Published in final edited form as:
*Cancer Causes Control*. 2013 January ; 24(1): 47–54. doi:10.1007/s10552-012-0088-6.

# CYP2C9 Variants Increase Risk of Colorectal Adenoma Recurrence and Modify Associations with Smoking but Not Aspirin Treatment

**Elizabeth L. Barry**[1], **Elizabeth M. Poole**[2,3], **John A. Baron**[1,4], **Karen W. Makar**[2], **Leila A. Mott**[1], **Robert S. Sandler**[4], **Dennis J. Ahnen**[5], **Robert S. Bresalier**[6], **Gail E. McKeown-Eyssen**[7], and **Cornelia M. Ulrich**[2,8]

[1]Dartmouth Medical School, Lebanon, NH

[2]Fred Hutchinson Cancer Research Center, Seattle, Washington

[3]Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[4]University of North Carolina, Chapel Hill, NC

[5]Denver Department of Veterans Affairs Medical Center and University of Colorado School of Medicine, Denver CO

[6]Department of Gastroenterology, Hepatology, and Nutrition, The University of Texas MD Anderson Cancer Center, Houston, TX

[7]Department of Public Health Sciences and Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada

[8]National Center for Tumor Diseases and German Cancer Research Center, Heidelberg, Germany

## Abstract

**Purpose—**The cytochrome P450 2C9 enzyme (*CYP2C9*) is involved in metabolism of endogenous compounds, drugs and procarcinogens. Two common nonsynonymous polymorphisms in *CYP2C9* are associated with reduced enzyme activity: *CYP2C9*2* (rs1799853, R144C) and *CYP2C9*3* (rs1057910, I359L).

**Methods—**We investigated whether *CYP2C9* genotype was associated with risk of colorectal adenoma and/or modified associations with aspirin treatment or cigarette smoking in a cohort of 928 participants in a randomized trial of aspirin chemoprevention. Generalized linear regression was used to compute relative risks (RRs) and 95% confidence intervals (95% CIs). Multiplicative interactions terms were used to assess effect modification.

**Results—***CYP2C9* genotype was associated with increased risks for adenoma recurrence of 29% (RR=1.29, 95% CI =1.09–1.51) for ≥1 variant allele (*CYP2C9**2 or *3) and 47% (RR=1.47, 95% CI=1.19–1.83) for ≥1 *CYP2C9**3 allele. The risk for advanced lesions or multiple (≥3) adenomas was increased by 64% (RR=1.64, 95% CI=1.18–2.28) for ≥1 variant allele (*CYP2C9**2 or *3) and 79% (RR=1.79, 95% CI=1.16–2.75) for ≥1 *CYP2C9**3 allele. Genotype modified associations with smoking, but not aspirin treatment. The adenoma risk was increased by 26% (RR=1.26, 95% CI=0.99–1.58) for former smokers and 60% (RR=1.60, 95% CI=1.19–2.15) for current smokers

Correspondence to: Elizabeth L. Barry, Department of Community and Family Medicine, Dartmouth Medical School, 46 Centerra Parkway, Suite 300, Lebanon, NH 03766. Phone: 603-650-3475; Fax: 603-650-3473. Elizabeth.L.Barry@dartmouth.edu.

among wild-type individuals, but there was no increased risk among individuals with ≥1 variant allele (*CYP2C9**2 or *3) ($P_{interaction}$=0.04).

**Conclusions—**Carriers of *CYP2C9* variants with lower enzyme activity have increased overall risk of colorectal adenoma but reduced adenoma risk associated with cigarette smoking. These results may be due to effects on the synthesis of endogenous eicosanoids and/or reduced activation of procarcinogens in smoke by *CYP2C9* variants.

### Keywords

colorectal neoplasia; CYP2C9; smoking; aspirin; polymorphism

## Introduction

As the second leading cause of cancer deaths in the United States, colorectal cancer is a major public health problem (1). Thus, it is important to investigate how genetic and environmental factors, and their interactions, contribute to colorectal neoplasia. The human cytochrome P450 (CYP) superfamily includes 57 genes encoding enzymes involved in the metabolism of xenobiotics, including drugs and environmental toxins, as well as numerous endogenous compounds (2). *CYP2C9* is thought to be involved in the inactivation of 10–30% of clinically useful drugs, the synthesis of important endogenous signaling molecules (i.e., arachidonic acid epoxides), as well as the activation of pro-carcinogenic compounds in tobacco smoke to carcinogenic metabolites (2–7). Notably, common polymorphisms in the *CYP2C9* gene modify its enzymatic activity (8–11) and thereby influence response to drugs, such as warfarin (12), and may also modify the effects of endogenous compounds and toxins from cigarette smoke. Previous research suggests that *CYP2C9* polymorphisms may be associated with risk of colorectal adenomas or cancer and/or modify the protective effect of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) (13–19). In the present study, we investigated associations of two common *CYP2C9* polymorphisms with risk of adenoma recurrence and interactions with aspirin treatment and smoking status among participants in a randomized clinical trial of aspirin and folate for the prevention of adenoma recurrence.

