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Impact of genetic polymorphisms on adenoma recurrence and toxicity in a COX2 inhibitor (celecoxib) trial: results from a pilot study

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Abstract

Objective—Chemoprevention trials have shown that celecoxib reduces adenoma recurrence but can cause cardiovascular toxicity. In this pilot study, we evaluated associations between genetic variation in several candidate pathways (e.g. prostaglandin synthesis) and adenoma recurrence and cardiovascular and gastrointestinal toxicities.

Methods—Genotyping analysis was carried out on 117 Israeli colorectal adenoma patients who participated in the Prevention of Colorectal Sporadic Adenomatous Polyps trial. Reassessment followed after 3 years on celecoxib and after 2 years from termination of treatment with celecoxib. Efficacy (absence of colorectal adenomas) was measured by colonoscopy at years 1, 3, and 5.

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Conflicts of interest

There are no conflicts of interest.

Toxicities were assessed by investigators during celecoxib treatment and by self-report post-treatment. A linkage disequilibrium-based selection algorithm ($r^2 \geq 0.90$, MAF $\geq 4\%$) identified 255 tagSNPs in 25 analyzed candidate genes. Genotyping was performed by using Illumina GoldenGate technology.

Results—Multiple genetic variants were associated with adenoma recurrence and toxicity. Genetic variability in *COX1*, *COX2*, and *ALOX12/15* genes played a role in adenoma recurrence, particularly among patients on placebo. More gene variants (especially variants in *PGES*, *CRP*, *SRC*, and *GPX3*) were associated with increased risk for cardiovascular toxicity and symptoms, compared with gastrointestinal toxicity and symptoms. The increased risk for cardiovascular toxicity/symptoms associated with the *SRC* gene variants (rs6017996, rs6018256, rs6018257) ranged from 6.61 (95% confidence interval 1.66–26.36, $P < 0.01$) to 10.71 (95% confidence interval 1.96–58.60, $P < 0.01$).

Conclusion—Genetic polymorphisms in multiple inflammation-related genes appear to interact with celecoxib on adenoma recurrence and its attendant toxicity, particularly cardiovascular toxicity/symptoms. Larger studies validating these pharmacogenetic relationships are needed.

Keywords

colorectal adenoma; colorectal cancer; cyclooxygenase-2 inhibitor; polymorphism; toxicity

Introduction

A number of chemoprevention trials have been conducted to assess the efficacy and safety of selective cyclooxygenase (COX)-2 inhibitors and the risk for adenoma recurrence in patients with a history of colorectal adenomas [1–3]. In the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) and Adenoma Prevention Celecoxib (APC) trials, celecoxib was associated with a significant reduction in adenoma recurrence [1,2], whereas rofecoxib significantly reduced adenoma recurrence in the Adenomatous Polyp Prevention on Vioxx (APPROVe) trial [3]. In the APC trial, both low dose (200 mg) and high dose (400 mg) celecoxib were associated not only with reduced risk for adenoma recurrence (33 and 45%, respectively) but also with significantly reduced adenoma size and number compared with placebo [2]. Likewise, in the PreSAP trial, celecoxib taken at a dose of 400 mg daily significantly reduced the occurrence of colorectal adenomas by 36% within 3 years after polypectomy, compared with placebo [1].

However, the enthusiasm generated by the success of selective COX2 inhibitors for colorectal adenoma recurrence was tempered by their dose-dependent cardiovascular toxicity [4–7]. For celecoxib, patients taking 200 and 400 mg daily in the APC trial had a 2.6- and 3.4-fold increased risk for adverse cardiovascular events, respectively, compared with those on placebo, whereas a nonstatistically significant 1.3-fold increased risk for adverse cardiovascular events was reported in the PreSAP trial. Another safety endpoint was gastrointestinal toxicity, which is a major limiting factor for the use of COX1 acetylators, such as aspirin [8–10], but is not associated with celecoxib use [1,2,11]. The final results from the APPROVe trial [3] and the APC and PreSAP trials [11,12] revealed that the drugs

are effective in reducing adenoma recurrence, particularly advanced adenomas, but the toxicities, mainly cardiovascular, remain.

Heritable factors play an important role in colorectal polyp formation and colorectal carcinogenesis [13–16]. We and others have previously published results suggesting that polymorphisms in inflammation and other related genes can modify an individual's response to NSAIDs and consequently the chemopreventive effect of NSAIDs on colorectal adenoma and cancer [17–26]. In this exploratory pilot study, we evaluated whether genetic polymorphisms in several genes related to inflammation modify the effects of celecoxib on recurrence in patients with adenomatous polyps. We also analyzed whether these polymorphisms are related to cardiovascular and gastrointestinal toxicities/symptoms. In an era of personalized medicine, and given the risk/benefit profile of the COX2 inhibitors, this information could potentially help in tailoring chemopreventive treatment to those who will benefit the most while limiting adverse effects.

Methods

Study design and data collection

Patients from the PreSAP trial, recruited in Israel, who consented for genotyping analyses were included in this pilot study. A subset of 117 blood samples from participants of the PreSAP trial was collected at the Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel [1]. The study was carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Briefly, participants were eligible if they were 30 years or older and had undergone a colonoscopy within 3 months before enrollment, which showed either one adenoma that was 6 mm or larger or two to 10 adenomas of any size. Methods have been described in detail elsewhere [1]. Photographic documentation showing that the cecum was reached, the colon was clean, and the adenomas that were removed were of the size charted was required for inclusion. All patients underwent follow-up colonoscopies as described previously [1]. All participants included in the PreSAP trial had to show 80% adherence to the 1 month run-in placebo medication and had to be willing to abstain from long-term use of all NSAIDs, including COX2 inhibitors, other than the blinded study drug, with the exception of cardioprotective doses of oral aspirin (defined as 162.5 mg/day or 325 mg every other day). Adenoma recurrence was defined as recurrence within 3.5 years after study initiation.

