomial family. Unadjusted (univariate) relative risks (RRs) are presented because the small numbers in some groups limited the number of covariates appropriate for the models. Where indicated, adjusted relative risks were calculated by including age, sex, aspirin treatment group, and mean follow-up time as covariates. Interactions were evaluated using product interaction terms and Wald statistics (age, sex, treatment assignments) among the subjects when compared with the GG ODC genotype.

### Main Association of ODC Genotype

The frequency of adenoma occurrence, which was approximately 45% overall, was similar (between 41.2% and 45.7%) among subjects in the three ODC genotype subgroups (Table 3). As indicated by the relative risks, there was virtually no association between the ODC genotype and the risk of any adenoma. However, when the analysis was limited to advanced lesions (found in 10% of subjects), there was a statistically significant 30% reduction in risk associated with the AA genotype relative to the GG genotype (RR = 0.70, 95% confidence interval [CI] = 0.29 to 1.69). Among heterozygotes, there was a nonstatistically significant 10% reduction in risk of advanced lesions compared with the GG genotype.

To avoid possible confounding by population stratification, we also conducted analyses limited to non-Hispanic whites (88% of subjects). In this population, there were nonstatistically significant risk reductions for advanced lesions among heterozygotes (RR = 0.86, 95% CI = 0.57 to 1.30) and among AA homozygotes (RR = 0.18, 95% CI = 0.03 to 1.20).

### Interaction Analysis

In the subset of 973 subjects who underwent genotyping, aspirin treatment (low and high doses combined) was associated with nonstatistically significant risk reductions for all adenomas (RR = 0.91, 95% CI = 0.78 to 1.05) and for advanced lesions (31%) ($P = .02$). In addition, subjects with the AA genotype had slightly longer (approximately 1.5 months) follow-up times on average than subjects with either the GG or GA genotypes. No statistically significant differences were found in other characteristics (age, sex, treatment assignments) among the subjects when grouped by ODC genotype.
As previously reported for the trial as a whole, the lower dose of aspirin (81 mg) was more effective than the higher dose (325 mg) at reducing the risk of colorectal adenoma (13). The pattern was also seen in the subset of subjects included in this analysis (Table 6). The lower dose of aspirin treatment was associated with statistically significant risk reductions for all adenomas (RR = 0.81, 95% CI = 0.68 to 0.96; P = .02) and nonstatistically significant risk reductions for advanced lesions (RR = 0.65, 95% CI = 0.40 to 1.06; P = .08), whereas the higher dose was not associated with risk reductions. In the analysis stratified by genotype, neither the low nor the high dose of aspirin treatment was associated with risk reductions among subjects with the GG genotype. Among heterozygotes, the lower aspirin dose was associated with statistically significant risk reductions for all adenomas (RR = 0.65, 95% CI = 0.49 to 0.85; P = .002) and for advanced lesions (RR = 0.27, 0.11 to 0.69; P = .006), whereas the higher dose showed smaller, nonstatistically significant reductions. Among AA homozygotes, both aspirin doses were also associated with risk reductions for all adenomas and advanced lesions, with more pronounced reductions in the higher dose group. Possibly because of the small numbers of AA subjects, however, the corresponding relative risks were not statistically significantly different. Essentially identical results were obtained when these analyses were restricted to non-Hispanic whites (data not shown).

**DISCUSSION**

In this analysis of a randomized aspirin trial, we found that ODC genotype modified the effect of aspirin treatment on the occurrence of new colorectal adenomas. Although aspirin did not reduce adenoma risk among subjects with the ODC GG genotype, aspirin treatment conferred a statistically significant risk reduction among subjects with at least one ODC A allele.

We began this analysis with the hypothesis that the ODC AA genotype would be associated with a reduced risk of occurrence of new colorectal adenomas, as reported by Martinez et al. (10). In that study, the odds of adenoma occurrence among AA homozygotes was about half of that for heterozygotes and GG homozygotes, a result that reached borderline statistical significance (P = .05). Although our data provided no evidence for an association between the AA genotype and reduced overall adenoma occurrence/total (%) RR (95% CI) * Occurrence/total (%) RR (95% CI) Occurrence/total (%) RR (95% CI)‡

<table>
<thead>
<tr>
<th>ODC genotype</th>
<th>All adenomas</th>
<th>Advanced lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occurrence/total (%)</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>All adenomas</td>
<td>231/522 (44.3)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>GG</td>
<td>175/383 (45.7)</td>
<td>1.03 (0.89 to 1.22)</td>
</tr>
<tr>
<td>GA</td>
<td>28/68 (41.2)</td>
<td>0.93 (0.69 to 1.25)</td>
</tr>
<tr>
<td>AA</td>
<td>56/122 (10.7)</td>
<td>1.00 (referent)</td>
</tr>
</tbody>
</table>

