

Research Article

Variants Downstream of the Ornithine Decarboxylase Gene Influence Risk of Colorectal Adenoma and Aspirin ChemopreventionElizabeth L. Barry¹, Leila A. Mott¹, Robert S. Sandler³, Dennis J. Ahnen⁴, and John A. Baron^{1,2,3}**Abstract**

Increased mucosal polyamine levels and ornithine decarboxylase (ODC) activity are associated with an increased risk of colorectal neoplasia and aspirin treatment reduces risk. Previous studies suggest that a single-nucleotide polymorphism (SNP) in the promoter of the *ODC* gene (rs2302615) may be associated with adenoma risk and/or response to aspirin chemoprevention. However, a comprehensive investigation of common genetic variation in the region of *ODC* gene is lacking. Using a tag SNP approach, we investigated associations between genotype or haplotype and adenoma risk among a cohort of 792 non-Hispanic white participants in a randomized trial of aspirin. Generalized linear regression was used to compute relative risks (RR) and 95% confidence intervals (95% CI) adjusted for age and sex. The false discovery rate was used to account for multiple testing. Interaction terms were used to assess whether genotype modified the effect of aspirin treatment. Of 15 SNPs analyzed, seven were statistically significantly associated with adenoma risk. However, in multiple SNP regression models, only two of these, located downstream of the gene, were independently associated with risk: rs11694911 (RR = 1.29; 95% CI, 1.08–1.53; $P = 0.005$) and rs2430420 (RR = 1.20; 95% CI, 1.03–1.40; $P = 0.022$). In addition, there was evidence that rs2430420 and rs28362380 modified the effect of aspirin treatment, whereas the previously investigated SNP, rs2302615, had no statistically significant main effect or interaction with aspirin treatment. Our findings suggest that common genetic variants located downstream (3') of the *ODC* gene influence risk of colorectal adenoma and may also impact the efficacy of aspirin chemoprevention. *Cancer Prev Res*; 4(12); 2072–82. ©2011 AACR.

Introduction

Colorectal cancer is the second leading cause of cancer death in the United States (1) and is potentially preventable. Modification of diet and lifestyle factors as well as the use of chemoprevention strategies in combination with screening may reduce the burden of this disease (2, 3). Substantial evidence from meta-analyses of randomized clinical trials indicate that aspirin is effective for prevention of colorectal cancer (4) and adenomas (the precursor to most cancers; ref. 5) and potentially cost-effective (6), although its effect may be modest. However, in a recent randomized clinical trial, a combination of the ornithine decarboxylase (ODC) inhibitor difluoromethylornithine (DFMO) and the non-

steroidal anti-inflammatory drug (NSAID) sulindac was remarkably effective in reducing the occurrence of colorectal adenomas: all adenomas were reduced by 70% and advanced lesions by more than 90% (7). ODC is a key regulatory enzyme for the synthesis of polyamines, small highly regulated molecules that are essential for cell growth and for the regulation of numerous processes including gene expression and ion channel activity (8). ODC catalyzes the first step in polyamine biosynthesis, the conversion of ornithine to putrescine (9). Although the association of increased polyamine synthesis (10, 11) and inflammation (12, 13) with colorectal carcinogenesis has been recognized for some time, the striking results of the DFMO/sulindac trial highlight the potential for an effective combination chemoprevention strategy (14). Given the mandate to use pharmacogenomics to personalize cancer treatment (15) and presumably prevention, it will be important to investigate the potential effect of common genetic variation on such a strategy.

Several previous genetic epidemiologic studies of a single-nucleotide polymorphism (SNP) in the *ODC* gene (rs2302615) suggested that the variant allele may be associated with a decreased risk for adenoma recurrence and/or an enhanced response to aspirin use or treatment (16–18). However, in the DFMO/sulindac trial, the variant allele appeared to be associated with a reduced response to

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treatment (19). In addition, it was associated with reduced survival in a cohort of colorectal cancer patients (20). Regardless, a major limitation of this prior work is that only one common SNP in the *ODC* gene has been investigated to date. The goal of the present study was to extend this work to investigate associations with adenoma risk and response to aspirin chemoprevention of common genetic variation throughout the *ODC* gene and adjacent chromosomal regions.

Materials and Methods

Study design and population

We conducted a cohort analysis of the association between *ODC1* genotypes and colorectal adenoma recurrence among participants in the Aspirin/Folate Polyp Prevention Study, a double-blind, placebo-controlled, randomized clinical trial of aspirin and/or folic acid for the prevention of colorectal adenoma recurrence (21, 22). Human subject committees at each of the clinical centers approved the study protocol and materials distributed to participants and all participants provided written informed consent. The study design and main findings have been described in detail previously (21, 22). In brief, eligible participants had no history of colorectal cancer or any familial cancer syndrome but had a recent history of one or more histologically confirmed colorectal adenoma and a complete colonoscopy within 3 months prior to enrollment with all polyps removed from the bowel. Subjects, who agreed to avoid NSAID use during their participation in the study, were randomized to aspirin treatment (placebo, 81, or 325 mg daily) and independently to folic acid treatment (placebo or 1 mg daily) in a 3 × 2 factorial design. Aspirin treatment was continued for an average of almost 3 years (33 months) until a follow-up colonoscopy was conducted. The principal outcome of the study was the occurrence of at least one adenoma during randomized treatment. To maximize outcome ascertainment, we included findings from colonoscopies that were conducted at least 1 year after randomization and on or before September 28, 2001, as described in the publication of the main study findings (21). Thus, the actual follow-up period ranged from 19 to 59 months postrandomization. A single, blinded, study pathologist provided uniform review of all clinical samples removed from the large bowel.

