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# Genetic Variation in *UGT* Genes Modify the Associations of NSAIDs with Risk of Colorectal Cancer: Colon Cancer Family Registry

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## Abstract

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with reduced risk of colorectal neoplasia. Previous studies have reported that polymorphisms in NSAID-metabolizing enzymes central to NSAID metabolism including UDP-glucuronosyltransferases (UGT) and cytochrome P450 (CYP) 2C9 may modify this protective effect. We investigated whether 35 functionally relevant polymorphisms within *CYP2C9* and *UGT* genes were associated with colorectal cancer risk or modified the protective effect of NSAIDs on colorectal cancer susceptibility, using 1,584 colorectal cancer cases and 2,516 unaffected sibling controls from the Colon Cancer Family Registry. A three-SNP genotype in *UGT1A6* (G-A-A; Ala7-Thr181-Arg184) and the Asp85 variant in *UGT2B15* increased the risk of colorectal cancer (OR 3.87; 95% CI 1.04-14.45 and OR 1.34; 95% CI 1.10-1.63, respectively). We observed interactions between *UGT1A3* Thr78Thr (A>G) and NSAID use (p-interaction=0.02), a three-SNP genotype within *UGT2B4* and ibuprofen use (p-interaction=0.0018), as well as *UGT2B15* Tyr85Asp (T>G) and aspirin use (p-interaction=0.01). The interaction with the *UGT2B4* and the *UGT2B15*

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polymorphisms were noteworthy at the 25% FDR level. This study highlights the need for further pharmacogenetic studies to identify individuals who might benefit from NSAID use as part of developing effective strategies for prevention of colorectal neoplasia.

### Keywords

*UGT*; *CYP2C9*; colorectal cancer; ibuprofen; aspirin; non-steroidal anti-inflammatory drugs; NSAIDs

## Introduction

Several studies, including randomized trials, have reported protective effects of non-steroidal anti-inflammatory drugs (NSAIDs) on colorectal carcinogenesis as well as on the etiology of other tumors, illustrating the chemopreventive potential of NSAIDs (Flossmann and Rothwell, 2007; Cross et al., 2008; Chia et al., 2012; Rothwell et al., 2010; 2012a). Many questions remain, however, unanswered, especially regarding dosage, treatment duration and long-term benefit of NSAID use (Potter, 2012). NSAIDs reduce inflammation through inhibition of prostaglandin synthesis (by blocking prostaglandin-endoperoxide synthases 1 (COX-1) and 2 (COX-2)) and thus affect pathways that are relevant for carcinogenesis such as proliferation, apoptosis and angiogenesis (Ulrich et al., 2006). In support, recent meta-analyses among >24,000 individuals showed a 37% reduction of colorectal cancer and a 19% reduction of cancer mortality overall among individuals using aspirin (Rothwell et al., 2012a). Interestingly, data on molecular tumor pathology of colorectal cancers suggest that the preventive effect of aspirin may be limited to a subset of tumors carrying mutations in PI3KCA (Ogino et al., 2013; Tougeron et al., 2013). Furthermore, the effectiveness of NSAIDs depends on the bioavailability of the active drug compound. Inter-individual differences in drug metabolism may modify therapeutic effects, which can be a direct consequence of polymorphisms within related genes (Ulrich et al., 2006; Cross et al., 2008). Polymorphisms in genes of drug-metabolizing enzymes have long been known to have functional impacts on pharmacokinetics, particularly on bioavailability (Weber and Hein, 1979; Ciotti et al., 1997; Takahashi et al., 1998). The metabolism of NSAIDs primarily involves glycine N-acyltransferase and uridine 5′ diphosphate glucuronosyl transferases (e.g. UGT1A6) and cytochrome P450 2C9 (CYP2C9) (Hutt et al., 1986; Leemann et al., 1993). Thus, interactions between polymorphisms in *CYP2C9* or the *UGT* gene families and NSAID use may modify the risk of colorectal cancer.

Previous studies have investigated the effect of selected polymorphisms (*CYP2C9*2, CYP2C9*3, UGT1A6*2*) (Rettie et al., 1994; Haining et al., 1996; Sullivan-Klose et al., 1996; Ciotti et al., 1997; Steward et al., 1997; Takahashi et al., 1998; Gill et al., 1999; Bigler et al., 2001; Chan et al., 2005; Samowitz et al., 2006; Bae et al., 2011) that appear to have functional consequences on colorectal cancer risk. Their findings suggest interactions between polymorphisms in drug-metabolizing enzymes and NSAID use, highlighting the potential of pharmacogenetics to tailor chemoprevention. Variants in *UGT* genes modified the risk of colorectal adenoma dependent on the use of NSAIDs (Bigler et al., 2001; Chan et al., 2005). UGT enzymes are phase II drug metabolizing enzymes, which modify xenobiotic

or endobiotic compounds through glucuronidation. UGT1A6 variant enzymes were reported to display lower activity, resulting in a prolonged exposure to the active drug and consequently reduced colorectal adenoma risk (Bigler et al., 2001; Samowitz et al., 2006; Chan et al., 2011). However, only little is known about other *UGT* polymorphisms and their interaction with NSAIDs in colorectal cancer susceptibility.