*RR = relative risk; CI = confidence interval.
†Interaction between ODC genotype and aspirin treatment, P = .04 (two-sided Wald test).
‡Combined 81 and 325 mg aspirin treatment groups.
§Interaction between ODC genotype and aspirin treatment, P = .02 (two-sided Wald test).
occurrence, this genotype was associated with a nonstatistically significant 30% reduction in the occurrence of new advanced lesions. Data for advanced adenomas were not reported by Martínez et al. (10). Notably, in both our study and that of Martínez et al., the statistical power to detect associations with the AA genotype was similarly low as a consequence of the low prevalence of the ODC A allele. Thus, larger studies will be required to determine whether ODC genotype is a marker of overall risk of adenoma. Interestingly, in the present study, a lower proportion of subjects with at least one A allele than of subjects with two G alleles had advanced adenomas at baseline (25% versus 31%, respectively, \( P = .02 \)). These data provide additional support for a relationship between ODC genotype and the occurrence of advanced adenomas.

A second hypothesis, also based on the Martínez et al. study (10), is that there is an interaction between ODC genotype and the effect of aspirin on the occurrence of new adenomas. In that study, the association of the AA genotype with a decreased risk of adenoma recurrence appeared to be stronger among aspirin users than among nonusers. However, in the present study, the association between the ODC A allele and the risk of adenoma occurrence appeared to be opposite among subjects treated with placebo (higher risk) compared with subjects treated with aspirin (lower risk).

In the current study, the protective effect of aspirin treatment on risk of adenoma was confined to subjects with at least one A allele. However, when heterozygotes and AA homozygotes were analyzed separately, the numbers in the AA group were too small to rule out chance. In addition, we found no clear gene dose response for the beneficial effect of the ODC A allele on the association of aspirin treatment with reduced risk of adenoma. Although this apparent lack of a dose response may be due to the limited power and wide confidence intervals in the AA group, it is also possible that a single A allele is sufficient for the biologic effect (e.g., responsiveness to aspirin).

The mechanism by which ODC genotype may influence the effectiveness of aspirin treatment in adenoma prevention is not known. Martínez et al. (10) suggested that the ODC polymorphism and aspirin may each act independently of the other to reduce adenoma risk by suppressing synthesis and activating catabolism, respectively, of colonic mucosal polyamines. This hypothesis is based on their experiments using a human colon cancer cell line, in which the activity of the ODC A allele promoter was selectively suppressed by expression of the wild-type adenomatous polyposis coli gene and by the transcriptional regulator mitotic arrest deficient 1. In addition, although there was no effect of aspirin on ODC expression or activity, aspirin treatment induced the expression and activity of spermidine–spermine

### Table 5. Association of ornithine decarboxylase (ODC) genotype with risk of adenoma occurrence stratified by aspirin treatment group

<table>
<thead>
<tr>
<th>ODC genotype</th>
<th>All subjects</th>
<th>Placebo</th>
<th>Aspirin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occurrence/total (%)</td>
<td>RR (95% CI)</td>
<td>Occurrence/total (%)</td>
</tr>
<tr>
<td>GG</td>
<td>231/522 (44.3)</td>
<td>1.0 (referent)</td>
<td>72/169 (42.6)</td>
</tr>
<tr>
<td>GA or AA</td>
<td>203/451 (45.0)</td>
<td>0.96 (0.88 to 1.17)</td>
<td>83/157 (52.9)</td>
</tr>
<tr>
<td>GG</td>
<td>56/522 (10.7)</td>
<td>1.0 (referent)</td>
<td>16/169 (9.5)</td>
</tr>
<tr>
<td>GA or AA</td>
<td>43/451 (9.5)</td>
<td>0.89 (0.61 to 1.30)</td>
<td>22/157 (14.0)</td>
</tr>
</tbody>
</table>

*Combined 81 and 325 mg aspirin treatment groups.
†RR = relative risk; CI = confidence interval. Unadjusted relative risks are shown.
‡Interaction between ODC genotype and aspirin treatment, \( P = .04 \) (two-sided Wald test).
§Interaction between ODC genotype and aspirin treatment, \( P = .02 \) (two-sided Wald test).