Exclusion criteria were known history of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, inflammatory bowel disease, or invasive cancer within 5 years before enrollment. Patients with renal, hepatic, or bleeding disorders, as well as those with known hypersensitivity to COX2 inhibitors, NSAIDs, salicylates, or sulfonamides, were also excluded.

Toxicity/symptoms were categorized as cardiovascular or gastrointestinal and assessed by investigators for the on-treatment period (all events that began or intensified from after the first dose of the study drug to up to 3 years after the first dose of the study drug). After 3

years, information on post-treatment toxicity was collected for another 2 years by participant self-report.

Cardiovascular toxicity and symptoms included angina, dyspnea, coronary artery stenosis, hypercoagulation, cerebrovascular disease, myocardial infarction or ischemia, peripheral vascular disease, circulatory collapse, hypertension, ventricular and atrial fibrillation, venous thrombosis, or thromboembolism. Gastrointestinal toxicity was defined as gastrointestinal bleeding, gastritis or duodenitis, upper or lower gastrointestinal ulceration, and hemorrhage or gastrointestinal symptoms (vomiting, diarrhea, constipation, heartburn, reflux, abdominal pain).

TagSNP selection and genotyping

On the basis of prior knowledge of the biological pathways of celecoxib activity, we categorized genes into primary genes (very strong prior hypothesis), secondary genes (strong hypothesis), and tertiary genes (moderately strong hypothesis) of interest. Primary genes of interest are those involved in prostaglandin synthesis: *PTGS1* (*COX1*), *PTGS2* (*COX2*), and prostaglandin E2 synthase (*PGES*) genes, as well as those encoding the epidermal growth factor receptor (*EGFR*) and thromboxane synthase (*TBXAS*). Secondary genes of interest included genes encoding enzymes of the arachidonate and leukotriene synthesis pathways (*PLA2A*, *ALOX12*, *ALOX15*, *ALOX5*, *FLAP*), interleukin23 (*IL23R*), and C-reactive protein (*CRP*), as well as genes involved in EGFR signaling (*SRC*, *EP2*, *EP4*), the prostaglandin dehydrogenase gene (*PGDH*), and the ornithine decarboxylase gene (*ODCI*), a gene that has previously been implicated in NSAID pharmacogenetics [27]. Tertiary genes of interest included a group of genes involved in oxidative regulation of prostaglandin synthesis (*GPX1*, *GPX2*, *GPX3*, *GPX4*, *SEPP*) and those involved in colorectal carcinogenesis (*WNT6*, *WNT10A*, *HER2*).

A linkage disequilibrium-based tagSNP selection algorithm ($r^2 \geq 0.90$, MAF $\geq 4\%$) was applied; this algorithm identified 255 tagSNPs in 25 analyzed candidate genes [28]. The analyzed single-nucleotide polymorphisms (SNPs) are listed in Supplementary Table 1, <http://links.lww.com/FPC/A612>.

Genotyping of lymphocyte DNA was performed at TGen, Phoenix, Arizona. We used an Illumina genotyping platform (Illumina GoldenGate bead-based genotyping technology; Illumina Inc., San Diego, California, USA). Intraplate and interplate replicates and blinded duplicates were included (at 5%) as quality control measures on the overall Illumina platform of multiple study populations (> 6000 samples). Data from 30 CEPH trios (Coriell Cell Repository, Camden, New Jersey, USA) genotyped using HapMap were used to confirm reliability and reproducibility. Genotypes were excluded from analyses under the following criteria: GenTrain score less than 0.4, 10% GC score less than 0.25, AB T Dev greater than 0.1239, call frequency less than 0.85, replicate errors greater than 2, P-P-C errors greater than 2, less than 85% concordance with blinded or nonblinded duplicates, and Hardy–Weinberg P -values less than 0.0001.

Statistical analysis

Genetic associations were investigated on the basis of three models: dominant, codominant, and log-additive models. The primary endpoint was adenoma recurrence and secondary endpoints were the occurrences of cardiovascular and gastrointestinal symptoms and toxicities. We observed only minor differences between the three models; thus, we only report results from the dominant model.

Cox proportional hazards regression analysis was used to investigate the association (hazard ratio) of genotype (variant vs. wild type) with time (days) to adenoma recurrence. To examine the main associations of SNPs with risk for adenoma recurrence, all Cox regression models were adjusted for the relevant covariates including treatment, sex, and age. Further analyses were stratified by treatment (placebo vs. celecoxib). Hazard ratios (HRs) and corresponding 95% confidence intervals (95% CI) were calculated. Kaplan–Meier curves were created for SNPs, which showed statistical significance ($P < 0.05$) in Cox regression analysis.

Age-adjusted and sex-adjusted logistic regression models were stratified by treatment and used to estimate the odds ratios (ORs) and corresponding 95% CIs for the association between genotypes for cardiovascular and gastrointestinal toxicities (binary endpoints).