The *UGT1A* gene family consists of four common exons and at least 13 variable exons, resulting in many shared sequences and consequently shared polymorphisms within this gene family giving rise to nine functional UGT1A enzymes (Mackenzie et al., 2005). In addition, several UGT enzymes share substrate specificity (Kuehl et al., 2005). Therefore, in order to study both the effect of *UGT1A* and *UGT2B* polymorphisms on risk of colorectal cancer and their potential to modify the protective effect of NSAID use on colorectal cancer risk, pharmacogenetic investigations of the *UGT* loci need to be performed in a targeted and comprehensive manner. Due to the complex structure of the *UGT* loci, genetic variation within this region is insufficiently covered on standard genome-wide association platforms. Consequently, GWAS consortia cannot provide thorough information to improve our understanding of the impact of *UGT* gene polymorphisms on cancer risk and many other phenotypes. Thus, many of the *UGT* variants reported here are being studied for the first time for an association with colorectal cancer risk.

We conducted a matched case-sibling control study, based on 1,584 colorectal cancer cases and 2,516 healthy controls, investigating a selection of putatively functional single nucleotide polymorphisms (SNPs) in ten genes of phase I (CYP2C9) and phase II (UGT) drug metabolizing enzymes in relation to risk of colorectal cancer. We also investigated combined genotypes across *UGT* genes, as multiple ‘hits’ in this detoxification machinery may be required to have an impact on colorectal carcinogenesis. Finally, a focus of this study was interactions between targeted polymorphisms and NSAID use in colorectal cancer risk.

## Materials and Methods

### Study Population

The study population has been described previously (Newcomb et al., 2007). Briefly, colorectal cancer cases were recruited for the Colon Cancer Family Registry (CCFR) from six registry centers. The CCFR cases were patients and affected relatives diagnosed with primary invasive colorectal cancer between 1998 and 2002 who were interviewed within five years of diagnosis. Controls were siblings without a colorectal cancer diagnosis at the time of enrollment. Although eligibility requirements varied slightly across registry centers, participants typically were required to be between the ages of 20 and 74 (Newcomb et al., 2007). Standard questionnaires were used to collect epidemiologic data from CCFR participants regarding demographic characteristics, medical history, NSAID use, family history of cancer, smoking history, diet, physical activity, height, and weight. NSAID use was defined as regular use in the two years prior to study enrollment. Blood and tissue samples were collected according to standardized procedures. Individuals were excluded from this study if the case did not have at least one matched unaffected sibling as a control or if an individual's sex determined by genotyping did not match reported sex on the

questionnaire. Only individuals self-reported as Caucasian and collected through population-based recruitment, were included in these analyses. Informed consent was obtained from all participants. The Institutional Review Boards at each CCFR site approved the study. Blood samples were collected according to standardized procedures as described earlier (Newcomb et al., 2007) and DNA was extracted from peripheral blood leukocytes and quantified using the PicoGreen kit (Invitrogen, Paisley, United Kingdom).

## Selection of polymorphisms and genotyping

Polymorphisms were selected based on a candidate gene approach with the aim of assessing the association of the complex genetic variation within *UGT1A* and *UGT2B* gene families, as well as *CYP2C9*, on both risk and effect modification of NSAID use on colorectal cancer. We selected 35 polymorphisms with minor allele frequencies of at least 3% in ten genes to capture genetic variants in *UGT* genes with previously demonstrated functional impact or with known amino acid changes (Haining et al., 1996; Ciotti et al., 1997; Takahashi et al., 1998; Krishnaswamy et al., 2005a; Thomas et al., 2006).

TagSNPs were specifically chosen to capture common non-synonymous variation in the *UGT1A* locus. We used extensive resequencing data from 92 Caucasian individuals (Thomas et al., 2006) to determine the LD structure of the locus and identify nsSNPs with minor allele frequencies greater than 3%. Outside of the *UGT1A* locus, the candidate genes *CYP2C9, UGT2B4, UGT2B7* and *UGT2B15* were chosen based on earlier functional work supporting their role in NSAID metabolism (Gill et al., 1999; Kuehl et al., 2005; Kuehl et al., 2006). With regards to the *UGT2B* genes, *UGT2B4* and *UGT2B7* were included because of their demonstrated glucuronidation of ibuprofen (Kuehl et al., 2005) and salicylic acid (Kuehl et al., 2006). In these experiments, there was little evidence that *UGT2B15* or *UGT2B17* could form acyl and phenolic glucuronides of salicylic acid at detectable levels. Furthermore, there were no non-synonymous SNPs in *UGT2B17* with minor allele frequencies >3%. We included *UGT2B15* due to its demonstrated, albeit low, glucuronidation of ibuprofen and naproxen in vitro (Kuehl et al., 2006).