SNP selection and genotyping

To provide comprehensive coverage of the *ODC1* gene and adjacent potentially regulatory regions, SNPs within 10 kb upstream and 10 kb downstream of the gene were chosen for genotyping using data from 4 sources. Two of the sources are publically available databases: the HAPMAP - CEU population (Utah residents with Northern and Western European ancestry from the CEPH collection from NCBI release #36; see ref. 23) and the NIEHS SNPs (National Institute of Environmental Health Sciences Environmental Genome Project; see ref. 24). In addition, some SNPs were chosen that were not included in either of the public

databases, based on frequency data obtained from more comprehensive genotyping of CEPH samples ($N = 90$) and a sample of 81 participants in the DFMO/sulindac trial (ref. 7; data shared by Drs. Eugene Gerner and Patricia Thompson from the Arizona Cancer Center, Tucson, AZ). Because "binning" of SNPs with $r^2 \geq 0.8$ was inconsistent across the 4 sets of genotyping data, all SNPs with a minor allele frequency (MAF) of 3% or greater were genotyped and tag SNPs that were identified after genotyping of the current population were chosen for analyses (see below).

Genomic DNA was isolated as previously described (17, 25). Genotyping was conducted by McGill University and Genome Quebec Innovation Center (Montreal, Quebec, Canada) using Sequenom iPLEX Gold technology according to the manufacturer's instructions (Sequenom) using a single panel. The oligos used are available upon request. Of a total of 31 SNPs selected for genotyping, 2 were in GC-rich regions and failed the initial validation step and therefore were not genotyped (rs2302616 and rs28742580). The remaining 29 SNPs had call rates ranging from 98.14% to 100% (median = 99.73%) and were in Hardy-Weinberg equilibrium ($P > 0.05$ using a χ^2 test for the comparison between observed and expected genotype counts among non-Hispanic white subjects with no adenoma recurrence). Concordance rates among 44 blinded duplicate samples were 100% for all SNPs except for one (rs13000913), which had one error for a concordance rate of 97.7%. The sample success rate was 99.0%; samples that could not be called at more than 3 of the 29 SNPs were deemed to have failed and were dropped from the analysis data set. Of the successful samples, 93.2% could be called at all SNPs and another 5.8% could be called at all but one SNP. Of the 29 SNPs that were successfully genotyped, 13 were excluded from the analyses because they were in high linkage disequilibrium ($r^2 \geq 0.8$) with a tag SNP selected using Haploview Tagger (26) and one was excluded because it had an MAF less than 3% in our data set. The excluded SNPs are as follows: rs2463463, rs4669584, rs28362433, rs28362422, rs2357550, rs1405948, rs3752661, rs2302613, rs2302614, rs12616336, rs7608353, rs7558559, rs2009741, and rs6432097. Thus, a total of 15 tag SNPs are included in the statistical analyses presented here along with rs2302615, which we genotyped previously (17). Notably, rs2302615 is not in high linkage disequilibrium ($r^2 \geq 0.8$) with any of the newly genotyped SNPs and thus meets the criteria for inclusion in these analyses as an independent tag SNP.

Statistical analysis

Of the total of 920 participants from the Aspirin/Folate Polyp Prevention Study with both genetic data and trial endpoint data available for this analysis, 128 (13.9%) reported a race/ethnicity other than non-Hispanic white. Genotype was statistically significantly associated with race for 8 of the 15 tag SNPs analyzed in our data set (not shown) and race/ethnicity may be associated with outcome (see ref. 25). Therefore, we limited our analyses to participants self-identified as "white, not of Hispanic origin" ($N = 792$)

because we did not have adequate numbers of other racial/ethnic groups to appropriately address population stratification (see Fig. 1). The outcome assessed in these analyses was the recurrence of one or more adenomas during randomized aspirin treatment. Because the asymptomatic nature of this outcome prohibited time-to-event analysis, findings were assessed at follow-up colonoscopy. Risk ratios and 95% confidence intervals (95% CI) used to estimate the association between genotype and adenoma recurrence were calculated with an underdispersed generalized linear regression model using the Poisson distribution as an approximation to the binomial family. Minimally adjusted (for age and sex) relative risks are shown in the tables. Several levels of genotype association analyses were conducted, as described below.

For SNP-level analyses, genotypes were included in the regression equation one at a time using an additive genetic model, providing per-allele relative risks and the Wald test *P* values for the tables. To account for multiple statistical testing of 15 SNPs, false discovery rate (FDR) *q* values were calculated (27) using the R statistical package (see ref. 28). Using this FDR method, we control the proportion of type I errors made rather than the probability of making even a single type I error (i.e., 5% probability with the classical Bonferroni method of multiple testing correction, which assumes independence of tests and thus is not appropriate for genetic analyses within and around a single gene). We used an FDR threshold of 20%, which has been suggested for candidate gene studies (29), such that up to 20% of the declared discoveries are expected to be false. In secondary analyses of statistically significantly associated SNPs, genetic models (additive, dominant, and recessive) were examined for best fit using maximum likelihood-based statistics.

For haplotype-level analyses, used to assess the combined effect of correlated SNPs, linkage disequilibrium blocks were defined with Haploview (26) using the default algo-

rithm based on confidence bounds on D' (ref. 30; see Fig. 2). Phased haplotype pairs and probabilities were estimated with Powermarker v3.25 (31) using the EM algorithm (32). Generalized linear regression was used to estimate the haplotype association with risk for adenoma recurrence taking haplotype uncertainty (probability) into account. The haplotype was modeled as a continuous variable wherein the number of copies of each allele was multiplied by its probability to obtain a continuous variable. The most common haplotype was used as the reference group and omitted from the model. For each haplotype, the model provides an estimate of the risk associated with each additional copy of the specified haplotype. The most frequent haplotypes (with frequencies above 3%) were analyzed individually and the remaining rare haplotypes were pooled. The Wald test *P* values were calculated for each individual haplotype, and a likelihood ratio test *P* value was calculated for the joint contribution of all haplotypes to the model.