### Table 6. Association of low- and high-dose aspirin treatment with risk of adenoma occurrence stratified by ornithine decarboxylase (ODC) genotype

<table>
<thead>
<tr>
<th>Treatment</th>
<th>All subjects</th>
<th>GG</th>
<th>GA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>RR (95% CI)</td>
<td>N (%)</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>Placebo</td>
<td>155/326 (47.6)</td>
<td>1.0 (referent)</td>
<td>72/169 (42.6)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Aspirin (81 mg)</td>
<td>126/328 (38.4)</td>
<td>0.81 (0.68 to 0.96)</td>
<td>72/178 (40.5)</td>
<td>0.95 (0.74 to 1.21)</td>
</tr>
<tr>
<td>Aspirin (325 mg)</td>
<td>153/319 (48.0)</td>
<td>1.01 (0.85 to 1.19)</td>
<td>87/175 (49.7)</td>
<td>1.17 (0.92 to 1.47)</td>
</tr>
<tr>
<td>Advanced lesions</td>
<td>38/326 (11.7)</td>
<td>1.0 (referent)</td>
<td>16/169 (9.5)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Aspirin (81 mg)</td>
<td>25/328 (7.6)</td>
<td>0.65 (0.40 to 1.06)</td>
<td>18/178 (10.1)</td>
<td>1.07 (0.56 to 2.02)</td>
</tr>
<tr>
<td>Aspirin (325 mg)</td>
<td>36/319 (11.3)</td>
<td>0.97 (0.63 to 1.49)</td>
<td>22/175 (15.6)</td>
<td>1.33 (0.72 to 2.44)</td>
</tr>
</tbody>
</table>

*No. of subjects with an adenoma or advanced lesion divided by the total number of subjects in that group.
†RR = relative risk; CI = confidence interval. Unadjusted relative risks are shown.
N-acetyltransferase, an enzyme that is involved in polyamine catabolism. It is possible that the combination of these two effects, via the ODC polymorphism and aspirin, may reduce the risk of colorectal neoplasia to a much greater degree than either alone. Alternatively, or in addition, aspirin may act directly on ODC itself or on a regulator of ODC expression or activity. In vivo studies on the mechanism underlying these observations are needed.

In the current study, the overall frequency of the ODC genotypes observed was similar to that seen in other studies involving US populations (10,12,15). However, as reported previously (12,16), there were statistically significant differences in ODC allele frequencies among different races and ethnic groups. In the present study, the prevalence of the A allele was the lowest in American Caucasians (0.25), a proportion similar to that reported previously (12,16). However, the prevalence of the A allele in American Hispanics (0.33) was much higher than previously reported in non-American Hispanics (0.18) (12,16). The prevalence of the A allele was high in African Americans (0.34), in agreement with a previous report (12,16), and was highest in American Asians (0.63), although this result was based on a very small sample size. ODC genotyping of a large number of Japanese and Chinese Asians supports these data (O’Brien TG: unpublished observations). This variability in genotype frequencies suggests the potential for confounding of our results due to population stratification if race or ethnicity is also associated with a substantial difference in disease frequency. Although there are little data on the risk of adenoma recurrence by race and ethnicity, the incidence of colorectal cancer does vary by race and ethnicity (17). However, because our results did not change when the analyses were restricted to non-Hispanic whites, it is unlikely that they are due to confounding by population stratification.

There were some limitations to the current analyses. We had limited power to study advanced lesions due to the small number of these lesions in subjects under regular surveillance. In addition, as discussed above, the low number of AA homozygotes limited our ability to study associations in these subjects.

However, the study also had several strengths. An important advantage of our study is that aspirin treatment was randomly assigned to the participants during the trial, whereas in the study of Martinez et al. (10), aspirin use was assessed only by a baseline questionnaire asking whether aspirin had been used in the previous month. Another strength of the current study is that a single pathologist reviewed all lesions from the subjects.

There are suggestions that variants in other genes may also modify the effect of aspirin or NSAIDs on risk of colorectal neoplasia. These genes include those that encode enzymes involved in NSAID metabolism, such as uridine diphosphate glucuronosyltransferase 1A6 and cytochrome P450 2C9 (18,19), and prostaglandin synthesis and metabolism, such as cyclooxygenases-1 and -2, prostacyclin synthase, and arachidonate 5-lipoxygenase (20,21,22). However, there is weak or conflicting evidence for the involvement of variants in these genes in colorectal neoplasia (23).

In conclusion, our results do not provide support for the hypothesis that the G315A ODC polymorphism is a genetic marker for colorectal adenoma occurrence, although we did find suggestions of an association with advanced adenomas. Our findings do suggest that ODC genotype may be an important predictor of the response to aspirin use for adenoma chemoprevention. Future studies should examine the association between ODC genotype and colorectal cancer (rather than adenoma occurrence). Due to the very small numbers of ODC AA homozygotes, larger numbers of subjects would be required to address these hypotheses with sufficient power.

References


NOTES

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