TaqMan-based assays were performed for the *CYP2C9* polymorphisms R144C (rs1799853) and I359L (rs1057910), the *UGT1A6* polymorphisms T181A (rs2070959) and R184S (rs1105879), the *UGT2B4* polymorphism D458E (rs13119049), the *UGT2B15* polymorphism D85Y (rs1902023), and the *UGT2B7* polymorphism Y268H (rs7439366) 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). The *CYP2C9* and *UGT1A6* assays contained 3.75 ng of DNA, 2.5 μl of 2X TaqMan Universal PCR Mix (No AmpErase UNG), 300 nmol/L forward primer, 300 nmol/L reverse primer, 100nmol/L FAM-labeled MGB probe, 100nmol/L VIC-labeled MGB probe and double-distilled water to a final volume of 5 μl. Cycling conditions were 50°C 2min, 95°C 10min, 40 × 95°C, 15sec; 60°C, 1 min. The *UGT2B15* polymorphism D85Y (rs1902023) was genotyped as above but with 900nM primer and 200nM probe concentrations and the PCR was extended to 50 cycles. *UGT2B4* and *UGT2B7* genotyping reactions used TaqMan core reagents (cat#4304439), 5ng DNA, 200nM primers, 5mM

(*UGT2B4*) and 4mM (*UGT2B7*) MgCl2, 50nM (*UGT2B4*) and 200nM (*UGT2B7*) probes, and 50 PCR cycles. All assays were validated on 30 CEPH family trios purchased from the Coriell Cell Repository (CEPH HAPMAPPT01).

Five polymorphisms (*UGT1A5* A158G (rs12475068), *UGT1A6* S7A (rs6759892), *UGT1A9* -275T>A (rs6714486), *UGT2B4* 3′UTR (rs1131878) and *UGT2B4* 5′UTR (rs1966151)) were genotyped using the Illumina GoldenGate platform and the Veracode BeadXpress Reader (Illumina, Inc. San Diego, CA) as described previously (Kleinstein et al., 2013). Call rates for all five genotypes exceeded 98%. The concordance for blinded duplicates was 100%.

The promoter repeat polymorphism in *UGT1A1* (rs34815109) was genotyped using the GeneScan assay, as previously described (Thomas et al., 2006). The exon 1 polymorphisms in *UGT1A3* and *UGT1A7* were genotyped using direct sequencing, as described (Thomas et al., 2006).

For quality control, all assays were validated using the 30 CEPH family trios prior to running study samples. Each assay batch contained negative and positive controls and 5% of the total number of samples were re-genotyped to confirm reproducibility. All genotypes were also reviewed independently by two technicians. Laboratory staff was blinded to case-control status for all assays.

Two polymorphisms (rs28898617 and rs6431625), both located in *UGT1A3* were not in Hardy-Weinberg Equilibrium and thus were excluded from further analyses.

## Statistical Analysis

The associations between polymorphisms and the risk of colorectal cancer were assessed with conditional logistic regression models estimating odds ratios (OR) and 95% confidence intervals (CI) (Horvath and Laird, 1998; Gauderman et al., 1999). All models used kinship as the matching variable and adjusted for age and sex. For each polymorphism, the risk of colorectal cancer, rectal cancer, and colon cancer (combining proximal and distal) was estimated using a co-dominant model unless any cell had fewer than 30 individuals, in which case a dominant model was used. A recessive model was not estimated as the sample sizes were too small for this analysis. A trend test was performed using the log-additive model to investigate dose-dependent associations with the variant allele. Combined analyses of all polymorphisms within each gene and haplotype analyses were also conducted. For the combined genotype analyses, genotype combinations within each gene were analyzed both individually and in a dominant analysis, where those with at least one variant allele in a genotype combination were compared with those carrying only the major alleles. For the haplotype analyses, haplotype frequencies in cases and controls were inferred with the SAS/Genetics software module. For haplotypes with fewer than 5 individuals per cell, haplotypes were combined in one category (HRare). The analyses were carried out using the expectation-maximization algorithm to generate maximum likelihood estimates of haplotype frequencies.

We also investigated polymorphism interactions with NSAID, aspirin or ibuprofen use between current users vs. never/former users. In this study population, current NSAID use was more strongly inversely associated with colorectal cancer risk than other aspects of NSAID use and thus we chose it as the primary variable for interaction analysis. The reference groups were comprised of individuals who were homozygous for the wild-type allele(s) and never/former users of NSAIDs. Because use of NSAIDs may be associated with other known risk factors for colorectal cancer, NSAID interactions were adjusted for smoking (continuous pack-years), body mass index (BMI; continuous), and physical activity (four-level ordinal variable based on average MET hours) in addition to age (continuous) and sex. We assessed the interaction between genotype and the use of both aspirin and ibuprofen on a multiplicative scale using the cross-product of NSAID exposure and genotype; the likelihood-ratio test was used to determine the statistical significance of the interaction.