A P -value of up to 0.05 was considered statistically significant. P -values were not adjusted for multiple comparisons, as this was the first exploratory study addressing this topic and generating hypotheses for further follow-up. For adenoma recurrence, genes with P -values between 0.05 and 0.10 are listed in Supplementary Table 2, <http://links.lww.com/FPC/A612>.

All statistical analyses were carried out using SAS (V9.1.2; SAS Institute Inc., Cary, North Carolina, USA).

Results

The mean age of study participants was 62.7 ± 9.9 years. The majority were male (65%) of Ashkenazi Jewish origin (73%) and 59% were on celecoxib during the PreSAP study period. Adenoma recurred in 39% of the patients (Table 1). In all, 42% ($n = 49$) and 19% ($n = 22$) of the participants experienced gastrointestinal toxicity/symptoms and cardiovascular toxicity/symptoms, respectively.

Adenoma recurrence

Genetic variability in the studied pathways played a larger role in adenoma recurrence among patients on placebo compared with those on celecoxib (16 vs. 4 statistically significant associations; Table 2). Variants of the *PGDH* and *IL23R* genes were more likely to be associated with adenoma recurrence among patients on celecoxib, whereas variants of the *COX* and *ALOX* genes were more relevant among patients in the placebo arm. Of note, however, is the *IL23R* variant rs6683455 (T > C near 5'UTR), which was associated with an increased risk for adenoma recurrence overall (HR 2.40, 95% CI 1.32–4.38, $P < 0.01$), both among patients on celecoxib (HR 2.85, 95% CI 1.20–6.77, $P = 0.02$) and among those on placebo (HR 2.51, 95% CI 1.06–5.96, $P = 0.04$).

Among the primary genes of interest, four variants of *COX1* (rs10306164, rs1236913, rs1330344, rs3119773) and two of *COX2* (rs4648268, rs6489469) were associated with significantly increased risk for adenoma recurrence among patients on placebo, with the highest increase in risk observed for the *COX1* rs3119773 variant (1890 T > C intron 2; HR 4.58, 95% CI 1.69–12.44, $P > 0.01$). Although we observed a greatly increased risk for adenoma recurrence among placebo patients with the *COX1* variants rs10306110 and rs10306122, the results should be cautiously interpreted because of very small sample sizes. We also observed a statistically significantly increased risk for adenoma recurrence in both treatment arms for several variants (Table 2 and Fig. 1). Among the primary genes of interest, we observed that the *COX2* rs5275 variant was significantly associated with adenoma recurrence in the dominant (C > T 3'UTR; HR 2.42, 95% CI 1.12–5.22, $P = 0.02$; Table 2) as well as in the codominant model (Fig. 1a).

Variants of some primary genes of interest were associated with borderline significantly reduced risk for adenoma recurrence overall (*COX2* rs5277, V102V exon 3; HR 0.45, 95% CI 0.18–1.14, $P = 0.09$) and among patients on celecoxib (*EGFR* rs11770531 and *EGFR* rs11760524; Supplementary Table 2, <http://links.lww.com/FPC/A612>). Three variants of the *ALOX* genes (rs2073438, rs2292350, rs4796535) were associated with adenoma recurrence among patients on placebo. The *ALOX12* rs2292350 variant (156G > A intron) was associated with decreased risk (HR 0.41, 95% CI 0.17–0.98, $P = 0.05$), whereas the other two variants were associated with increased risk. The *ALOX15* rs11568141 variant was associated with a three-fold increased risk for adenoma recurrence, albeit of borderline statistical significance (HR 3.14, 95% CI 0.99–10.02, $P = 0.053$; Supplementary Table 2, <http://links.lww.com/FPC/A612>). Two *PGDH* gene variants (rs7349744, HR 0.48, 95% CI 0.26–0.89, $P = 0.02$; rs1365613, HR 0.53, 95% CI 0.28–0.99, $P = 0.05$) were inversely associated with risk for adenoma recurrence in both treatment arms combined (Table 2).

Gastrointestinal toxicity and symptoms

Few genetic variants showed an increased risk for gastrointestinal symptoms and toxicity in the celecoxib arm. However, risks were substantially elevated for one variant of *ALOX15* (rs2255888) and two variants of the *EP4* gene (rs4133101, rs13186505) with 3.4- to 5.5-fold increased risks. Of the primary genes of interest, only the *EGFR* rs2072454 variant (OR 2.43, 95% CI 1.10–5.63, $P = 0.04$) was associated with a statistically significantly increased risk for gastrointestinal toxicity/symptoms (Table 3).

Cardiovascular toxicity and symptoms

Compared with gastrointestinal toxicity and symptoms, a larger number of genetic variants were associated with cardiovascular toxicity and symptoms, especially among patients on celecoxib (Table 4). Among the primary genes of interest, two variants of the *PGES* gene (rs2241271 and rs2302821) were associated with increased risks (four- and eight-fold, respectively) among patients. Four other gene variant genotypes, one in the *CRP* gene (rs1800947) and three in the *SRC* gene (rs6017996, rs6018256, rs6018257, ranging from seven- to 11-fold), were associated with significantly increased risk for cardiovascular toxicity/symptoms among patients on celecoxib. The *CRP* rs1800947 variant conferred the highest risk (OR 15.14, 95% CI 2.54–90.12, $P < 0.01$). Overall, five variant genotypes, of

which three were in the *IL23R* gene (rs10789229, rs11465810, rs1884444), were associated with significantly reduced risk for cardiovascular toxicity/symptoms among patients on celecoxib.