## Materials and Methods

### Study Design and Population

We performed an observational (prospective cohort) analysis of the association between *CYP2C9* genotypes and risk of colorectal neoplasia among participants in the Aspirin/Folate Polyp Prevention Study, a placebo-controlled, randomized clinical trial of aspirin and/or folic acid for the prevention of colorectal adenoma recurrence. The parent study design and main findings have been described in detail previously (20, 21). In brief, 1121 participants, who were recruited from 9 clinical centers between 1994 and 1998, had a recent history of one or more histologically confirmed colorectal adenoma and a complete colonoscopy within 3 months prior to enrollment with all known polyps removed from the bowel. Participants agreed to avoid NSAID use during their participation in the study and 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. Per protocol, aspirin treatment was to be continued until a follow-up colonoscopy was performed three years after the baseline colonoscopy. Compliance was excellent. Endpoint data included an assessment of size, number, histology and location of all colorectal lesions detected during randomized aspirin treatment. A single, blinded, study pathologist provided uniform review of all clinical samples removed from the large bowel.

### Genotyping

Of the 1048 randomized subjects with adenoma outcome data, 928 were genotyped (88.5%); 120 subjects could not be genotyped due to lack of subject consent or local IRB approval. TaqMan-based assays were performed on two common non-synonymous polymorphisms, *CYP2C9*2* (R144C, rs1799853) and *CYP2C9*3* (I359L, rs1057910), at the Fred Hutchinson Cancer Research Center using the Applied Biosystems 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Genotypes were assigned using the Allelic Discrimination Software (Applied Biosystems SDS Software, version 2.3). Primers and cycling conditions are available upon request. For quality control, 7% of the total number of samples were re-genotyped to validate genotype identification protocols. Call rates for all four genotypes exceeded 99% and all were in Hardy-Weinberg equilibrium ($p>0.05$). Concordance for blinded duplicates was 100%. Laboratory staff were blinded to case-control status for all assays.

### Statistical Analysis

The principal outcome was a finding of at least one traditional adenoma at a follow-up colonoscopy after at least one year of randomized treatment and up until the end of randomized aspirin treatment. A secondary "high-risk" outcome was defined as a finding of at least one advanced lesion (a traditional adenoma ≥ 1cm in size, or with villous histology or high grade dysplasia, or cancinoma) and/or multiple adenomas (≥ 3 traditional adenomas). Notably, lesions in the serrated pathway are not included in these outcomes (i.e. serrated or sessile serrated adenomas with or without dysplasia). Risk ratios and 95% confidence intervals used to estimate the association between genotype and outcome were calculated with generalized linear regression models using the Poisson distribution as an approximation to the binomial family and corrected for over or under dispersion. Models were adjusted for age and sex. Genotypes were analyzed using a dominant genetic model due to the limited number of variant homozygotes. For the combined genotype analyses, the variant genotype was defined as having ≥ 1 variant allele of either type (*CYP2C9**2 or *3) to maximize power. We also evaluated whether aspirin treatment or smoking status interacted with genotype to modify associations with risk of colorectal neoplasia using multiplicative interaction terms in the regression models and Wald tests. Smoking was defined by self-report as "one cigarette or more each day for a year or more". Analyses that included aspirin treatment were conducted according to the intention-to-treat principle. Comparisons of selected subject or study characteristics between the groups with and without adenoma recurrence or between groups with wildtype vs. variant genotypes used Pearson $\chi^2$ tests for categorical variables or two sample t tests for continuous variables. Race/ethnicity was collected by self-report; analyses were performed on all races and on non-Hispanic whites only. All statistical tests were two-sided and considered significant at a value of $P<0.05$. Stata (version 10) was used for all analyses.

## Results

Demographic and other selected characteristics of genotyped participants at enrollment in the Aspirin/Folate Polyp Prevention Study are presented in Table 1 overall (total) and by the primary outcome (adenoma recurrence). Among the 925 genotyped participants, 377 (40.8%) had a recurrence of one or more colorectal adenomas during an average follow-up period of 32.8 months. A higher proportion of participants with adenoma recurrence were current or former smokers (17.1 and 44.5%, respectively) compared to those without adenoma recurrence (12.1 and 41.9%, respectively)(P=0.03). As previously reported for the entire study population (20), individuals in this genotyped subset randomized to treatment with 81 mg/day of aspirin appeared less likely to have adenoma recurrence (30%) compared

with those randomized to the placebo arm (35.8%) or to treatment with 325 mg/day aspirin (34.2%)(P=0.09).