All tests of statistical significance were two-sided at $\alpha$=0.05. The false discovery rate (FDR) of Benjamini and Hochberg was used to correct for multiple testing (Benjamini and Hochberg, 1995; Benjamini et al., 2001). Most of the investigated polymorphisms were selected based on functional significance at an *in vitro* or *in silico* levels, thus we used the FDR at 25%. Analyses were conducted using SAS Version 9.3 for Windows (SAS Institute Inc., Cary, NC).

## Results

Characteristics of the study population are presented in Table 1. We observed no statistically significant differences between cases and unaffected sibling controls by age; regular NSAID, ibuprofen, or aspirin use; physical activity; or BMI. Cases were more likely to be male ($p<0.01$). A summary of genotyped polymorphisms and respective minor allele frequencies is given in Supplementary Table 1. Allele frequencies were consistent with previous studies in Caucasian populations (Bigler et al., 2001; Chan et al., 2005; Dura et al., 2012).

### SNP risk estimates

Out of 18 analyzed polymorphisms, one showed a significant association with colorectal cancer risk (Supplementary Table 2). A non-synonymous polymorphism in the *UGT2B15* gene (rs1902023, T>G; *UGT2B15*2*), which leads to a tyrosine to aspartic acid change at position 85, statistically significantly increased the risk of colorectal cancer (OR 1.34; 95% CI 1.10-1.63, p-value=0.02) in individuals with the TG genotype (Table 2). The risk of colorectal cancer for homozygous carriers of the variant allele was not statistically significant (OR 1.27; 95% CI 0.97-1.67). Similarly, in an additive analysis, no significant association was observed. No other polymorphisms were statistically significantly associated with risk of colorectal cancer.

As previous studies have shown that haplotypes or genotype combinations of *UGT* polymorphisms may have a larger impact on enzyme activity than a single nucleotide change, we also investigated haplotypes within each *UGT* gene and across the *UGT1A* locus, as well as combined genotypes within each genotyped gene for association with colorectal

cancer risk (Supplementary Tables 3, 4 and 5). Although none of the haplotypes were statistically significantly associated, a three-SNP genotype within *UGT1A6* was associated with risk of colorectal cancer (Table 3). Individuals who were homozygous for the major alleles of the polymorphisms rs2070959 (A>G; Thr181Ala) and rs1105879 (A>C; Arg184Ser) and, additionally, homozygous for the rs6759892 (T>G; Ser7Ala) minor G-allele of the *UGT1A6* gene, i.e. the *UGT1A6*3* allele (Bock et al., 2005), were at a 3.9-fold higher risk of colorectal cancer (OR 3.87; 95% CI 1.04-14.45) than individuals carrying only the major alleles of all three polymorphisms (*UGT1A6*1*). This association was noteworthy at the 25% FDR level.

## NSAID interactions

Several of the investigated variants showed a significant ($p<0.05$) interaction with the modification of colorectal cancer risk by NSAID use; however, some of these results were based on small cell sizes and should be considered tentative.

The *UGT1A3* Thr78Thr (rs17868336; A>G) variant showed a statistically significant interaction with NSAID use (p-interaction=0.02), increasing the risk of non-NSAID users with the homozygous minor G-allele genotype by 50% (OR 1.57; 95% CI 1.06-2.34, Table 4.a) in comparison to non-users with the major A-allele.

A stratified analysis by tumor site showed a statistically significant interaction between two of the *UGT1A6* polymorphisms (Ser7Ala and Arg184Ser) and NSAID use for risk of rectal cancer (p-interaction=0.03, Table 4.b, and p-interaction=0.02, Table 4.c respectively).

We also investigated whether haplotypes across the *UGT1A* locus, within any of the investigated *UGT* genes, where several polymorphisms were genotyped (i.e. *UGT1A3*, *UGT1A6, UGT1A7, UGT2B4* and *CYP2C9*) or combined genotypes within any of the investigated genes interacted with NSAID use, or specifically, ibuprofen or aspirin in colorectal cancer predisposition. No interactions were observed with overall NSAID use; however, the use of ibuprofen was associated with a higher risk of colorectal cancer in individuals who were homozygous for the major alleles of all three investigated *UGT2B4* polymorphisms (rs1966151, A>G, 5′UTR; rs13119049, A>T, Asp458Glu; rs1131878, A>G, 3′UTR; OR 2.31; 95% CI 1.07-4.97), whereas ibuprofen users who carried at least one variant allele at any of the three loci tended to be at lower risk (OR 0.73; 95% CI 0.48-1.12) of colorectal cancer than non-users with only major alleles (p-interaction=0.0018, Table 5). When we accounted for multiple testing based on FDR, this association was noteworthy at the 25% FDR level.