Multiple outcomes

Some variants were associated with multiple outcomes (Supplementary Table 3, <http://links.lww.com/FPC/A612>). Among the primary genes of interest, the *COX1* rs10306164 variant was associated with increased risks for adenoma recurrence and cardiovascular toxicity/symptoms among patients on placebo. Two *GPX3* variants (rs3828599, rs736775) were associated with increased risk for cardiovascular but reduced risk for gastrointestinal toxicity/symptoms.

Discussion

In this initial study screening for inflammatory pathways we observed that genetic variability was associated with adenoma recurrence, as well as cardiovascular and gastrointestinal toxicities/symptoms. Although we did not have sufficient statistical power in this pilot study to formally investigate gene–treatment interactions, several of the investigated variants showed significant associations with adenoma recurrence or toxicities only in patients of one treatment group (either placebo or celecoxib). Thus, our strategy has the potential to identify complex biological links among these variants and their associated pathways. We identified several variants that showed associations with adenoma recurrence or that modified the protective or chemotoxic effects of celecoxib. However, a detailed functional follow-up analysis for all variants is beyond the scope of this work and we anticipate that the associations presented herein will be further evaluated and validated in larger trials, such as the APC trial. We thus focus only on the most promising variants of the following genes: for adenoma recurrence, variants of *COX1*, *COX2*, and *ALOX12/15* genes were more relevant among patients on placebo compared with patients on celecoxib. Multiple SNPs (especially variants of *PGES*, *CRP*, *SRC*, and *GPX3*) were associated with an increased risk for cardiovascular toxicity and symptoms.

NSAIDs are among the most widely used drugs [29]. However, it is well established that their use is associated with significant adverse gastrointestinal and cardiovascular side effects. Selective NSAIDs such as the COX2 inhibitors have been shown to possess much improved gastrointestinal tolerability and fewer adverse gastrointestinal effects, but their cardiovascular toxicity is well recognized [4,5,30]. COX2 inhibitor-induced cardiovascular toxicity may include the induction of myocardial infarction, thrombosis, and pulmonary edema; blood pressure destabilization; and death [5,7,30]. Studies have shown that patients at an increased risk for cardiovascular disease or with a history of cardiovascular disorders or other morbid illnesses were at a higher risk of experiencing adverse cardiovascular events after receiving COX inhibitors [31,32]. Therefore, it is important to define the risks to the gastrointestinal and cardiovascular systems associated with COX2 inhibitor therapy and to identify the factors that can contribute to risks of adverse events. In this context, not much is known about the impact of genetic susceptibility and its potential to predict toxicity in patients treated with NSAIDs and COX2 inhibitors, in particular.

The efficacy and toxicity of NSAIDs are a direct consequence of the inhibition of the prostaglandin H synthase (PTGS) enzymes (often referred to as COX enzymes) [29]. NSAID toxicity has been shown to be dose and treatment duration dependent and is compound specific [29,33]. The exact mechanism of the apparently increased ‘COX inhibitor’-associated cardiovascular risk is still controversial, although accumulating evidence suggests that it involves blood pressure elevation and an effect on endothelial function, both of which are likely to be related to the choice of the drug and the dose and duration of treatment [34]. Although NSAIDs inhibit both COX1 and COX2, the inhibition of COX2 can inhibit the production of protective prostaglandins and result in reduced prostacyclin (PGI₂) production, a vasodilator and modulator of platelet activation, without reducing COX1-dependent thromboxane (TXA₂) production, which contribute to platelet aggregation and vasoconstriction [35,36].

Genetic polymorphisms in inflammation-related pathways, particularly in prostaglandin synthesis, can potentially help discern patients who will benefit most from NSAID therapy while minimizing the attendant toxicities [17,37]. However, few studies have reported on the associations between genetic polymorphisms and the risks of NSAID-induced adverse events, mainly for aspirin [17]. For two *COX1* SNPs, A842G and C50T, an increased sensitivity to aspirin has been observed. Lower prostaglandin synthesis capacity has been shown but lacked statistical significance in the association with gastrointestinal toxicity (e.g. bleeding peptic ulcer) [38]. There are some known genetic variants for aspirin resistance, the thromboxane A₂ receptor gene (*TXA2*), the platelet activating factor acetylhydrolase gene (*PA-FAH*), and the gene for coagulation factor XIII (*F13B*) [39,40]; however, data on the frequency of gastrointestinal events in these variants are still lacking and further large-scale clinical trials are required. In the present exploratory study we observed a significantly increased risk for adenoma recurrence in carriers of the *COX1* rs10306110 (A918G) variant allele. This association was restricted to the placebo group. The rs10306110 variant is linked to the nonsynonymous *COX1* polymorphism rs3842787 (P17L; $r^2 = 1.0$), which causes a proline to leucine amino acid change at position 17 of the COX1 protein. Interestingly, the altered protein has been associated with modifications in the effects of aspirin and NSAID use on colorectal polyp risk [41], changed bioavailability of aspirin, as well as increased bleeding in patients undergoing elective coronary angiography [42,43].