Overall, among all genotyped participants, 213 (23%) had one or more *CYP2C9*2* variant alleles (minor allele frequency (MAF)=12.2%), 101 (10.9%) had one or more *CYP2C9*3* variant alleles (MAF=5.5%) and 298 participants (32.3%) had one or more variant allele of either type (combined genotype) (Table 1). Notably, a higher proportion of individuals with adenoma recurrence had a *CYP2C9*3* variant genotype (15.1%) compared to those without adenoma recurrence (8%) (P=0.002), whereas the proportion of *CYP2C9*2* variant genotype was similar between the two groups (P=0.30). When the CYP2C9 combined genotype was examined, a higher proportion of individuals with adenoma recurrence had one or more variant alleles (38.3%) compared to those without adenoma recurrence (28.1%) (P=0.001).

As shown in Table 2, most subject and study characteristics did not differ by CYP2C9 genotype, including age, sex, aspirin or folate treatment groups and mean follow-up time. However, the *CYP2C9*2 genotype was associated with race/ethnicity: a higher proportion of individuals with one or more variant alleles were non-Hispanic white (94.4%) compared to the proportion of wild type individuals that were non-Hispanic white (84%) (P=0.001). In addition, the CYP2C9*3 genotype appeared to be associated with smoking status, such that a higher proportion of individuals with one or more variant alleles were former (45.5%) or current smokers (20.8%) compared to the proportion of wild type individuals that were former (42.6%) or current smokers (13.3%)(P=0.05).

We examined the association of *CYP2C9* genotypes with risk of colorectal neoplasia during the period of randomized aspirin treatment by assessing the occurrence of any adenoma or of advanced lesions/multiple adenomas (Table 3). The combined variant genotype (one or more variant allele of either type) was associated with statistically significant increased risks of 29% for any adenoma (RR=1.29, 95% CI=1.09–1.51, P=0.002) and 64% for advanced lesions or multiple adenomas (RR=1.64; 95% CI=1.18–2.28, P=0.003). The increase in risk was mostly due to the *CYP2C9*3* genotype: having one or more variant allele was associated with increased risks of 47% (RR=1.47, 95% CI=1.19–1.83, P<0.001) for any adenoma and 79% (RR=1.79, 95% CI=1.16–2.75, P=0.008) for advanced lesions or multiple adenomas. However, the *CYP2C9*2* variant was also associated with non-statistically significant increased risks, especially for the “high-risk” findings (advanced lesions or multiple adenomas). As shown in Table 3, very similar results were observed when the analyses were restricted to non-Hispanic whites (86.2% of the study population).

We also investigated whether *CYP2C9* genotype modified the effect of aspirin treatment or the association of smoking status with risk of any adenoma (Table 4). Among all genotyped participants, the lower dose of aspirin treatment (81 mg) reduced risk by 19% (RR=0.81, 95% CI=0.67–0.98, P=0.03) whereas the higher dose (325 mg) appeared ineffective (RR=0.94 RR, 95% CI=0.78–1.13, P=0.48) (not shown). When aspirin treatment was stratified by *CYP2C9* genotype (Table 4), similar results were obtained regardless of genotype and there was no evidence for an interaction of genotype and aspirin treatment. Notably, the results were similar when this analysis was restricted to non-Hispanic whites (Table 4) or to the *CYP2C9*3* genotype (not shown). In addition, CYP2C9 genotype also did not modify the effect of folate treatment (not shown).

On the other hand, there was evidence for an interaction between smoking status and *CYP2C9* genotype (Table 4). Among participants with the wild-type CYP2C9 genotype, smoking was associated with increased adenoma risks of 26% (RR=1.26, 95% CI=0.99–1.58) for former smokers and 60% (RR=1.60, 95% CI=1.19–2.15) for current smokers.

However, in participants with one or more variant alleles of either type, smoking status was not associated with increased risks: RR=0.80 (95% CI=0.62–1.03) in former smokers and RR=1.03 (95% CI=0.72–1.50) in current smokers. In this stratified analysis, the combined CYP2C9 variant genotype (one or more variant allele of either type) was associated with a 67% increased risk of adenoma among non-smokers (RR=1.67, 95% CI= 1.30–2.16, Table 4 footnote) compared to the 29% increased risk seen in all subjects (Table 3). Similar results were seen when the analyses were restricted to non-Hispanic whites. The interaction between smoking status and *CYP2C9* genotype was statistically significant for all participants (P=0.04) as well as for non-Hispanic whites (P=0.04). When this analysis was restricted to the *CYP2C9*3* genotype, similar results were seen, although the interaction was not statistically significant (not shown).

## Discussion

In this prospective analysis of participants from a randomized clinical trial of aspirin for colorectal adenoma chemoprevention, *CYP2C9* genotype was associated with increased risks of any adenoma of 29% for ≥1 variant of either type (*CYP2C9**2 or *3) and 47% for ≥1 *CYP2C9*3* variant. Moreover, the associations with "high-risk" findings (multiple adenomas (≥3) and/or advanced lesions), appeared larger: risks increased by 64% for ≥1 variant of either type (*CYP2C9**2 or *3) and 79% ≥1 *CYP2C9*3* variant. In addition, although *CYP2C9* genotype didn't modify the protective effect of aspirin on adenoma risk, it substantially modified the association with smoking such that former or current smoking was only associated with increased risk of adenoma among participants with the wild-type *CYP2C9* genotype.