Additionally, the coding variant Tyr85Asp in the *UGT2B15* (*UGT2B15*2*) gene showed a statistically significant interaction with aspirin use (p-interaction=0.01). Individuals who were homozygous for the minor G-allele and used aspirin were at higher risk of colorectal cancer (OR 1.71; 95% CI 1.07-2.73), than non-users who carried only the major T-allele (Figure 1 and Table 6). This association was noteworthy at the 25% FDR level.

## Discussion

Colorectal cancer is a heterogeneous disease and targeted prevention strategies are required to reduce the public health burden of the disease (Colussi et al., 2013). In light of extensive data on the preventive effects of aspirin and other NSAIDs on risk of cancer, there is growing interest in determining whether genetic variation in NSAID-metabolizing enzymes can be used to predict the protective effect of NSAIDs on cancer development and on complications such as gastrointestinal bleedings (Derry and Loke, 2000; Ulrich et al., 2006; Cross et al., 2008; Cuzick et al., 2009; Chia et al., 2012; Kraus et al., 2013; Rothwell at al., 2010; 2012a; 2012b; 2013). Since metabolizing enzymes can modify, conjugate, and/or excrete endobiotic or xenobiotic compounds, including the detoxification of carcinogens and metabolism of chemopreventive or chemotherapeutic compounds, genetic variability in these enzymes may thus alter the toxicity or efficacy of xenobiotics and consequently alter cancer susceptibility.

In this large population-based study of sibling pairs in the CCFR, we investigated whether polymorphisms within genes of phase I (*CYP2C9*) and phase II (*UGT*) drug-metabolizing enzymes were associated with or modified the protective effect of NSAIDs on colorectal cancer risk. In addition to previously investigated polymorphisms, we targeted several previously unstudied polymorphisms in *UGT* genes in this study.

Variants in four genes (*UGT1A3, UGT1A6, UGT2B4* and *UGT2B15*) were statistically significantly associated with colorectal or rectal cancer risk either in individual analysis, combined genotype analysis, or in combination with NSAID use. The interaction between the *UGT2B15* Tyr85Asp polymorphism and aspirin use (which was also associated with risk overall) as well as between a three-SNP genotype in *UGT2B4* and ibuprofen use were noteworthy at the 25% FDR level.

This study is the first to report the association of the *UGT1A6*3* polymorphism, a three-SNP genotype (Ala7, Thr181, Arg184), with increased colorectal cancer risk; previous studies had focused on a two-SNP genotype within *UGT1A6* (Thr181Ala, Arg184Ser, i.e. *UGT1A6*8* also referred to as *UGT1A6*2* in some studies) (Bigler et al., 2001; Chan et al., 2005; McGreavey et al., 2005; Samowitz et al., 2006). Nonetheless, these results require further validation, as the association was based on few individuals and thus stratification by tumor location was not possible. *In vitro* investigations of the *UGT1A6*3* variant enzyme showed no significant change of the enzymatic activity compared to the wild type enzyme *UGT1A6*1* (Krishnaswamy et al., 2005b). However, the family of *UGT1A* genes represents a complex locus consisting of four common exons and at least 13 variable exons with many shared sequences and polymorphisms among the *UGT1A* genes (Mackenzie et al., 2005). The complexity of the *UGT1A* region is further increased by its LD structure; many SNPs are linked to each other, thus making it difficult to identify the causal variation and the related gene or enzyme product. The Ser7Ala polymorphism is in complete LD with other polymorphisms in the *UGT1A* locus, including three polymorphisms located in the 5′UTR of the gene, associated with 50% decreased gene expression of *UGT1A6*, but not with lower protein levels or reduced enzyme activity (Krishnaswamy et al., 2005a; Thomas et al., 2006), thus the functional consequence of these polymorphisms remains to be elucidated.

Previous studies have shown that the *UGT1A6*8* allele (two-SNP genotype: Ala181 and Ser184) modified the protective effect of aspirin on colon or colorectal adenoma; however, no study has shown similar effects on the risk of colon carcinoma (Bigler et al., 2001; Chan et al., 2005; McGreavey et al., 2005; Samowitz et al., 2006; Thompson et al., 2009), concordant with our observations. It has been proposed earlier that the protective effect of NSAIDs may occur earlier during carcinogenesis and may thus be relevant for adenoma prevention rather than for prevention of carcinomas (McGreavey et al., 2005). However, recent meta-analyses (Rothwell et al., 2012a; 2012b) strongly suggest that the preventive effect of NSAIDs exists across all stages of colorectal neoplasia. Nevertheless, further investigation regarding dose, treatment duration and long-term benefit of NSAID use are required to optimize its use in cancer prevention (Cuzick et al., 2009; Potter, 2012).