For gene-level analyses, a multiple SNP test was used to assess which SNPs in this chromosomal region were independently associated with risk of adenoma recurrence in an exploratory data-driven analysis. Starting with a composite regression equation containing all 16 tag SNPs, a step-down selection process was used in which SNP variables were removed from the equation one at a time in order of decreasing likelihood ratio test *P* values until only SNPs with values of $P < 0.05$ remained. A step-up approach gave the same results. A global multiple degrees of freedom likelihood ratio test was used to assess the statistical significance of the joint contribution of all independently associated SNPs to the model. In addition, a composite genetic risk score was created for independently associated SNPs by summing the number of risk genotypes over these loci.

We also evaluated whether aspirin treatment interacted with ODC genotypes to modify associations with adenoma

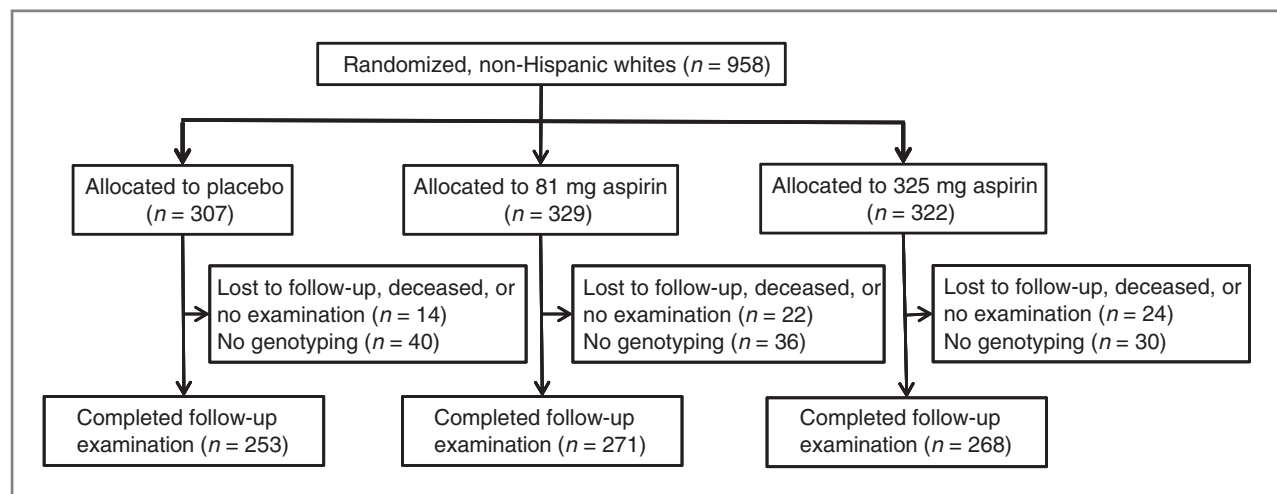
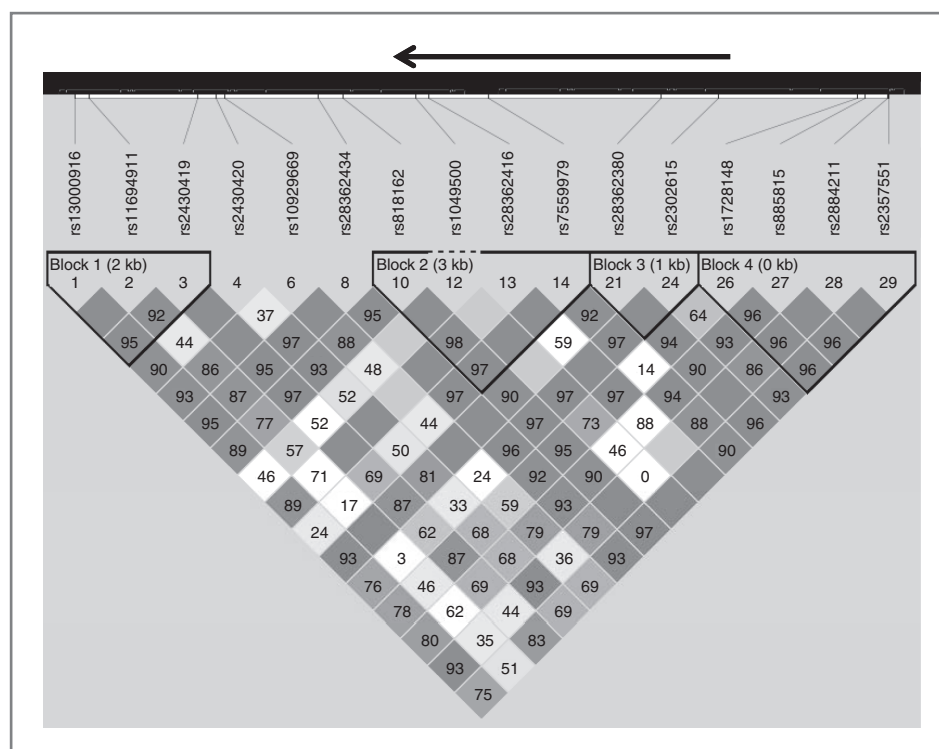


Figure 1. Flow diagram showing the numbers of participants from the Aspirin/Folate Polyp Prevention Study who were included in this secondary analysis involving ODC genotyping. The non-Hispanic whites shown here represent 85% of the total randomized population ($n = 1,121$).

Figure 2. The linkage disequilibrium block structure of the 19.33 kb genotyped region is shown as determined using Haploview (26). The heavy black arrow on top shows the location of the ODC gene (transcribed region). D' values are shown for each pairwise comparison (dark blocks without values are in complete linkage disequilibrium whereas lighter boxes without values are "inconclusive"; ref. 26).



risk using interaction terms in the single SNP regression models and Wald tests. Stratified analyses (by aspirin treatment group or by genotype) were used to obtain stratum-specific estimates of relative risk and CIs. We did not account for multiple testing in these analyses, as we had limited power.

Analyses that included study treatment with aspirin or folate were conducted according to the intention-to-treat principle. All statistical tests were 2-sided and considered significant at a value of $P < 0.05$, except as otherwise indicated above. Stata (version 10) was used for all analyses, except as described above.