In our study, we examined several polymorphisms in other genes related to prostaglandin synthesis: *PTGS2* (*COX2*) and *PGES* genes and those involved in other related pathways (arachidonate and leukotriene). COX2 is a key enzyme in eicosanoid metabolism, which converts arachidonic acid released from membrane phospholipids into PGG₂ and PGH₂. Eicosanoids have important functions in a variety of physiologic and pathologic conditions, including inflammation, cardiovascular homeostasis, reproduction, and carcinogenesis [44]. The fate of PGH₂ and the distribution of prostanoids (prostaglandins, thromboxane, and prostacyclin) formed by it are cell-specific and depends upon specific receptor-dependent signaling pathways defining their biological responses [45]. COX2-derived prostanoid products have divergent effects on the molecular and cellular mechanisms underlying disease pathogenesis; for example, through its interaction with the E-prostanoid receptor (EP4) on macrophages, COX2-derived PGE₂ suppresses chemokine production [46]. Our

data showed that variants of the *EP4* gene (rs4133101, rs13186505) were significantly associated with an increased risk for gastrointestinal toxicity in the celecoxib treated group. The biological relevance of these SNPs in the *EP4* gene remains to be determined.

Inhibition of COX2 can also theoretically increase the flux of arachidonic acid through the lipoxygenase pathways. The 12-lipoxygenase, 15-lipoxygenase, and 5-lipoxygenase have key roles in leukotriene synthesis and the development of atherosclerosis [47]. Both ALOX12 and ALOX15 have been reported to contribute to low-density lipoprotein oxidation and play a role in atherogenesis [48]. A study by Mehrabian *et al.* [49] demonstrated that the gene for 5-lipoxygenase is a major contributor to atherosclerosis in mice, whereas in humans, variant 5-lipoxygenase genotypes-tandem promoter repeats of Sp-1 binding motifs identify a subpopulation of individuals with increased risk for atherosclerosis [50]. Thus, a polymorphism in the 5-lipoxygenase gene promoter could be related to atherosclerosis in humans. Although the *ALOX15* (rs4796535) variant was associated with a two-fold increased risk for adenoma recurrence, we did not observe any associations between this variant and cardiovascular toxicity.

To the best of our knowledge, this is the first study analyzing a large number of genetic variants to reveal associations between genetic polymorphisms and clinical outcomes among colorectal adenoma patients treated with COX2 inhibitors. A strength of our study is that we examined a comprehensive number of genes related to inflammation and colorectal carcinogenesis. Nevertheless, our study is limited by the small sample size available for genotyping analyses, because of which only strong signals reached significance. This reduces the statistical power to detect differences in the subgroup analyses. In addition, the small sample size increases the likelihood of false-positive results.

Conclusion

Our study reveals that genetic variability in prostaglandin and leukotriene syntheses (especially in *COX1*, *COX2*, and *ALOX12/15* genes) may play a role in adenoma recurrence. Many variant genotypes (particularly variants in *PGES*, *CRP*, *SRC*, and *GPX3*) were associated with increased risk for cardiovascular toxicity among patients on celecoxib. Few variant genotypes were associated with gastrointestinal toxicity in either group of patients. The associations suggested in this exploratory study were, in part, quite strong. Follow-up in additional trial populations should be undertaken to confirm or disprove these associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Polymorphisms significantly associated with adenoma recurrence. The single nucleotide polymorphisms (a) rs5275 (*COX2*), (b) rs4796535 (*ALOX15*), (c) rs6683455 (*IL23R*), (d) rs1365613 (*PGDH*), (e) rs7349744 (*PGDH*), and (f) rs1050450 (*GPX1*) were significantly associated with adenoma recurrence in both treatment groups.

Table 1

Baseline characteristics

	Frequency [<i>n</i> (%)]
Age (years) [mean (SD)]	62.7 ($\pm$ 9.9)
Sex	
Male	76 (65)
Female	41 (35)
Ethnicity	
Ashkenazi Jewish origin	85 (73)
Sephardic Jewish origin	20 (17)
Mixed Jewish origin (Ashkenazi + Sephardic)	6 (5)
Any other origin	5 (4)
Unknown	1 (1)
Treatment	
Celecoxib	69 (59)
Placebo	48 (41)
Adenoma recurrence ^a	
Yes	46 (39)
No	71 (61)
Gastrointestinal symptoms/toxicity ^{b,c}	
Yes	49 (42)
No	68 (58)
Cardiovascular symptoms/toxicity ^{b,d}	
Yes	22 (19)
No	95 (81)

^a Adenoma recurrence is defined as recurrence within 3.5 years after enrollment into the study.

^b Some participants had more than one symptom/toxicity.

^c Gastrointestinal toxicity and symptoms were defined as gastrointestinal bleeding, gastritis or duodenitis, upper or lower gastrointestinal ulceration, hemorrhage, or gastrointestinal symptoms (vomiting, diarrhea, constipation, heartburn, reflux, abdominal pain).

^d Cardiovascular toxicity and symptoms included angina, dyspnea, coronary artery stenosis, hypercoagulation, cerebrovascular disease, myocardial infarction or ischemia, peripheral vascular disease, circulatory collapse, hypertension, ventricular and atrial fibrillation, venous thrombosis, or thromboembolism.