There is evidence from recent tissue microarray experiments that the *CYP2C9* enzyme is broadly expressed in human tissues, including moderate expression in the colon (22), and is the most abundantly expressed epoxygenase in several human malignant neoplasms (23). The stronger association of *CYP2C9* genotype with "high-risk" findings (advanced and multiple adenomas) in the current study is noteworthy because of the potential clinical importance of these lesions, given their association with greater risk for future advanced neoplasia and cancer (24). Importantly, we observed similar results when our analyses were restricted to non-Hispanic whites, mitigating concerns about potential effects of population stratification. In addition, the reduction in enzyme activity for the *CYP2C9*3* variant is more substantial than for the *CYP2C9*2* variant (8–10), so the stronger associations observed with the *CYP2C9*3* variant genotype in the current work is consistent with a role for this enzyme in modulating adenoma risk. However, others have suggested that associations with the *CYP2C9*2* genotype may actually be due to linkage disequilibrium with the *CYP2C8*3* variant rather than a true effect of the *CYP2C9* polymorphism (see (11, 17, 25, 26)).

A number of previous studies have investigated associations of these *CYP2C9* polymorphisms with colorectal adenomas and cancer. Among previous studies of *CYP2C9* genotype and adenoma risk, Chan et al. also reported a statistically significant increase in risk of distal adenoma associated with combined (*CYP2C9**2 or *3) variant genotype in the Nurses Health Study (14). However, there were only modest, non-statistically significant increased risks in several other studies (13, 15, 17) and one report of decreased risk in a small Scottish case-control study (19).

Among these prior studies, one reported that CYP2C9 genotype modified the protective association with aspirin, although it was not clear if this interaction was statistically significant (13), whereas two others noted no interaction (14, 15). Our trial, with its larger size and randomized aspirin intervention, was well suited to address this issue, which was a

major impetus for the current work (27, 28). However, since the CYP2C9 enzyme is thought to have a relatively minor role in metabolizing aspirin (10), it is perhaps not surprising that we did not find an interaction. Only one of the previously mentioned studies investigated the interaction between genotype and smoking on adenoma prevalence, and no association was found (14). Of studies investigating associations with colorectal cancer risk, the two largest reported either no association (16) or a decreased risk associated with the *CYP2C9*2* genotype (18), and no interaction with aspirin (16) or smoking (18).

The apparent association of CYP2C9*3 genotype with smoking status in our study population may be a chance finding or may reflect a previously unrecognized role for this enzyme in metabolizing nicotine. Although a large genome wide association study meta-analysis did not identify genetic variation in the vicinity of this gene to be associated with smoking behaviors (29), it is possible that there were effects that were not detected at a genome wide significance level. Interestingly, the CYP2C9 gene is located on chromosome 10q24 and a marker in the adjacent 10q25 region was associated with smoking behavior in a study of twins from Finland (30). In addition, another member of the CYP2 gene family, CYP2A6, has been associated with smoking behaviors and lung cancer risk (31).

The mechanism by which *CYP2C9* genotype may increase adenoma risk is not known. However, the *CYP2C9* enzyme may play a role in the metabolism of arachidonic acid to eicosanoids, specifically to epoxyeicosatrienoic acid, and may ultimately influence carcinogenesis through effects on cellular proliferation and inflammation (2, 3, 8). In addition to effects on endogenous modulators, CYP2C9 may be involved in the metabolism of environmental carcinogens impacting colorectal carcinogenesis. The attenuated risk associated with cigarette smoking among participants with the variant genotype in the current study could be explained by a role for CYP2C9 in activation of procarcinogenic chemicals such as polycyclic aromatic hydrocarbons found in tobacco smoke (4–7, 32) (33). The increased adenoma risk associated with smoking observed in our population is consistent with the results of published meta-analyses for colorectal adenoma (34) and cancer (35, 36). Since smoking appears to increase risk of certain histologically and molecularly defined subsets of colorectal cancer (37, 38)), which can also result in differential interactions (see(39)), it will be important to explore associations with *CYP2C9* genotype among these subsets in future studies. Likewise, it seems possible that differences in the types of pre-cancerous lesions examined and/or smoking status among different study populations may explain some of the heterogeneity observed in published studies of associations between *CYP2C9* genotype and adenoma risk and this will be an important avenue for future exploration.

## Acknowledgments

The authors are grateful to the co-investigators, study coordinators and participants in the Aspirin/Folate Polyp Prevention Study who made this research possible and to Bayer for providing the aspirin and placebo tablets for the clinical trial.

**Grant Support:**

This work was supported by grants from the National Institutes of Health, National Cancer Institute: R01 CA59005 to JA Baron; R03 CA136026 to CM Ulrich.