Results

Demographic and other selected characteristics of non-Hispanic white participants from the Aspirin/Folate Polyp Prevention Study with genotype and outcome data who were included in the present analysis (see Fig. 1) are presented in Table 1. Among the 792 participants, 370 (46.7%) had a recurrence of one or more colorectal adenomas during follow-up. The mean age was almost 58 years and the majority of participants were males (64%). Approximately 39% of participants had a family history of colorectal cancer among first-degree relatives. On average, participants were followed for 32.8 months from randomization to their follow-up colonoscopy. As observed in the full analyses of the trial (21, 22), individuals who were randomized to 81 mg/d of aspirin were less likely to have a recurrence than those randomized to the placebo arm ($P = 0.04$), whereas treatment with 325 mg/d aspirin ($P = 0.83$) or 1 mg/d folate

($P = 0.51$) was not significantly associated with the outcome. In addition, the main effect of aspirin in this subset of 792 participants (RR = 0.77; 95% CI, 0.63–0.94; $P = 0.009$ for 81 mg aspirin and RR = 0.99; 95% CI, 0.82–1.19; $P = 0.89$ for 325 mg aspirin) was similar to that for the entire cohort (21).

We first examined associations of common SNPs in the vicinity of the *ODC1* gene with risk of adenoma recurrence in single SNP analyses (Table 2). The region analyzed encompassed about 23 kb in total: including approximately 7 kb upstream (5') and 8 kb downstream (3') of the 8 kb transcribed region of the *ODC1* gene. In Table 2, MAFs and gene locations are shown for the SNPs that were included in the statistical analyses, including 15 newly genotyped SNPs and rs2302615, which was genotyped previously (17). Results for 7 SNPs were statistically significant at a value of $P < 0.05$. Of these, 5 were associated with increased risk [rs11694911 (RR = 1.29; 95% CI, 1.10–1.51), rs2430420 (RR = 1.17; 95% CI, 1.05–1.31), rs10929669 (RR = 1.22; 95% CI, 1.04–1.43), rs1049500 (RR = 1.38; 95% CI, 1.10–1.73), and rs2357551 (RR = 1.13; 95% CI, 1.01–1.27)] and 2 were associated with decreased risk [rs13000916 (RR = 0.89; 95% CI, 0.80–0.99) and rs818162 (RR = 0.86; 95% CI, 0.76–0.97)] of adenoma recurrence. After accounting for multiple comparisons using a 20% FDR threshold ($q < 0.2$), all 7 associations were still statistically significant. The previously investigated SNP, rs2302615, was not associated with risk when the analysis was restricted to non-Hispanic whites, in agreement with our previous results for all participants (17). Although an additive genetic model was used for all SNPs in the analyses shown in Table 2, other models

Table 1. Selected characteristics of the study population^a

Characteristics	Total	No. of adenoma	Adenoma recurrence	P ^b
Subjects, n (%)	792 (100)	422 (53.3)	370 (46.7)	
Age at enrollment, y, mean ± SD	57.83 ± 9.53	56.4 ± 9.5	59.4 ± 9.3	<0.001
Sex, n (%)				
Male	507 (64.0)	250 (59.2)	257 (69.5)	0.003
Female	285 (36.0)	172 (40.8)	113 (30.5)	
Family history of colorectal cancer, ^c n (%)				
No	397 (60.9)	218 (61.8)	179 (59.9)	0.24
Yes	255 (39.1)	135 (38.2)	120 (40.1)	
Follow-up time, ^d mo, mean ± SD	32.8 ± 3.6	32.6 ± 3.1	33.0 ± 4.1	0.14
Aspirin treatment group, n (%)				
Placebo	253 (31.9)	128 (30.3)	125 (33.8)	
81-mg aspirin	271 (34.2)	161 (38.2)	110 (29.7)	0.04
325-mg aspirin	268 (33.8)	133 (31.5)	135 (36.5)	0.83
Folate treatment group, n (%)				
Placebo	358 (45.2)	196 (46.5)	162 (43.8)	
1-mg folate	367 (46.3)	192 (45.5)	175 (47.3)	0.51
Not randomized to folate	67 (8.5)	34 (8.1)	33 (8.9)	0.55

^aOnly participants self-identifying as "white, not of Hispanic origin" were included.

^bTests for comparison between group with no adenoma and group with adenoma recurrence using 2-sample *t* test for continuous variables and Pearson χ^2 test for categorical variables.

^cFamily history data are missing for 140 subjects.

^dMonths between randomization and follow-up colonoscopy.

(i.e., dominant or recessive) provided a better fit for several of the SNPs with a significant association suggesting larger effect sizes (data not shown).

We also examined the association of adenoma recurrence with common haplotypes found within 4 linkage blocks identified in this segment of the chromosome (see Table 3) to assess the combined effect of correlated SNPs. In the first block, which contains 3 SNPs, a haplotype (GTC) found in 10.6% of the study population (8.6% controls, 13.0% cases) was associated with a 33% increased risk (RR = 1.33; 95% CI, 1.12–1.57; $P = 0.001$) of adenoma recurrence (per copy of the haplotype) compared with the most common haplotype in this block (TCG). Another haplotype (GCA), occurring in 24% of the study population (23.1% controls, 25.5% cases), was associated with a 14% increased risk that was borderline statistically significant (RR = 1.14; 95% CI, 1.00–1.30; $P = 0.06$). A test of the overall association of genetic variation in block 1 with adenoma recurrence was statistically significant ($P = 0.011$). In the second block, which contains 4 SNPs, one haplotype (CCCT) found in 23.3% of individuals (25.2% controls, 21.4% cases), was associated with a 15% decreased risk (RR = 0.85; 95% CI, 0.73–0.98; $P = 0.029$) and another (GTCC) found in 4.1% of individuals (2.7% controls, 5.7% cases) was associated with a 27% increased risk (RR = 1.27; 95% CI, 1.01–1.61; $P = 0.044$) compared with the most common haplotype (GCCT). Overall, genetic variation in this block was also statistically significantly associated with adenoma recurrence ($P = 0.012$). Genetic variation in block

3 was not associated with the outcome ($P = 0.63$). For the fourth block, one haplotype (CAAA) found in 26.1% of individuals (23.8% controls, 28.9% cases) was associated with a 17% increased risk (RR = 1.17; 95% CI, 1.02–1.33; $P = 0.021$) per copy of the haplotype compared with the most common haplotype in this block (GAAG), but the test of overall association was not statistically significant ($P = 0.19$).