In the present study we observed an association of a *UGT2B15* variant with increased risk of colorectal cancer, which also interacted with aspirin use in colorectal cancer predisposition, as well as novel interactions between *UGT2B4* polymorphisms and ibuprofen. Both the UGT2B4 and the UGT2B15 enzymes are primarily involved in the metabolism of sex hormones. Polymorphisms within these genes have been associated with increased breast and prostate cancer risk, respectively (MacLeod et al., 2000; Park et al., 2004; Low et al., 2010; Grant et al., 2013). While UGT2B4 has been shown to metabolize NSAIDs at quite high rates, UGT2B15-mediated glucuronidation was observed only at low rates (Kuehl et al., 2005; 2006). *In vitro* studies of the UGT2B15 variant enzyme displayed similar substrate specificity between the Tyr85 and the Asp85 enzymes; however, the Asp85 variant enzyme had a faster turn-around time (i.e. higher Vmax) (Levesque et al., 1997). This may lead to a reduced internal dose of the active drug compound accompanied by reduction of its preventive effect. The previously reported associations with cancer risk are probably due to changed sex steroid metabolism resulting in altered tissue exposure to hormones capable of stimulating cell proliferation (Yong et al., 2011). However, the strong interaction between the *UGT2B15* polymorphism and aspirin use in relation to colorectal cancer risk, which remained significant after accounting for multiple testing, suggests a meaningful role of the enzyme in NSAID metabolism. Nevertheless, it seems that larger structural genetic variation such as copy number variations (CNV) have a stronger impact on cancer risk as previously reported in recent study. It was shown that a large CNV that leads to the deletion of the *UGT2B17* gene, significantly decreased the risk of rectal cancer (Angstadt et al., 2013), but not colon cancer.

Concordantly with a recent meta-analysis which covered 13 studies, we did not observe an association between any of the *CYP2C9* polymorphisms and risk of colorectal cancer (Liang et al., 2012). While an interaction of the polymorphisms with NSAID use on colorectal cancer risk was reported previously, in this study no interaction was observed (Bigler et al., 2001; Samowitz et al., 2006).

There are several strengths to this study. The case–unaffected sibling control design helps avoid false positives that can result from population stratification and increases the power to detect gene–NSAID interactions. The relatively large overall sample size in this study made it possible to examine combined genotypes, as well as interaction analysis for NSAID use. There are several potential limitations to our study. Current NSAID use was only modestly

associated with reduced CRC risk in our study population, which may limit the power for detection of gene-NSAID interaction. The family-based study design likely reduced the power of the main effect analyses. Furthermore, despite the large sample size, interaction analysis with combined genotypes or specific NSAIDs resulted in small cell sizes, reducing the precision of estimates and the power. When we tested the interactions for multiple comparisons at the 25% FDR level, only the interaction of the *UGT2B4* combined genotype with NSAID use and the *UGT2B15*2* with aspirin use were noteworthy. On the other hand, we investigated specific hypotheses with respect to putative gene-NSAID interactions and functional polymorphisms; in this setting adjustment for multiple comparisons is less critical. Finally, our study was based on the investigations of SNPs, thus we did not cover larger structural variants in *UGT* genes, which may have a stronger impact on colorectal cancer risk.

In summary, our results suggest that variation in four genes (*UGT1A3, UGT1A6, UGT2B4* and *UGT2B15*) modifies the risk of colorectal cancer either independent or in conjunction with NSAID use. Our results underscore the importance of pharmacogenetics as a tool to identify individuals who may benefit from NSAIDs as chemopreventives. In addition, our study results suggest that it appears to be more important to consider combinations of genotypes rather than individual polymorphisms to identify the interaction between genetic variability and the protective effect of NSAID use on colorectal cancer development, particularly for *UGT* families.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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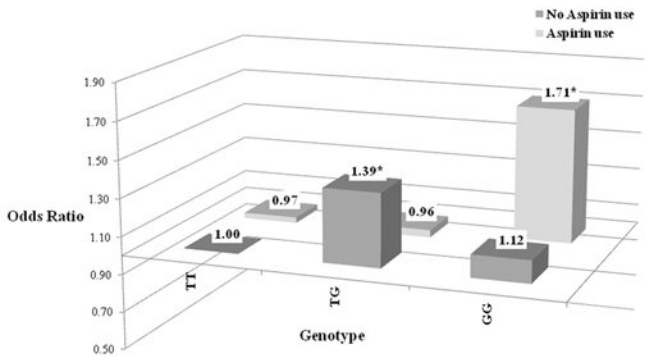