## References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60:277–300. [PubMed: 20610543]
2. Nebert DW, Russell DW. Clinical importance of the cytochromes P450. Lancet. 2002; 360:1155–62. [PubMed: 12387968]

3. Panigrahy D, Kaipainen A, Greene ER, Huang S. Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. Cancer Metastasis Rev. 2010; 29:723–35. [PubMed: 20941528]
4. Shou M, Krausz KW, Gonzalez FJ, Gelboin HV. Metabolic activation of the potent carcinogen dibenzo[a,h]anthracene by cDNA-expressed human cytochromes P450. Arch Biochem Biophys. 1996; 328:201–7. [PubMed: 8638931]
5. Yun CH, Shimada T, Guengerich FP. Roles of human liver cytochrome P4502C and 3A enzymes in the 3-hydroxylation of benzo(a)pyrene. Cancer Res. 1992; 52:1868–74. [PubMed: 1551116]
6. Mo SL, Zhou ZW, Yang LP, Wei MQ, Zhou SF. New insights into the structural features and functional relevance of human cytochrome P450 2C9. Part I Curr Drug Metab. 2009; 10:1075–126.
7. Shou M, Korzekwa KR, Crespi CL, Gonzalez FJ, Gelboin HV. The role of 12 cDNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo[a]pyrene and benzo[a]pyrene trans-7,8-dihydrodiol. Mol Carcinog. 1994; 10:159–68. [PubMed: 8043197]
8. Zhou SF, Zhou ZW, Huang M. Polymorphisms of human cytochrome P450 2C9 and the functional relevance. Toxicology. 2010; 278:165–88. [PubMed: 19715737]
9. Rokitta D, Fuhr U. Comparison of enzyme kinetic parameters obtained in vitro for reactions mediated by human CYP2C enzymes including major CYP2C9 variants. Curr Drug Metab. 2010; 11:153–61. [PubMed: 20359285]
10. Wang B, Wang J, Huang SQ, Su HH, Zhou SF. Genetic polymorphism of the human cytochrome P450 2C9 gene and its clinical significance. Curr Drug Metab. 2009; 10:781–834. [PubMed: 19925388]
11. Van Booven D, Marsh S, McLeod H, et al. Cytochrome P450 2C9-CYP2C9. Pharmacogenet Genomics. 2010; 20:277–81. [PubMed: 20150829]
12. Lindh JD, Holm L, Andersson ML, Rane A. Influence of CYP2C9 genotype on warfarin dose requirements--a systematic review and meta-analysis. Eur J Clin Pharmacol. 2009; 65:365–75. [PubMed: 19031075]
13. Bigler J, Whitton J, Lampe JW, Fosdick L, Bostick RM, Potter JD. CYP2C9 and UGT1A6 genotypes modulate the protective effect of aspirin on colon adenoma risk. Cancer Res. 2001; 61:3566–9. [PubMed: 11325819]
14. Chan AT, Tranah GJ, Giovannucci EL, Hunter DJ, Fuchs CS. A prospective study of genetic polymorphisms in the cytochrome P-450 2C9 enzyme and the risk for distal colorectal adenoma. Clin Gastroenterol Hepatol. 2004; 2:704–12. [PubMed: 15290664]
15. Hubner RA, Muir KR, Liu JF, et al. Genetic variants of UGT1A6 influence risk of colorectal adenoma recurrence. Clin Cancer Res. 2006; 12:6585–9. [PubMed: 17085674]
16. Samowitz WS, Wolff RK, Curtin K, et al. Interactions between CYP2C9 and UGT1A6 polymorphisms and nonsteroidal anti-inflammatory drugs in colorectal cancer prevention. Clin Gastroenterol Hepatol. 2006; 4:894–901. [PubMed: 16797247]
17. Chan AT, Zauber AG, Hsu M, et al. Cytochrome P450 2C9 variants influence response to celecoxib for prevention of colorectal adenoma. Gastroenterology. 2009; 136:2127–36. e1. [PubMed: 19233181]
18. Cleary SP, Cotterchio M, Shi E, Gallinger S, Harper P. Cigarette smoking, genetic variants in carcinogen-metabolizing enzymes, and colorectal cancer risk. Am J Epidemiol. 2010; 172:1000–14. [PubMed: 20937634]
19. Northwood EL, Elliott F, Forman D, et al. Polymorphisms in xenobiotic metabolizing enzymes and diet influence colorectal adenoma risk. Pharmacogenet Genomics. 2010; 20:315–26. [PubMed: 20375710]
20. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003; 348:891–9. [PubMed: 12621133]
21. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. Jama. 2007; 297:2351–9. [PubMed: 17551129]
22. Enayetallah AE, French RA, Thibodeau MS, Grant DF. Distribution of Soluble Epoxide Hydrolase and of Cytochrome P450 2C8, 2C9, and 2J2 in Human Tissues. Journal of Histochemistry & Cytochemistry. 2004; 52:447–54. [PubMed: 15033996]