Although the tag SNPs analyzed here were not in strong linkage disequilibrium (by definition, $r^2 < 0.8$), some correlation still exists between them. Thus, in addition to the single SNP and haplotype analyses described above, a multiple SNP analysis was used to assess which SNPs in this chromosomal region were independently associated with risk of adenoma recurrence. Starting with all SNPs in Table 2, those that were not statistically significantly associated with risk were successively removed from a composite regression model, until only 2 SNPs remained showing statistically significant independent associations with risk: rs11694911 (RR = 1.29; 95% CI, 1.08–1.53; $P = 0.005$) and rs2430420 (RR = 1.20; 95% CI, 1.03–1.40; $P = 0.022$) using dominant genetic models (which provided a better fit than additive or recessive models). The joint contribution of these 2 SNPs to adenoma risk was highly statistically significant ($P = 0.0003$) and the linkage disequilibrium between them was very low ($r^2 = 0.05$). In addition, there was no evidence for an interaction between the 2 SNPs: their combined effect was essentially the sum of their excess risks. Thus, having at

Table 2. Associations of ODC genotypes with risk of adenoma recurrence, Aspirin/Folate Polyp Prevention Study, 1994–2001

SNP	Position ^a	Location	Call rate	P_{HWE}	MAF	RR (95% CI) ^c	P	Q^d
rs13000916	10,490,304	3' flanking	0.995	0.09	0.449	0.89 (0.80–0.99)	0.031	0.07
rs11694911	10,490,649	3' flanking	0.991	0.96	0.106	1.29 (1.10–1.51)	0.002	0.027
rs2430419	10,493,239	3' flanking	1.000	0.59	0.247	1.09 (0.97–1.24)	0.15	0.27
rs2430420	10,493,677	3' flanking	1.000	0.86	0.324	1.17 (1.05–1.31)	0.005	0.027
rs10929669	10,493,883	3' flanking	0.997	0.61	0.115	1.22 (1.04–1.43)	0.017	0.054
rs28362434	10,496,095	3' flanking	1.000	0.82	0.183	0.94 (0.81–1.08)	0.36	0.52
rs818162	10,496,675	3' flanking	1.000	0.44	0.302	0.86 (0.76–0.97)	0.012	0.048
rs1049500	10,498,418	Exon 12 ^b	1.000	0.57	0.041	1.38 (1.10–1.73)	0.005	0.027
rs28362416	10,498,720	Intron 11	0.996	0.10	0.067	0.85 (0.67–1.07)	0.18	0.29
rs7559979	10,500,130	Intron 8	0.985	0.61	0.347	1.05 (0.93–1.17)	0.44	0.59
rs28362380	10,504,231	Intron 1	0.999	0.83	0.103	1.04 (0.88–1.23)	0.67	0.77
rs2302615 ^e	10,505,589	Intron 1	0.998	0.50	0.254	1.02 (0.90–1.15)	0.80	
rs1728148	10,508,908	5' flanking	0.999	0.79	0.451	0.92 (0.83–1.02)	0.12	0.24
rs885815	10,509,079	5' flanking	0.992	0.32	0.214	0.96 (0.84–1.09)	0.51	0.63
rs2884211	10,509,623	5' flanking	1.000	0.70	0.077	1.01 (0.83–1.23)	0.89	0.94
rs2357551	10,509,631	5' flanking	1.000	0.73	0.342	1.13 (1.01–1.27)	0.029	0.07

Abbreviations: HWE, Hardy–Weinberg equilibrium; RR, relative risk.

^aLocation on chromosome 2p25 according to NCBI Human Genome Map Build 36.

^bThe substitution is synonymous.

^cPer-allele relative risk and Wald test P value using an additive genetic model adjusted for age and sex.

^dFDR q values (27).

^ePreviously genotyped SNP, not included in multiple testing correction ($n = 765$; ref. 17).

least one risk allele at both loci (15% of the study population) was associated with a 53% increased risk (RR = 1.53; 95% CI, 1.24–1.90; $P < 0.001$) whereas having at least one risk allele at only one loci (45% of the study population) was associated with a 24% increased risk (RR = 1.24; 1.05–1.47 95% CI; $P = 0.012$) compared with having no risk alleles at either loci (40% of the study population, referent group).

Finally, we evaluated whether there was evidence for an interaction between ODC genotypes and aspirin treatment on risk for adenoma recurrence. Table 4 shows the association of each genotype with adenoma risk stratified by aspirin treatment group. Two SNPs (rs2430420 and rs28362380) had nominally statistically significant interactions, although these findings were not corrected for multiple testing. For rs2430420, which had an independent statistically significant main effect as described above, the variant allele was not associated with risk in the placebo group but was associated with an increased risk of 21% (RR = 1.21; 95% CI, 0.98–1.49) and 38% (RR = 1.38; 95% CI, 1.15–1.66) per allele in the 81- and 325-mg aspirin treatment groups, respectively. Conversely, when the effect of aspirin was stratified by rs2430420 genotype (Table 5), 81-mg aspirin treatment appeared to be protective among wild-type homozygotes, with a risk reduction of 32% (RR = 0.68; 95% CI, 0.50–0.94) compared to placebo, but not among heterozygotes/variant homozygotes (RR = 0.95; 95% CI, 0.75–1.20). Notably, there was no evidence

for interaction of aspirin treatment with genotype at the other SNP that was independently associated with risk (rs11694911). However, for rs28362380, which did not have a main effect on risk overall (see Table 2), each variant allele was associated with a 25% risk reduction in the placebo group (RR = 0.75; 95% CI, 0.53–1.04), a 39% risk increase in the 81 mg/d aspirin treatment group (RR = 1.39; 95% CI, 1.02–1.87), but virtually no change in risk in the 325-mg/d aspirin treatment group (RR = 1.03; 95% CI, 0.80–1.35; Table 4). Conversely, when the effect of aspirin was stratified by genotype (Table 5), 81-mg aspirin treatment was associated with a 25% risk reduction in wild-type homozygotes (RR = 0.75; 95% CI, 0.61–0.92) compared with placebo but not among heterozygotes/variant homozygotes (RR = 1.32; 95% CI, 0.85–2.06).