**Figure 1.**

## Table 1

**Selected characteristics of population-based Colon Cancer Family Registry kinships restricted to Caucasians (N=4100)**

| | Cases (N=1584)[a] N (%) | Controls (N=2516)[a] N (%) |
|---|---|---|
| **Age (y), mean (SD)** | 53.5±10.8 | 54.0±11.7 |
| **Gender** | | |
| Female | 774 (48.9) | 1390 (55.3) |
| Male | 810 (51.1) | 1126 (44.7) |
| **Family History of Cancer**[b] | | |
| Yes | 506 (32.5) | 771 (31.1) |
| No | 1051 (67.5) | 1708 (68.9) |
| **Center** | | |
| Ontario | 296 (18.7) | 491 (19.5) |
| USC (University of Southern California) | 319 (20.1) | 444 (17.7) |
| Australia | 317 (20.0) | 554 (22.0) |
| Hawaii | 6 (0.4) | 7 (0.3) |
| Mayo Foundation | 266 (16.8) | 502 (20.0) |
| Seattle | 380 (24.0) | 518 (20.6) |
| **Tumor site** | | |
| Proximal | 526 (33.2) | - |
| Distal | 460 (29.0) | - |
| Rectal | 523 (33.0) | - |
| **Regular Aspirin use**[c] | | |
| No | 1320 (84.1) | 2080 (84.1) |
| Yes | 250 (15.9) | 394 (15.9) |
| **Regular Ibuprofen use**[c] | | |
| No | 1444 (92.5) | 2255 (90.9) |
| Yes | 117 (7.5) | 226 (9.1) |
| **Regular NSAID use**[c] | | |
| No | 1234 (78.2) | 1916 (76.9) |
| Yes | 343 (21.8) | 577 (23.1) |
| **Physical activity** | | |
| Inactive | 375 (23.7) | 585 (23.3) |
| Less active | 424 (26.8) | 679 (27.0) |
| Active | 374 (23.6) | 564 (22.4) |
| Very active | 338 (21.3) | 548 (21.8) |
| **BMI [kg/m$^2$]±SD** | 27.4 ± 6.0 | 26.8 ± 5.5 |
| **Cigarette smoking (pack-years)±SD** | 12.9 ±19.5 | 11.7 ± 19.3 |

[a] Numbers may not add to total because of missing data.

[b] First degree relative

[c] Regular Aspirin, Ibuprofen and NSAID use defined as regular use of least two pills per week for at least one month.

**Table 2**

**Association between *UGT2B15* Tyr85Asp (rs1902023, *UGT2B15*2*) and risk of colorectal cancer**

| | Cases | Controls | OR[a,b] | 95% CI | p-value[c] |
|---|---|---|---|---|---|
| ***UGT2B15* (Tyr85Asp)** | | | | | |
| T/T | 403 | 693 | 1.00 | | |
| T/G | 792 | 1161 | 1.34 | 1.10 – 1.63 | |
| G/G | 354 | 586 | 1.27 | 0.97 – 1.67 | 0.017 |
| TG or GG | 1146 | 1747 | 1.33 | 1.10 – 1.62 | 0.019 |

[a] OR, odds ratio; CI, confidence interval.

[b] All analyses were adjusted for age and sex.

[c] Global p-value from log-likelihood ratio test.

**Table 3**

**Association between 3-SNP-genotypes (Ser7Ala, Thr181Ala, Arg184Ser) in *UGT1A6* and risk of colorectal cancer**

| *UGT1A6*[a] | Cases | Controls | OR[b,c] | 95% CI | p-value[d] | p-value[e] |
|---|---|---|---|---|---|---|
| TT/AA/AA | 477 | 775 | 1.00 | - | - | 0.002[f] |
| TG/AG/AC | 508 | 806 | 1.11 | 0.89 - 1.37 | 0.36 | |
| GG/GG/CC | 159 | 239 | 1.12 | 0.80 - 1.57 | 0.49 | |
| TG/AA/AA | 115 | 187 | 1.04 | 0.72 - 1.49 | 0.84 | |
| GG/AG/AC | 65 | 97 | 1.26 | 0.77 - 2.06 | 0.36 | |
| TG/AA/AC | 43 | 43 | 1.72 | 0.94 - 3.16 | 0.08 | |
| GG/AG/CC | 16 | 35 | 0.51 | 0.24 - 1.22 | 0.13 | |
| GG/AA/AA | 9 | 11 | 3.87 | 1.04 - 14.45 | 0.04 | |
| GG/AA/AC | 5 | 5 | 3.28 | 0.76 - 14.11 | 0.11 | |
| TT/AG/AC | 1 | 1 | n.a. | n.a. | n.a. | |
| GG/AA/CC | 0 | 1 | n.a. | n.a. | n.a. | |

[a] Sequence of variants: rs1105879/ rs2070959/ rs6759892

[b] OR, odds ratio; CI, confidence interval.

[c] All analyses were adjusted for age and sex.

[d] Wald test p-value.

[e] Global p-value from log-likelihood ratio test.

[f] Noteworthy at the 25% FDR level.