23. Enayetallah AE, French RA, Grant DF. Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms. J Mol Histol. 2006; 37:133–41. [PubMed: 16957870]
24. Martinez ME, Baron JA, Lieberman DA, et al. A pooled analysis of advanced colorectal neoplasia diagnoses after colonoscopic polypectomy. Gastroenterology. 2009; 136:832–41. [PubMed: 19171141]
25. Yasar U, Lundgren S, Eliasson E, et al. Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. Biochem Biophys Res Commun. 2002; 299:25–8. [PubMed: 12435384]
26. Yasar U, Bennet AM, Eliasson E, et al. Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. Pharmacogenetics. 2003; 13:715–20. [PubMed: 14646690]
27. Cross JT, Poole EM, Ulrich CM. A review of gene-drug interactions for nonsteroidal anti-inflammatory drug use in preventing colorectal neoplasia. Pharmacogenomics J. 2008; 8:237–47. [PubMed: 18195728]
28. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer. 2006; 6:130–40. [PubMed: 16491072]
29. The TobaccoGenetics Consortium. Genome-wide meta-analyses identify multiple loci associated with smoking behavior. Nat Genet. 2010; 42:441–7. [PubMed: 20418890]
30. Loukola A, Broms U, Maunu H, et al. Linkage of nicotine dependence and smoking behavior on 10q, 7q and 11p in twins with homogeneous genetic background. Pharmacogenomics J. 2008; 8:209–19. [PubMed: 17549066]
31. Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNB4 variation and smoking behaviors and lung cancer risk. J Natl Cancer Inst. 2011; 103:1342–6. [PubMed: 21747048]
32. Shimada T. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. Drug Metab Pharmacokinet. 2006; 21:257–76. [PubMed: 16946553]
33. Ding YS, Ashley DL, Watson CH. Determination of 10 carcinogenic polycyclic aromatic hydrocarbons in mainstream cigarette smoke. J Agric Food Chem. 2007; 55:5966–73. [PubMed: 17602652]
34. Botteri E, Iodice S, Raimondi S, Maisonneuve P, Lowenfels AB. Cigarette smoking and adenomatous polyps: a meta-analysis. Gastroenterology. 2008; 134:388–95. [PubMed: 18242207]
35. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. Jama. 2008; 300:2765–78. [PubMed: 19088354]
36. Tsoi KK, Pau CY, Wu WK, Chan FK, Griffiths S, Sung JJ. Cigarette smoking and the risk of colorectal cancer: a meta-analysis of prospective cohort studies. Clin Gastroenterol Hepatol. 2009; 7:682, 8 e1–5. [PubMed: 19245853]
37. Limsui D, Vierkant RA, Tillmans LS, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst. 2010; 102:1012–22. [PubMed: 20587792]
38. Boland CR, Goel A. Clearing the air on smoking and colorectal cancer. J Natl Cancer Inst. 2010; 102:996–7. [PubMed: 20587791]
39. Campbell PT, Curtin K, Ulrich CM, et al. Mismatch repair polymorphisms and risk of colon cancer, tumour microsatellite instability and interactions with lifestyle factors. Gut. 2009; 58:661–7. [PubMed: 18523027]

**Table 1**

Selected characteristics of the study population by adenoma recurrence

| Characteristics | | Total | No Adenoma | Adenoma Recurrence | P |
|---|---|---|---|---|---|
| No. of subjects (%) | | 925 (100) | 548 (9.2) | 377 (40.8) | |
| Mean age at ± SD, y enrollment | | 57.8 ± 9.5 | 56.6 ± 9.5 | 59.4 ± 9.2 | <0.001 |
| Sex, No. (%) | | | | | <0.001 |
| Male | | 593 (64.1) | 322 (58.8) | 271 (71.9) | |
| Female | | 332 (35.9) | 226 (41.2) | 106 (28.1) | |
| Race/ethnicity. No. (%) | | | | | 0.21 |
| Non-Hispanic white | | 799 (86.2) | 467 (85.2) | 332 (88.1) | |
| Non-Hispanic Black | | 49 (5.5) | 27 (4.9) | 22 (5.8) | |
| Hispanic | | 51 (5.5) | 35 (6.4) | 16 (4.2) | |
| Other | | 26 (2.8) | 19 (3.5) | 7 (1.9) | |
| Smoking status at enrollment[a] | | | | | 0.03 |
| Never | | 396 (43.0) | 252 (46.1) | 144 (38.4) | |
| Former | | 396 (43.0) | 229 (41.9) | 167 (44.5) | |
| Current | | 130 (14.0) | 66 (12.1) | 64 (17.1) | |
| Follow-up time (mean ± SD), mo | | 32.8 ± 3.8 | 32.7 ± 3.3 | 33.0 ± 4.3 | 0.24 |
| Aspirin treatment group, No. (%) | | | | | 0.09 |
| Placebo | | 303 (32.8) | 168 (30.7) | 135 (35.8) | |
| 81 mg/day aspirin | | 313 (33.8) | 200 (36.5) | 113 (30.0) | |
| 325 mg/day aspirin | | 309 (33.4) | 180 (32.9) | 129 (34.2) | |
| Folate treatment group, No. (%)[a] | | | | | 0.34 |
| Placebo | | 419 (49.6) | 256 (51.0) | 163 (47.7) | |
| 1 mg/day folate | | 425 (50.4) | 246 (49.0) | 179 (52.3) | |
| *CYP2C9*2*[a] | CC | 711 (77.0) | 429 (78.3) | 282 (77.0) | 0.30 |
| | TC | 200 (21.6) | 110 (20.1) | 190 (21.6) | |
| | TT | 13 (1.4) | 9 (1.6) | 4 (1.4) | |
| *CYP2C9*3* | AA | 824 (89.1) | 504 (92.0) | 320 (84.9) | 0.002 |
| | AC | 100 (10.8) | 43 (7.8) | 57 (15.1) | |