Table 2Polymorphisms associated with adenoma recurrence^a

SNP	Location	Both treatments ^b		Celecoxib ^c		Placebo ^c	
		HR (95% CI) ^d	P-value ^e	HR (95% CI) ^d	P-value ^e	HR (95% CI) ^d	P-value ^e
Primary genes of interest ^f							
<i>COX1</i> rs10306110	A>C - near gene-5'	0.36 (0.05-2.71)	0.32	NA ^g	NA ^g	15.46 (1.58-151.48)	0.02
<i>COX1</i> rs10306122	T>C - intron	2.46 (0.93-6.52)	0.07	1.97 (0.67-5.80)	0.22	15.58 (1.59-152.67)	0.02
<i>COX1</i> rs10306164	G>T - intron	1.50 (0.79-2.85)	0.22	0.87 (0.32-2.36)	0.79	2.53 (1.02-6.29)	0.05
<i>COX1</i> rs1236913	C>T - W8R	1.68 (0.71-3.98)	0.24	0.48 (0.06-3.55)	0.47	3.78 (1.32-10.80)	0.01
<i>COX1</i> rs1330344	G>A - near gene-5'	1.32 (0.69-2.52)	0.41	0.74 (0.30-1.82)	0.51	2.75 (1.12-6.75)	0.03
<i>COX1</i> rs3119773	T > C - intron	0.93 (0.39-2.22)	0.86	NA ^g	NA ^g	4.58 (1.69-12.44)	<0.01
<i>COX2</i> rs4648268	G>A - intron	1.52 (0.64-3.62)	0.34	0.43 (0.06-3.23)	0.41	3.73 (1.26-11.11)	0.02
<i>COX2</i> rs5275	T>C - 3'-UTR	2.42 (1.12-5.22)	0.02	2.07 (0.76-5.68)	0.16	3.09 (0.88-10.82)	0.08
<i>COX2</i> rs689469	G>A - 3'-UTR	3.00 (1.16-7.76)	0.02	2.28 (0.67-7.72)	0.19	6.99 (1.39-35.20)	0.02
<i>EGFR</i> rs7801956	G>A - intron	1.23 (0.60-2.53)	0.57	0.73 (0.25-2.14)	0.56	2.65 (1.03-6.86)	0.04
Secondary genes of interest ^f							
<i>ALOX12</i> rs11078659	G>A - intron	0.70 (0.36-1.36)	0.29	0.38 (0.16-0.89)	0.03	2.33 (0.70-7.81)	0.17
<i>ALOX12</i> rs2073438	G>A - intron	1.24 (0.68-2.26)	0.49	0.76 (0.33-1.74)	0.52	2.38 (1.00-5.68)	0.05
<i>ALOX12</i> rs2292350	G>A - intron	0.75 (0.40-1.40)	0.36	1.35 (0.50-3.63)	0.56	0.41 (0.17-0.98)	0.05
<i>ALOX15</i> rs4796535	A>G - intron	2.27 (1.17-4.39)	0.01	1.76 (0.72-4.28)	0.22	3.56 (1.33-9.56)	0.01
<i>IL23R</i> rs10889675	C>A - intron	0.90 (0.39-2.10)	0.81	NA ^g	NA ^g	3.08 (1.05-9.04)	0.04
<i>IL23R</i> rs6683455	T>C - near gene-5'	2.40 (1.32-4.38)	<0.01	2.85 (1.20-6.77)	0.02	2.51 (1.06-5.96)	0.04
<i>IL23R</i> rs7518660	G>A - intron	0.70 (0.37-1.33)	0.27	0.35 (0.14-0.88)	0.03	1.34 (0.54-3.32)	0.53
<i>PGDH</i> rs7349744	G>A - intron	0.48 (0.26-0.89)	0.02	0.32 (0.13-0.80)	0.01	0.72 (0.30-1.72)	0.45
<i>PGDH</i> rs1365613	A>G - intron	0.53 (0.28-0.99)	0.05	0.47 (0.19-1.13)	0.09	0.72 (0.29-1.79)	0.48
<i>PGDH</i> rs45567139	C>A - intron	2.19 (0.67-7.18)	0.20	1.01 (0.13-7.67)	0.99	12.44 (2.16-71.55)	<0.01
<i>PLA2A</i> rs9657930	A>C - intron	2.72 (1.01-7.34)	0.05	1.86 (0.41-8.34)	0.42	3.98 (0.84-18.74)	0.08
<i>SRC</i> rs6063022	C>T - near gene-5'	1.74 (0.96-3.15)	0.07	0.90 (0.38-2.12)	0.81	3.38 (1.35-8.50)	<0.01
Tertiary genes of interest ^f							
<i>GPX1</i> rs1050450	C>T - P200L	0.51 (0.27-0.96)	0.04	0.52 (0.20-1.33)	0.17	0.46 (0.19-1.09)	0.08

Significant associations (P -values<0.05) are given in bold.

CI, confidence interval; HR, hazard ratio; NA, not applicable; SNP, single-nucleotide polymorphism.

^a Adenoma recurrence was defined as recurrence within 3.5 years after study initiation.^b Adjusted for treatment, age, and sex.^c Adjusted for age and sex.

^d Risk ratios refer to the risk associated with heterozygous/homozygous variant genotypes compared with wild type (reference group).

^e All polymorphisms with *P*-values less than 0.05 in any group are shown here.

^f Primary genes of interest (very strong prior hypothesis): *COX1*, *COX2*, *EGFR*, *PGES*, *TBXAS*. Secondary genes of interest (strong hypothesis): *ALOX5*, *ALOX12*, *ALOX15*, *FLAP*, *CRP*, *SRC*, *EP2*, *EP4*, *PGDH*, *IL23R*, *ODC1*, *PLA2A*. Tertiary genes of interest (moderately strong hypothesis): *SEPP1*, *GPX1*, *GPX2*, *GPX3*, *GPX4*, *WNT6*, *WNT10A*, *HER2*.