Finally, although we previously reported a statistically significant interaction between rs2302615 and aspirin treatment (17), the interaction was not statistically significant in the current analyses which differed from the prior analyses in that they were restricted to non-Hispanic whites and were adjusted for age and sex and analyzed the aspirin treatment groups separately (Tables 4 and 5). However, when we precisely mimicked the previous analysis by assessing the combined aspirin treatment effect (81 and 325 mg doses together) stratified by genotype using a dominant genetic model, the interaction was still not statistically significant, although the results were similar (not shown). Specifically, in the current analysis, as in the prior analysis, there was no

Table 3. Association of ODC haplotypes with risk for adenoma recurrence, Aspirin/Folate Polyp Prevention Study, 1994–2001

Haplotype	Frequency (%) controls/cases	RR (95% CI)	<i>P</i> ^a	<i>P</i> ^b
Block 1^c				
TCG	47.2/41.6	1.00 (reference)		0.011
GCA	23.1/25.5	1.14 (1.00–1.30)	0.06	
GCG	21.0/19.2	1.02 (0.88–1.17)	0.84	
GTG	8.5/13.0	1.33 (1.12–1.57)	0.001	
Rare	0.2/0.7	1.41 (0.70–2.81)	0.34	
Block 2^d				
GCCT	33.3/37.3	1.00 (reference)		0.012
GCCC	31.0/29.7	0.91 (0.80–1.05)	0.20	
CCCT	25.2/21.4	0.85 (0.73–0.98)	0.029	
CCTT	7.3/5.8	0.82 (0.64–1.04)	0.11	
GTCC	2.7/5.7	1.27 (1.01–1.61)	0.044	
Rare	0.4/0.1	0.55 (0.13–2.32)	0.41	
Block 3^e				
TG	66.1/64.3	1.00 (reference)		0.63
TA	23.7/25.3	1.05 (0.93–1.20)	0.41	
CG	10.2/10.4	1.06 (0.89–1.27)	0.50	
Block 4^f				
GAAG	46.4/41.9	1.00 (reference)		0.19
CAAA	23.8/28.9	1.17 (1.02–1.33)	0.021	
CGAG	21.4/20.1	1.02 (0.88–1.18)	0.81	
CAGA	7.3/7.4	1.06 (0.86–1.31)	0.57	
Rare	0.9/1.6	1.27 (0.86–1.31)	0.33	

^aPer-haplotype relative risk and Wald test *P* value adjusted for age and sex.

^bLikelihood ratio test *P* values for joint test of all haplotypes in the block.

^cBlock 1 includes rs13000916, rs11694911, and rs2430419.

^dBlock 2 includes rs818162, rs1049500, rs28362416, and rs7559979.

^eBlock 3 includes rs28362380 and rs2302615.

^fBlock 4 includes rs1728148, rs885815, rs2884211, and rs2357551.

risk reduction in the wild-type homozygotes (RR = 1.04; 95% CI, 0.83–1.30), whereas a 17% risk reduction was observed among heterozygotes/variant homozygotes (RR = 0.83; 95% CI, 0.66–1.04; *P*_{int} = 0.15) that was only slightly smaller in magnitude than that reported previously (23% risk reduction, RR = 0.77; 95% CI, 0.63–0.95; *P*_{int} = 0.04; see ref. 17).

Discussion

We observed that several common genetic variants in or near the *ODC* gene are associated with risk of colorectal adenoma recurrence among non-Hispanic white participants in a randomized aspirin trial. After adjustment for multiple comparisons, significant associations between adenoma recurrence and genotype at 7 tag SNPs remained using an additive genetic model, including 5 downstream (rs13000916, rs11694911, rs2430420, rs10929669, and rs818162), 1 upstream (rs2357551), and 1 within the transcribed region (exon 12) of the *ODC* gene (rs1049500). In addition, common haplotypes in 3 of 4 haplotype blocks

were significantly associated with adenoma risk, as was overall variation in 2 of the blocks. However, there was no evidence for combined allelic effects within a block because the haplotype effect sizes were similar to those seen in the single SNP analyses. In a composite (multiple SNP) analysis, only 2 of the SNPs were independently associated with risk: having at least one variant allele was associated with increased risks of 20% for rs2430420 (RR = 1.20; 95% CI, 1.03–1.40; *P* = 0.022) and 29% for rs11694911 (RR = 1.29; 95% CI, 1.08–1.53; *P* = 0.005). In addition, in the 15% of the population with at least one variant allele at both loci, risk was increased by 53% compared with individuals without any variant alleles at these 2 loci (RR = 1.53; 95% CI, 1.24–1.90; *P* < 0.001). There was also evidence for an interaction between rs2430420 genotype and aspirin treatment because genotype was only associated with increased risk in aspirin-treated participants. Interestingly, another SNP (rs28362380) without a main effect on risk also appeared to interact with aspirin treatment.