| Characteristics | | Total | No Adenoma | Adenoma Recurrence | P |
|---|---|---|---|---|---|
| | CC | 1 (0.1) | 1 (0.2) | 0 | |
| Combined genotype[b] | CC[c], AA | 626 (67.8) | 394 (71.9) | 232 (61.7) | 0.001 |
| | TC, TT or AC, CC[d] | 298 (32.3) | 154 (28.1) | 144 (38.3) | |

*CYP2C9*2* = rs1799853, 430 C>T, R144C; *CYP2C9*3* = rs1057910, 1075 A>C, I359L.

[a] Smoking status is missing for 3 subjects; 81 subjects were not randomized to folate treatment; CYP2C9*2 genotype is missing for 1 subject.

[b] For the combined genotype analysis, the variant genotype was defined as having ≥ 1 variant allele of either type (*CYP2C9**2 or *3).

[c] Here “CC” is CYP2C9*2 homozygous wildtype genotype.

[d] Here “CC” is CYP2C9*3 homozygous variant genotype.



*Cancer Causes Control*. Author manuscript; available in PMC 2014 January 01.

**Table 2**

Selected characteristics of the study population by CYP2C9 genotypes

| Characteristics: | *CYP2C9*2*[a] | | | *CYP2C9*3* | | |
|---|---|---|---|---|---|---|
| | CC | TC/TT | P | AA | AC/CC | P |
| Mean age, ± SD, y | 57.6 ± 9.5 | 58.2 ± 9.3 | 0.42 | 57.8 ± 9.5 | 57.4 ± 9.6 | 0.71 |
| Sex, No. (%) | | | 0.59 | | | 0.70 |
| Male | 453 (63.7) | 140 (65.7) | | 530 (64.3) | 63 (62.4) | |
| Female | 258 (36.3) | 73 (34.3) | | 294 (35.7) | 38 (37.6) | |
| Race/ethnicity. No. (%) | | | 0.001 | | | 0.42 |
| Non-Hispanic white | 597 (84.0) | 201 (94.4) | | 708 (85.9) | 91 (90.1) | |
| Non-Hispanic Black | 43 (6.0) | 6 (2.8) | | 47 (5.7) | 2 (2.0) | |
| Hispanic | 45 (6.3) | 6 (2.8) | | 45 (5.5) | 6 (5.9) | |
| Other | 26 (3.7) | 0 (0) | | 24 (2.9) | 2 (2.0) | |
| Smoking status[a] | | | 0.16 | | | 0.05 |
| Never | 304 (42.9) | 92 (43.2) | | 362 (44.1) | 34 (33.7) | |
| Former | 296 (41.8) | 99 (46.5) | | 350 (42.6) | 46 (45.5) | |
| Current | 108 (15.3) | 22 (10.3) | | 109 (13.3) | 21 (20.8) | |
| Follow-up time (mean ± SD), mo | 32.8 ± 3.6 | 32.9 ± 4.3 | 0.91 | 32.8 ± 3.7 | 33.1 ± 3.8 | 0.38 |
| Aspirin group, No. (%) | | | 0.57 | | | 0.76 |
| Placebo | 238 (33.5) | 65 (30.5) | | 271 (32.9) | 32 (31.7) | |
| 81 mg/day aspirin | 242 (34.0) | 71 (33.3) | | 281 (34.1) | 32 (31.7) | |
| 325 mg/day aspirin | 231 (32.5) | 77 (36.2) | | 272 (33.0) | 37 (36.3) | |
| Folate group, No. (%)[a] | | | 0.78 | | | 0.68 |
| Placebo | 327 (49.9) | 91 (48.7) | | 373 (49.4) | 46 (51.7) | |
| 1 mg/day folate | 329 (50.1) | 96 (51.3) | | 382 (50.6) | 43 (48.3) | |

*CYP2C9*2* = rs1799853, 430 C>T, R144C; *CYP2C9*3* = rs1057910, 1075 A>C, I359L.

[a]Smoking status is missing for 3 subjects; 81 subjects were not randomized to folate treatment; CYP2C9*2 genotype is missing for 1 subject.