## Table 4

**Association between NSAID use and colorectal cancer and rectal cancer risk stratified by *UGT1A* genotypes**

**a) Association between NSAID use and colorectal cancer risk stratified by *UGT1A3* (Thr78Thr) genotypes**

| | No NSAID use | | | | NSAID use | | | |
|---|---|---|---|---|---|---|---|---|
| | CRC[a] Cases | Controls | OR[b,c] | 95% CI | CRC Cases | Controls | OR | 95% CI |
| ***UGT1A3* (Thr78Thr)** | | | | | | | | |
| A/A | 964 | 1527 | 1.00 | | 277 | 451 | 0.95 | 0.78 - 1.16 |
| A/G or G/G | 95 | 123 | 1.57 | 1.06 - 2.34 | 18 | 41 | 0.66 | 0.34 - 1.27 |
| p-interaction 0.02[d] | | | | | | | | |

**b) Association between NSAID use and rectal cancer risk stratified by *UGT1A6* (Ser7Ala)**

| | No NSAID use | | | | NSAID use | | | |
|---|---|---|---|---|---|---|---|---|
| | RC[e] Cases | Controls | OR | 95% CI | RC Cases | Controls | OR | 95% CI |
| ***UGT1A6* (Ser7Ala)** | | | | | | | | |
| T/T | 99 | 187 | 1.00 | | 37 | 55 | 1.33 | 0.75 - 2.37 |
| T/G or G/G | 222 | 342 | 1.40 | 0.94 - 2.08 | 41 | 83 | 0.77 | 0.42 - 1.41 |
| p-interaction 0.03[d] | | | | | | | | |

**c) Association between NSAID use and rectal cancer risk stratified by *UGT1A6* (Arg184Ser) genotypes**

| | No NSAID use | | | | NSAID use | | | |
|---|---|---|---|---|---|---|---|---|
| | RC Cases | Controls | OR | 95% CI | RC Cases | Controls | OR | 95% CI |
| ***UGT1A6* (Arg184Ser)** | | | | | | | | |
| A/A | 146 | 266 | 1.00 | | 46 | 67 | 1.23 | 0.76 - 1.99 |
| A/C or C/C | 217 | 328 | 1.25 | 0.89 - 1.77 | 36 | 82 | 0.66 | 0.37 - 1.15 |
| p-interaction 0.02[d] | | | | | | | | |

[a] CRC, Colorectal cancer

[b] OR, odds ratio; CI, confidence interval.

[c] All analyses were adjusted for age, sex, BMI, pack-years and physical activity.

[d] For multiplicative interaction term.

[e] RC, Rectal cancer

**Table 5**

**Association between ibuprofen use and colorectal cancer risk stratified by combined *UGT2B4* genotypes (5′UTR, Asp458Glu, 3′UTR)**

| | No Ibuprofen use | | | | Ibuprofen use | | | |
|---|---|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | **OR[a,b]** | **95% CI** | **Cases** | **Controls** | **OR[a,b]** | **95% CI** |
| ***UGT2B4* (5′UTR, Asp458Glu, 3′UTR)** | | | | | | | | |
| AA_AA_AA | 198 | 328 | 1.00 | - | 28 | 20 | 2.31 | 1.07 - 4.97 |
| any variant | 934 | 1408 | 1.12 | 0.86 - 1.50 | 67 | 157 | 0.73 | 0.48 - 1.12 |
| p-interaction 0.0018[c,d] | | | | | | | | |

[a] OR, odds ratio; CI, confidence interval.

[b] All analyses were adjusted for age, sex, BMI, pack-years and physical activity.

[c] p-value for multiplicative interaction term.

[d] Noteworthy at the 25% FDR level.

**Table 6**

**Association between aspirin use and colorectal cancer risk stratified by *UGT2B15*2* (Tyr85Asp) genotypes**

| | No Aspirin use | | | | Aspirin use | | | |
|---|---|---|---|---|---|---|---|---|
| | **Cases** | **Controls** | **OR[a,b]** | **95% CI** | **Cases** | **Controls** | **OR[a,b]** | **95% CI** |
| ***UGT2B15* (Tyr85Asp)** | | | | | | | | |
| T/T | 316 | 533 | 1.00 | | 54 | 88 | 0.97 | 0.63 – 1.50 |
| T/G | 604 | 866 | 1.39 | 1.11 – 1.74 | 112 | 191 | 0.96 | 0.68 – 1.34 |
| G/G | 259 | 465 | 1.12 | 0.82 – 1.53 | 58 | 61 | 1.71 | 1.07 – 2.73 |
| | | | | p-interaction 0.01[c,d] | | | | |

[a] OR, odds ratio; CI, confidence interval.

[b] All analyses were adjusted for age, sex, BMI, pack-years and physical activity.

[c] For multiplicative interaction term.

[d] Noteworthy at the 25% FDR level.