^g Maximum likelihood estimate does not exist.

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Table 3Polymorphisms significantly associated with gastrointestinal toxicity and symptoms^a

SNP	Location	Gastrointestinal toxicity/symptoms ^a					
		Both treatments ^b		Celecoxib ^c		Placebo ^c	
		OR (95% CI) ^d	P-value ^e	OR (95% CI) ^d	P-value ^e	OR (95% CI) ^d	P-value ^e
Primary genes of interest ^f							
<i>EGFR</i> rs2072454	C>T - N158N	2.43 (1.05-5.63)	0.04	2.51 (0.84-7.51)	0.10	1.86 (0.45-7.71)	0.39
Secondary genes of interest ^f							
<i>ALOX15</i> rs2255888	C>T - near gene-5'	2.28 (1.01-5.11)	0.05	3.49 (1.20-10.16)	0.02	1.20 (0.33-4.36)	0.79
<i>ALOX15</i> rs2619118	A>G - Intron	2.30 (0.85-6.2)	0.10	1.00 (0.29-3.51)	1.00	7.77 (1.34-45.07)	0.02
<i>EP4</i> rs4133101	C>T - near gene-5'	1.34 (0.55-3.29)	0.52	5.52 (1.13-27.06)	0.04	0.20 (0.04-1.01)	0.05
<i>EP4</i> rs13186505	G>A - near gene-5'	2.79 (1.23-6.32)	0.01	3.35 (1.11-10.10)	0.03	1.86 (0.53-6.61)	0.34
<i>FLAP</i> rs4254165	G>A - intron	0.30 (0.13-0.68)	<0.01	0.55 (0.19-1.55)	0.26	0.12 (0.03-0.50)	<0.01
<i>FLAP</i> rs3803278	A>G - intron	0.40 (0.18-0.89)	0.03	0.47 (0.16-1.38)	0.17	0.32 (0.09-1.10)	0.07
<i>PGDH</i> rs9312555	A>G - 3'-UTR	0.44 (0.19-1.03)	0.06	0.39 (0.12-1.22)	0.11	0.57 (0.15-2.18)	0.41
<i>SRC</i> rs7269342	T>C - intron	0.45 (0.20-1.00)	0.05	0.59 (0.20-1.74)	0.34	0.26 (0.07-0.96)	0.04
Tertiary genes of interest ^f							
<i>GPX3</i> rs3828599	C>T - Intron	0.43 (0.20-0.95)	0.04	1.09 (0.40-3.02)	0.87	0.11 (0.03-0.48)	<0.01
<i>GPX3</i> rs736775	T>C - near gene-3'	0.31 (0.13-0.71)	<0.01	0.50 (0.17-1.46)	0.20	0.14 (0.03-0.64)	0.01
<i>GPX3</i> rs8177406	A>G - 5'-UTR	0.37 (0.14-0.97)	0.04	0.21 (0.05-0.85)	0.03	0.55 (0.13-2.43)	0.43
<i>GPX3</i> rs8177447	G>A - intron	0.72 (0.32-1.62)	0.43	1.85 (0.64-5.36)	0.26	0.24 (0.06-0.95)	0.04
<i>SEPP1</i> rs13168440	T>C - intron	1.70 (0.58-4.92)	0.33	0.61 (0.11-3.40)	0.57	7.30 (1.19-44.85)	0.03

Significant associations (P -values<0.05) are given in bold.

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

^aMost frequent toxicities/symptoms ($n>5$): abdominal pain, heartburn, diarrhea, reflux, constipation, vomiting.^bAdjusted for treatment, age, and sex.^cAdjusted for age and sex.^dRisk ratios refer to the risk associated with heterozygous/homozygous variant genotypes compared with wild type (reference group).^eAll polymorphisms with P -values less than 0.05 in any group are shown here.^fPrimary genes of interest (very strong prior hypothesis): *COX1*, *COX2*, *EGFR*, *PGES*, *TBXAS*. Secondary genes of interest (strong hypothesis): *ALOX5*, *ALOX12*, *ALOX15*, *FLAP*, *CRP*, *SRC*, *EP2*, *EP4*, *PGDH*, *IL23R*, *ODC1*, *PLA2A*. Tertiary genes of interest (moderately strong hypothesis): *SEPP1*, *GPX1*, *GPX2*, *GPX3*, *GPX4*, *WNT6*, *WNT10A*, *HER2*.

Table 4

Polymorphisms significantly associated with cardiovascular toxicity and symptoms^a