**Table 3**

Risk of any adenoma recurrence (top) or advanced lesions/multiple adenomas (bottom) by CYP2C9 genotypes

| Outcome | Subjects | Genotype[b] | # with outcome / # total[c] | RR (95% CI)[d] | P[e] |
|---|---|---|---|---|---|
| Any adenoma | All races | Variant (combined) | 144/298 | 1.29 (1.09–1.51) | 0.002 |
| | | *CYP2C9*2* | 94/213 | 1.09 (0.91–1.31) | 0.33 |
| | | *CYP2C9*3* | 57/101 | 1.47 (1.19–1.83) | <0.001 |
| | Non-Hispanic whites | Variant (combined) | 135/277 | 1.27 (1.07–1.50) | 0.005 |
| | | *CYP2C9*2* | 90/201 | 1.09 (0.90–1.31) | 0.37 |
| | | *CYP2C9*3* | 51/91 | 1.43 (1.13–1.79) | 0.002 |
| Advanced lesions or multiple adenomas[a] | All races | Variant (combined) | 55/295 | 1.64 (1.18–2.28) | 0.003 |
| | | *CYP2C9*2* | 35/210 | 1.29 (0.89–1.85) | 0.18 |
| | | *CYP2C9*3* | 22/100 | 1.79 (1.16–2.75) | 0.008 |
| | Non-Hispanic whites | Variant (combined) | 53/274 | 1.84 (1.29–2.62) | 0.001 |
| | | *CYP2C9*2* | 34/198 | 1.38 (0.94–2.01) | 0.10 |
| | | *CYP2C9*3* | 21/90 | 1.94 (1.24–3.03) | 0.003 |

[a] An outcome of advanced lesions (adenomas ≥1cm, or with villous histology or high grade dysplasia, or arcinoma) or multiple adenomas (≥3); data is missing for two subjects with unknown adenoma size.

[b] For combined genotype analysis, variant (combined) = one or more variant (minor) allele at either *CYP2C9*2* (rs1799853, R144C) or *CYP2C9*3* (rs1057910, I359L). For single SNP analyses, both SNPs are coded with a dominant genetic model.

[c] This is the # of subjects with ≥1 variant allele with the specified outcome/total # of subjects with ≥1 variant allele.

[d] Relative risk (RR) adjusted for sex and age for comparisons with homozygous wild type; 95% confidence interval (95% CI).

[e] Wald test for the comparison to wild-type genotype.

**Table 4**

Interaction of *CYP2C9* genotype and aspirin treatment or smoking status on risk of any adenoma recurrence

| Subjects | Genotype | RR (95% CI)[b] | | | $P_{int}$[c] |
|---|---|---|---|---|---|
| | | Aspirin Treatment Group | | | |
| | | Placebo | 81 mg/day | 325 mg/day | |
| All races | Wild-type | 1.0 (referent) [86/210] | 0.79 (0.61–1.02) [68/214] | 0.95 (0.74–1.21) [78/202] | 0.85 |
| | Variant[a] | 1.0 (referent)[d] [49/93] | 0.81 (0.60–1.08) [45/99] | 0.87 (0.65–1.16) [50/106] | |
| Non-Hispanic Whites | Wild-type | 1.0 (referent) [71/170] | 0.81 (0.61–1.07) [58/178] | 0.94 (0.72–1.22) [67/173] | 0.97 |
| | Variant[a] | 1.0 (referent)[e] [44/85] | 0.83 (0.61–1.12) [44/96] | 0.91 (0.69–1.22) [47/96] | |
| | | Smoking Status | | | |
| | | Never | Former | Current | |
| All races | Wild-type | 1.0 (referent) [82/274] | 1.26 (0.99–1.58) [105/258] | 1.60 (1.19–2.15) [41/91] | 0.04 |
| | Variant[a] | 1.0 (referent)[f] [62/122] | 0.80 (0.62–1.03) [61/137] | 1.03 (0.72–1.50) [21/39] | |
| Non-Hispanic Whites | Wild-type | 1.0 (referent) [72/233] | 1.21 (0.95–1.56) [92/218] | 1.47 (1.05–2.08) [30/67] | 0.04 |
| | Variant[a] | 1.0 (referent)[g] [62/118] | 0.76 (0.58–0.99) [55/124] | 0.98 (0.67–1.43) [18/35] | |

[a] Combined genotype analysis where variant = one or more variant (minor) alleles at either *CYP2C9*2* (R144C, rs1799853) or *CYP2C9*3* (I359L, rs1057910).

[b] Relative risk (RR) adjusted for age and sex; 95% confidence interval (95% CI); [#/#] = [# subjects with adenoma/total # subjects]

[c] $P_{int}$ = P for interaction of genotype with aspirin treatment or smoking status.

[d] RR (95% CI) for variant vs. wild-type is 1.31 (1.00–1.72) among placebo subjects.

[e] RR (95% CI) for variant vs. wild-type is 1.28 (0.96–1.71) among placebo subjects.

[f] RR (95% CI) for variant vs. wild-type is 1.67 (1.30–2.16) among never smokers.

[g] RR (95%CI) for variant vs. wild-type is 1.65 (1.27–2.14) among never smokers.