These results provide additional support for the importance of the polyamine pathway in the development of

Table 4. Association of ODC genotypes with risk of adenoma recurrence stratified by aspirin treatment group, Aspirin/Folate Polyp Prevention Study, 1994–2001

SNP	RR (95% CI) ^a			<i>P</i> _{int}
	Placebo	81 mg/d Aspirin	325 mg/d Aspirin	
rs13000916	0.89 (0.75–1.07)	0.94 (0.76–1.15)	0.84 (0.71–1.00)	0.76
rs11694911	1.40 (1.06–1.85)	1.24 (0.92–1.67)	1.31 (1.00–1.70)	0.82
rs2430419	0.91 (0.73–1.12)	1.13 (0.89–1.42)	1.27 (1.05–1.55)	0.08
rs2430420	0.99 (0.82–1.20)	1.21 (0.98–1.49)	1.38 (1.15–1.66)	0.05
rs10929669	1.26 (0.94–1.69)	1.23 (0.92–1.66)	1.21 (0.94–1.56)	0.97
rs28362434	1.14 (0.91–1.44)	0.83 (0.62–1.11)	0.82 (0.65–1.04)	0.10
rs818162	0.94 (0.77–1.14)	0.77 (0.61–0.98)	0.84 (0.70–1.03)	0.45
rs1049500	1.42 (0.99–2.05)	1.53 (1.03–2.27)	1.16 (0.75–1.80)	0.63
rs28362416	0.63 (0.41–0.97)	0.79 (0.51–1.25)	1.19 (0.84–1.69)	0.08
rs7559979	1.01 (0.84–1.22)	1.15 (0.92–1.45)	0.98 (0.82–1.18)	0.50
rs28362380	0.75 (0.53–1.04)	1.39 (1.02–1.87)	1.03 (0.80–1.35)	0.03
rs2302615 ^b	1.11 (0.91–1.36)	0.94 (0.73–1.21)	0.97 (0.79–1.19)	0.56
rs1728148	0.88 (0.73–1.06)	0.94 (0.76–1.15)	0.93 (0.78–1.10)	0.91
rs885815	1.13 (0.91–1.40)	0.86 (0.65–1.13)	0.86 (0.69–1.09)	0.19
rs2884211	0.95 (0.68–1.34)	1.06 (0.74–1.52)	1.06 (0.76–1.47)	0.89
rs2357551	1.05 (0.86–1.28)	1.15 (0.93–1.43)	1.22 (1.02–1.46)	0.56

Abbreviation: *P*_{int} = *P* for interaction (between aspirin treatment and genotype, modeled additively).^aPer-allele relative risk using an additive genetic model adjusted for age and sex.^bPreviously genotyped SNP (17).

colorectal neoplasia, as common genetic variation in a potential regulatory region just downstream of the ODC gene is associated with risk of adenoma recurrence. Polyamines may play an important role in etiology of colorectal carcinogenesis, as DFMO treatment (in combination with sulindac) was highly effective in a chemoprevention trial (7). *ODC* gene variants have not been identified in genome-wide association studies (GWAS) of colorectal cancer conducted to date even though the effect sizes and MAFs seen

here are of the same order of magnitude as for the SNPs detected by these studies (33, 34). It is possible that an intermediate outcome, such as adenoma, may show stronger associations with genotype than cancer if the genotype effect is related to early events in adenoma initiation or progression. Thus, it may be worthwhile to conduct GWAS of colorectal adenomas, as opposed to cancer, in future studies to identify (additional) chemoprevention targets. However, it is also possible that the true genotype effect sizes

Table 5. Association of aspirin treatment with risk of adenoma recurrence stratified by ODC genotypes, Aspirin/Folate Polyp Prevention Study, 1994–2001

SNP	[No. of controls/cases] RR (95% CI)			<i>P</i> _{int}
	Placebo	81 mg/d aspirin	325 mg/d aspirin	
rs2430420				
GG	[58/58] 1.0 (referent)	[76/40] 0.68 (0.50–0.94)	[75/50] 0.81 (0.61–1.09)	
GA/AA	[70/67] 1.0 (referent)	[85/70] 0.95 (0.75–1.20)	[58/85] 1.22 (0.98–1.53)	0.06
rs28362380				
TT	[98/106] 1.0 (referent)	[136/84] 0.75 (0.61–0.92)	[105/110] 0.97 (0.80–1.18)	
TC/CC	[30/19] 1.0 (referent)	[24/26] 1.32 (0.85–2.06)	[28/25] 1.24 (0.80–1.94)	0.06
rs2302615 ^a				
GG	[72/59] 1.0 (referent)	[78/65] 0.97 (0.75–1.25)	[71/76] 1.11 (0.87–1.42)	
AG/AA	[49/64] 1.0 (referent)	[75/44] 0.65 (0.47–0.88)	[54/58] 0.96 (0.73–1.26)	0.25

Abbreviation: *P*_{int} = *P* for interaction (between aspirin treatment and genotype, modeled dominantly).^aPreviously genotyped SNP (17).

are smaller than we see here and, therefore, it will be important to replicate these findings in other studies.

Whether the SNPs (rs2430420 and rs11694911) identified in this study are themselves causal or are in linkage disequilibrium with unmeasured causal variants is not known. However, our results suggest that there are at least 2 causal variants near the *ODC* gene that independently impact risk and whose effects are additive with each other. These results highlight the potential importance of genetic variation in noncoding regions, which is likely to be involved in the regulation of gene expression, and the gaps in our knowledge about mechanisms for such effects. These findings are consistent with the results of numerous GWAS studies conducted to date, where the vast majority (about 80%) of "hits" (disease- or trait-associated SNPs) were from noncoding introns and intergenic regions (35). To our knowledge, the function of these SNPs (rs2430420 and rs11694911) has not been investigated and potential gene regulatory mechanisms are not known. Notably, no SNPs were in linkage disequilibrium ($r^2 \geq 0.8$) with these 2 SNPs in our data set of 28 other SNPs genotyped within 23 kb of the *ODC* gene. Also, no SNPs in the HAPMAP CEU data set of 41 SNPs genotyped within 50 kb of the *ODC* gene were in linkage disequilibrium with rs11694911 (note that rs2430420 is not in the HAPMAP data set and so could not be included in this type of analysis).