SNP	Location	Cardiovascular toxicity/symptoms ^a					
		Both treatments ^b		Celecoxib ^c		Placebo ^c	
		OR (95% CI) ^d	P-value ^e	OR (95% CI) ^d	P-value ^e	OR (95% CI) ^d	P-value ^e
Primary genes of interest ^f							
COX1 rs10306164	G>T - intron	1.29 (0.44-3.79)	0.65	0.24 (0.03-2.11)	0.20	5.21 (1.04-26.04)	0.04
EGFR rs4947984	G>A - intron	2.33 (0.8-6.84)	0.12	1.28 (0.29-5.69)	0.75	6.24 (1.08-36.13)	0.04
EGFR rs4947986	G>A - intron	0.23 (0.07-0.74)	0.01	0.32 (0.08-1.34)	0.12	0.12 (0.01-1.10)	0.06
EGFR rs3752651	T>C - intron	0.25 (0.07-0.92)	0.04	0.36 (0.07-1.88)	0.23	0.14 (0.02-1.31)	0.08
PGES rs2302821	A>C - 3'-UTR	3.84 (1.02-14.53)	0.05	8.09 (1.49-44.04)	0.02	1.04 (0.09-11.90)	0.97
PGES rs2241271	C>T - intron	2.48 (0.92-6.68)	0.07	4.24 (1.08-16.54)	0.04	1.19 (0.25-5.68)	0.83
Secondary genes of interest ^f							
ALOX12 rs11078659	G>A - intron	0.62 (0.22-1.79)	0.38	0.23 (0.06-0.92)	0.04	3.52 (0.38-32.48)	0.27
ALOX15 rs11568141	G>A - intron	5.38 (1.51-19.11)	<0.01	2.71 (0.50-14.72)	0.25	20.57 (1.85-228.80)	0.01
CRP rs1800947	G>C - intron	6.86 (1.9-24.79)	<0.01	15.14 (2.54-90.12)	<0.01	2.29 (0.30-17.61)	0.42
FLAP rs4075131	A>G - intron	1.55 (0.58-4.14)	0.38	0.59 (0.16-2.15)	0.42	6.76 (1.33-34.42)	0.02
FLAP rs4293222	C>G - intron	0.82 (0.29-2.27)	0.70	0.24 (0.06-0.97)	0.04	4.59 (0.79-26.82)	0.09
FLAP rs3803278	A>G - intron	2.06 (0.75-5.63)	0.16	5.7 (1.22-26.51)	0.03	0.71 (0.16-3.12)	0.65
IL23R rs10789229	T>C - intron	0.27 (0.1-0.74)	0.01	0.16 (0.04-0.62)	<0.01	0.54 (0.12-2.40)	0.42
IL23R rs10889675	C>A - intron	4.48 (1.26-15.95)	0.02	2.24 (0.34-14.83)	0.40	9.25 (1.43-59.73)	0.02
IL23R rs11465810	A>C - intron	0.22 (0.07-0.75)	0.01	0.12 (0.02-0.63)	0.01	0.52 (0.09-2.96)	0.46
IL23R rs1884444	G>T - Q94H	0.7 (0.24-2.03)	0.51	0.20 (0.05-0.83)	0.03	5.13 (0.56-47.01)	0.15
IL23R rs6693831	C>T - intron	2.57 (0.94-7)	0.06	1.62 (0.43-6.18)	0.48	5.08 (1.00-25.72)	0.05
PGDH rs45535235	G>A - near gene-3'	0.24 (0.06-0.87)	0.03	0.28 (0.05-1.46)	0.13	0.17 (0.02-1.5)	0.11
PGDH rs45567139	C>A - intron	11.88 (1.86-75.76)	<0.01	6.39 (0.73-55.9)	0.09	NA ^g	NA ^g
PLA2A rs5637	G>A - S98S	3.41 (1.14-10.19)	0.03	0.79 (0.14-4.31)	0.78	25.31 (3.32-193.01)	<0.01
SRC rs6017916	A>C - near gene-5'	0.45 (0.16-1.27)	0.13	0.96 (0.26-3.53)	0.95	0.10 (0.01-0.98)	0.04
SRC rs6017996	A>G - intron	2.36 (0.87-6.41)	0.09	6.61 (1.66-26.36)	<0.01	0.71 (0.17-3.03)	0.64
SRC rs6018256	C>T - intron	3.34 (1.07-10.45)	0.04	10.71 (1.96-58.60)	<0.01	1.17 (0.19-7.11)	0.87
SRC rs6018257	C>T - intron	3.1 (0.94-10.2)	0.06	8.51 (1.71-42.26)	<0.01	0.61 (0.06-6.27)	0.68
Tertiary genes of interest ^f							
GPX3 rs3828599	C>T - intron	3.02 (1.1-8.27)	0.03	2.84 (0.73-11.08)	0.13	3.46 (0.72-16.58)	0.12
GPX3 rs736775	T>C - near gene-3'	5.72 (1.24-26.43)	0.03	6.44 (0.76-54.39)	0.09	5.18 (0.56-48.23)	0.15
GPX3 rs3763013	A>G - near gene-3'	3.75 (1.16-12.11)	0.03	4.13 (0.81-21.03)	0.09	3.36 (0.61-18.52)	0.16

Significant associations (*P*-values<0.05) are given in bold.

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

^a Most frequent toxicity/symptom ($n>5$): chest pain.

^b Adjusted for treatment, age, and sex.

^c Adjusted for age and sex.

^d Risk ratios refer to the risk associated with heterozygous/homozygous variant genotypes compared with wild type (reference group).

^e All polymorphisms with P -values less than 0.05 in any group are shown here.

^f Primary genes of interest (very strong prior hypothesis): *COX1*, *COX2*, *EGFR*, *PGES*, *TBXAS*. Secondary genes of interest (strong hypothesis): *ALOX5*, *ALOX12*, *ALOX15*, *FLAP*, *CRP*, *SRC*, *EP2*, *EP4*, *PGDH*, *IL23R*, *ODC1*, *PLA2A*. Tertiary genes of interest (moderately strong hypothesis): *SEPP1*, *GPX1*, *GPX2*, *GPX3*, *GPX4*, *WNT6*, *WNT10A*, *HER2*.

^g Maximum likelihood estimate does not exist.