The mechanism by which common genetic variants in or near the *ODC* gene may affect the efficacy of aspirin chemoprevention is also not known. However, it has been suggested previously that aspirin, in addition to inhibiting the prostaglandin pathway, may also induce spermidine/spermine-*N*-acetyltransferase (SSAT) activity (16), which catalyzes the first step in polyamine catabolism or excretion from the cell (36, 37). Aspirin and some other NSAIDs, including sulindac, have been shown to stimulate polyamine catabolism in colon cancer cells via induction of SSAT activity (38, 39). It has been suggested that polyamines may play a role in the relationship between inflammation and carcinogenesis (40, 41). Interestingly, in our randomized aspirin trial, 81 mg/d aspirin was effective in reducing the recurrence of colorectal adenoma whereas 325 mg/d was not (21). The reason for this is not clear but may relate to differential pro- and anticarcinogenic effects of aspirin at different doses, which could involve differential effects on the polyamine pathway, although this is speculation. Our results hint that the interaction between *ODC* genotype and aspirin may differ by aspirin dose, which could lend support to this idea and could be explored in future studies.

Previous work has focused only on a SNP (rs2302615) in the promoter region of the *ODC* gene, near binding sites for transcription factors, that appears to affect transcriptional activity (16, 42, 43). Among participants in the Wheat Bran Fiber Trial, individuals homozygous for the variant allele had an approximately 50% reduced risk of colorectal adenoma recurrence and appeared to have an enhanced risk reduction in response to aspirin use compared with individuals that were homozygous wild type, although these associations did not reach statistical significance (16). Sim-

ilar results were found among participants in the United Kingdom Colorectal Adenoma Prevention Trial (18). However, in our previous analysis of all subjects in the Aspirin/Folate Polyp Prevention Study (regardless of race/ethnicity), we found that genotype was not associated with a main effect on risk of adenoma recurrence (17) consistent with the current analysis of non-Hispanic whites. Although we previously reported a statistically significant interaction between genotype and aspirin treatment in an unadjusted analysis using all subjects (17), the interaction is no longer statistically significant after adjusting for age and sex and including only non-Hispanic whites in the current analysis (see Table 5). The main difference was the adjustment for age, as the magnitude of the effect was not attenuated and the interaction was still significant after adjustment for sex and restriction to non-Hispanic whites. Notably, a published meta-analysis analysis of these 3 studies (16–18) used raw (unadjusted) data (18). In light of our findings, a more rigorous analysis of this SNP (rs2302615) may be warranted. Finally, it is worth noting that this previously studied SNP is not correlated with the either of the 2 SNPs (rs2430420 and rs11694911) identified here to be independently associated with adenoma risk ($r^2 = 0$ and 0.01, respectively).

There are some limitations to the current analyses. Because of the size of the study population, we had limited power, especially for investigating interactions with aspirin treatment. Also, because of the limited sample size and power, we did not investigate associations with advanced lesions (which occur much more rarely) or include minorities (individuals with a race or ethnicity other than non-Hispanic whites). In addition, the study was conducted on individuals with a history of adenoma who may be at increased risk relative to the general population undergoing screening or surveillance colonoscopy, potentially limiting the generalizability of the findings. However, as adenoma prevalence in the general (middle aged or older) population is high, likely between 25% and 50% (44, 45), this is unlikely to be a major limitation. Finally, multiple comparisons were conducted, which increases the likelihood that some of our findings may be due to chance. Although the analyses of the SNP main effects were adjusted for multiple comparisons (and were still statistically significant), the tests for interactions with aspirin were not. Thus, we can be less certain that the interactions discovered are not due to chance. However, this concern is mitigated to some extent by the fact that these tests were chosen *a priori*, based on previous studies and a biological rationale and not as part of a data-dredging exercise.

This study also had several notable strengths. A single study pathologist reviewed all lesions from trial participants, ensuring uniform endpoint ascertainment. Data on environmental exposures and subject characteristics were collected in a detailed and uniform manner at the time of participant enrollment. Aspirin treatment was randomly assigned, thereby ensuring uniform exposure and minimizing concerns about differences between the treated and placebo groups, which could confound the results. Subject

compliance with study procedures was excellent (21), including pill taking and avoidance of outside use of the study agents. Finally, a comprehensive tag SNP approach was used to capture common genetic variation throughout the *ODC* gene and adjacent chromosomal regions. This was important for capturing genetic variation in potential regulatory regions that are likely to influence gene expression through effects on transcriptional or translational efficiency.

This work suggests several potential lines of investigation for future studies. First, these findings need to be replicated in other populations and with larger samples sizes. It would be especially useful to investigate these effects in other populations with well-characterized or randomized aspirin treatment with different doses, as well as in studies of colorectal cancer. If these results are replicated, it will be important to try to identify the causal SNPs by exploring the functionality of the SNPs identified here, especially rs2430420 and rs11694911, and by sequencing this chromosomal region to ascertain unmeasured or rare variants in linkage disequilibrium with these SNPs that may themselves be the causal variants. In addition, given the strong evidence basis for both aspirin and DFMO in colorectal chemoprevention, it will be valuable to genotype these SNPs in individuals that are treated with these agents in future clinical studies to assess their impact on efficacy. Finally, given the strong association of genetic variation in this region with race and ethnicity, it will also be worthwhile

to investigate these effects in minority populations, especially African Americans and Hispanics.

Disclosure of Potential Conflicts of Interest

J.A. Baron and Dartmouth College hold a use patent for aspirin chemoprevention in the colorectum. J.A. Baron is a consultant/advisory board member in Bayer Health Care LLC, R.S. Sandler is a consultant/advisory board member in Pozen, and D.J. Ahnen is a consultant/advisory board member in SLA Pharma. No potential conflicts of interest were disclosed by other authors.

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Variants Downstream of the Ornithine Decarboxylase Gene Influence Risk of Colorectal Adenoma and Aspirin Chemoprevention

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